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**Diagnosis of inflammatory bowel disease: Potential role of molecular biometrics**

M’Koma AE. Advances and challenges in IBD Diagnosis

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**Abstract**

Accurate diagnosis of predominantly colonic inflammatory bowel disease (IBD) is not possible in 30% of patients. For decades, scientists have worked to find a solution to improve diagnostic accuracy for IBD, encompassing Crohn’s colitis (CC) and ulcerative colitis. Evaluating protein patterns in surgical pathology colectomy specimens of colonic mucosal and submucosal compartments, individually, has potential for diagnostic medicine by identifying integrally independent, phenotype-specific cellular and molecular characteristics. Mass spectrometry (MS) and imaging (I) MS are analytical technologies that directly measure molecular species in clinical specimens, contributing to the in-depth understanding of biological molecules. The biometric-system complexity and functional diversity is well suited to proteomic and diagnostic studies. The direct analysis of cells and tissues by Matrix-Assisted-Laser Desorption/Ionization (MALDI) MS/IMS has relevant medical diagnostic potential. MALDI-MS/IMS detection generates molecular signatures obtained from specific cell types within tissue sections. Herein discussed is a perspective on the use of MALDI-MS/IMS and bioinformatics technologies for detection of molecular-biometric patterns and identification of differentiating proteins. I also discuss a perspective on the global challenge of transferring technologies to clinical laboratories dealing with IBD issues. The significance of serologic-immunometric advances is also discussed.

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**Key words**: Inflammatory bowel disease; Diagnosis; Advances and challenges;MALDI-MS/IMS; Molecular biometrics; Immunometrics

**Core tip:** Pouch surgery (the restorative proctocolectomy andileal pouch-anal anastomosis for the curative surgical treatment of ulcerative colitis and familial adenomatous polyposis) replaces the colon and rectum after proctocolectomy with a pouch constructed from the distal small bowel (ileum) and sutured to the anal canal above the dentate/pectinate line preserving the anal sphincters. The operation restores gut continuity, defecation, deferral, and discrimination, if the diagnosis is correct, which is unpredictable in 30% of the colonic-inflammatory bowel disease-patients. Mass spectrometry and imaging mass spectrometry are groundbreaking, non-invasive analytical technologies with the ability to directly measure individual molecular species in complex clinical specimens. These technologies provide quantitative and qualitative analysis of cellular systems, and allow differentiation between disease and normal molecules from the same organ. These characteristics offer diagnostic and prognostic value for clinical medicine.

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**INTRODUCTION**

***Inflammatory bowel disease***

Inflammatory bowel disease (IBD) comprises Crohn’s colitis (CC) and ulcerative colitis (UC), a group of diseases of the gastrointestinal (GI) tract characterized by chronic relapsing and remitting inflammation[[1](#_ENREF_1),[2](#_ENREF_2)]. IBD affects as many as 1.6 million persons in the United States and 2.2 million in Europe. The incidence is increasing worldwide[1-5]. In spite of advances in IBD-therapy, IBD hospitalizations and surgery rates in the United States have increased significantly since 1990[[6]](#_ENREF_6). IBD is one of the five most prevalent GI disease burdens in the United States, with annual overall health care costs of more than $1.7 billion[7,8]. One to two of every 1000 people in developed countries are affected with IBD[[9]](#_ENREF_9), and global rates seem to be increasing[1,10-12], attributable to the rapid modernization and Westernization of the population[1](#_ENREF_1). These chronic diseases result in significant morbidity and mortality, compromising quality of life and life expectancies. While there is no drug for cure for these diseases, the last three decades have seen major advances in the molecular understanding intestinal immune responses and how they relate to IBD. This, in turn, has led to the development and refinement of several new treatments. Most significant has been the development of restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis (IPAA). The pelvic pouch surgery allows for the removal of the entire colon while maintaining transanal fecal continence without a permanent diverting loop ileostomy. The success of RPC (judged by the entire removal of a diseased colon while preserving gastrointestinal continuity, bowel evacuation, continence and fertility) restores physiological function and greatly improves patient health quality of life (HQoL). Successful RPC also frees the healthcare system from the immense burden of current lifelong, non-curative treatments. These outcomes are dependent on a correct diagnosis and meticulous surgical techniques available at well-established IBD centers[13-15].

The etiology of IBD poorly understood. The general consensus holds that IBD is an automatic dysfunction triangle of antigen and antibody reaction against mucosal response to commensal bacteria. The fundamental question is why the immune system responds aggressively to harmless, ever-present bacteria, releasing complex mixes of cytokines, chemokines and other substances that cause inflammation. One possible explanation is that the gut immune system is compromised because of defects in the barrier function of the gut luminal epithelium[[16](#_ENREF_16)]. Although the etiology of IBD is at present not delineated, histopathologic and clinical assessments demonstrate that CC and UC, the two major classifications of IBD, are indeed distinct entities and have different causes and discrete mechanisms of tissue damage and treatment[16-21]. UC results in inflammation and ulcerations in the mucosal and to a lesser degree submucosal linings of the colon and rectum. CC differs in that it may result in inflammation deeper within the intestinal wall (transmullary) and can occur in any parts of the digestive system (including the mouth, esophagus, stomach, duodenum, small intestine, colon and rectum). Further, Crohn’s may also involve other organs outside the GI system through fistulization[22,23]. Crohn's is diagnosed in at least four patients per 100000 in the United States, and the incidence and prevalence is rising worldwide[1,10-12].

***Diagnosis challenges in IBD***

The current standard of care for IBD treatment is based on steroids and immunosuppressant agents, including glucocorticoids (GCs), aminosalicylates, cyclosporine, methotrexate and biologic agents such as anti-TNF-α and IL1-β. The correct IBD diagnosis is crucial for providing correct, evidence-based treatment, since treatment response and complications differ significantly among UC and CC patients[[24](#_ENREF_24)]. The absence of specific phenotypes indicating the particular disease condition challenges pathologist interpretation and categorization of tissue morphology, subsequently leading to difficulties in diagnosis and consistent standard of care[[25]](#_ENREF_25). However, despite advances in our understanding of the genetic[16,26], immunologic[26,27], and environmental[1,24,28] influences that may trigger complex IBD pathologies, to date there is no single indicator sensitive enough to accurately and consistently delineate CC and UC. The available data indicate that genetic factors determine an individual’s susceptibility to developing IBD, and environmental factors elicit cellular responses that drive disease progression. Histological evaluation and interpretation of tissue provides insights that directly impact care[[25]](#_ENREF_25). Pathologists rely mainly on microscopic visual inspection and interpretation of stained and/or dyed tissue sections to identify the disease state of a patient sample[29,30]. Inherently, these procedures possess a significant degree of subjectivity[[31]](#_ENREF_31) and are fraught with problems[31,32]. Rigorous training in pathology subspecialties has attempted to improve the standard of care and avoid unnecessary mistakes[[33]](#_ENREF_33). Despite these extremely thorough standards, inevitable situations arise in which objectivity cannot be guaranteed and where significant disagreement occurs between specialists[[34]](#_ENREF_34). This challenge is common for IBD patient populations[13,15,35,36] To date, there is no single, absolute diagnostic test[37,38]. A diagnosis should neither be based on nor excluded by any one variable or result[[39]](#_ENREF_39). The consensus statement on the diagnosis, management and surveillance of both CC[[40]](#_ENREF_40)and UC[41](#_ENREF_41) recommend that “multiple” tissue biopsies from at list five sites around the colon and rectum should be collected for support of a reliable diagnosis. Of these six sites a minimum of two samples from each should be sampled[40,41]. Although the procedure is reliable, it is invasive and uncomfortable to the patients.

***Inaccurate diagnosis in IBD and consequences***

When IBD predominantly involves the colon, differentiation between CC from UC is often challenging. Inaccurate diagnoses are estimated to occur in 30% of IBD patients[[42](#_ENREF_42)[,43]](#_ENREF_43). In most cases the diagnostic uncertainty arises from the overlap of clinical and histologic features, making CC appear like UC[[44]](#_ENREF_44" \o "Geboes, 2009 #4020). This scenario is particularly relevant to young children, a population in which IBD consists of up to 80%. The differentiation between UC and CC relies on a compilation of clinical, radiologic, endoscopic, and histopathologic interpretations[[40]](#_ENREF_40); a compilation that is not always accurate. An estimated 15% of IBD patients are indistinguishable and are labeled as ‘‘indeterminate colitis’’ (IC)[45-47]. In addition, another 15% of the colonic IBD cases that undergo pouch surgery resulting from a definitive UC diagnosis (based on the pathologist’s initial designation of endoscopic biopsies and colectomy specimen) will have their original UC diagnosis changed to CC based on the postoperative follow-up when clinical and histopathology changes indicate development of CC in the ileal pouch[15,35,36,48,49]. One-half of these patients will require pouch excision or diversion[[49]](#_ENREF_49).

Because of the unpredictable nature of IBD, side effects of medications, and potential complications, some of which may end in sudden incapacitation, IBD is becoming a global health concern. Distinguishing between CC and UC is critical to therapy. The clinical experience suggests that identifying patients with CC and positive outcomes after pouch surgery is arduous. Thus, RPC should be contraindicated for CC patients, whereas ileal pouch-anal anastomosis (IPAA) is standard acceptable care for patients with UC and IC who are predicted likely to develop UC. Inevitably, pouch complications are significantly higher in patients with CC (± 64%) and IC (± 43%) *vs* patients having UC (± 22%) (*P < 0.05*)[[46](#_ENREF_46),[47](#_ENREF_47),[49](#_ENREF_49)]. This diagnostic dilemma and the potential morbidity from a wrong diagnosis and unnecessary and/or inappropriate surgical interventions underscore the importance of research strategy focused at improving diagnosis of the colitides using molecular biometrics[[42](#_ENREF_42),[50](#_ENREF_50)-[52](#_ENREF_52)].

***Clinico-histopathologic findings in Crohn’s colitis***

Crohn’s colitis is recognized to encompass a heterogeneous group of disorders[[38](#_ENREF_38)]. Usually CC is segmental with deep inflammation where the disease activity is transmural, with lymphoid composite extending to the sub-serosa. The Montreal classification[[53](#_ENREF_53)] and the Paris pediatric modification[[54](#_ENREF_54)] have brought consistency to definitions of subtypes of CC and of colitides. It is noteworthy that both the Montreal and Paris classifications rely on the location of gross disease, *i.e.*, visible lesions with more than a few aphthous ulcers. Patterns of macroscopic involvement, rather than microscopic, have been useful traditionally in predicting clinical course, as exemplified by the tendency of small bowel disease, particularly, to stricture over time. Despite the fact that microscopic involvement does not define subtypes of CC, the role of histology in the diagnosis of CC does differ according to the anatomic location of macroscopic disease[[38](#_ENREF_38)].

Histologic features useful for the diagnosis of CC have been reviewed by Griffiths, (Table 1)[[38](#_ENREF_38)] but, according to Van Assche et al. presented at The second European evidence-based Consensus on the diagnosis and management of Crohn’s colitis[[**40**](#_ENREF_40)], there are no data available as to how many of these features must be present to allow a firm diagnosis[[40](#_ENREF_40)]. Focal (discontinuous) chronic (lymphocytes and plasma cells) inflammation and patchy chronic inflammation, focal crypt irregularity (discontinuous crypt distortion) and granulomas (not related to crypt injury) are the generally accepted microscopic features which allow a diagnosis of CC[[40](#_ENREF_40" \o "Van Assche, 2010 #4015)]. Within one histologic section, inflammation may be immediately adjacent to an uninflamed microscopic “skip area”. Mucosal changes may resemble ulcerative or infectious colitis with infiltration of the crypts by polymorphonuclear leukocytes (cryptitis or crypt abscesses), and distortion of crypt architecture. Granulomas (collections of monocytes/macrophages) in the lamina propria (not associated with crypt injury) are a corroborating feature of suspected Crohn’s after exclusion of identifiable infectious etiology, but reported prevalence in mucosal biopsies at time of first diagnosis varies. The likelihood of finding granuloma is a function of the number of specimens taken, the number of sections examined, and the definition of a granuloma. Granulomas occur more commonly in the submucosa than the mucosa[[55](#_ENREF_55" \o "Rubio, 2007 #4018)]. Hence, they are observed in 60% of surgical specimens but relevant to the question of histology for diagnosis, in only 20%-40% of mucosal biopsies[[55](#_ENREF_55" \o "Rubio, 2007 #4018)]. Moreover, according to Griffiths *et al*[[38](#_ENREF_38)] data indicating clinical significance or prognostic value of presence or absence of granulomata are lacking.

***Clinico-histopathologic findings in ulcerative colitis***

The classic microscopic features in untreated UC (and CC hard criteria) used for diagnosis, as outlined by Robert Odze[[56](#_ENREF_56" \o "Odze, 2003 #4265)], and are depicted in Table 2. Clinically, the hallmark of UC is hematochezia[[57](#_ENREF_57),[58](#_ENREF_58)]. Additional clinical presentations include rectal tenesmus and incontinence, abdominal pain, severe inflammation of the rectum (proctitis), leukocytosis, hospitalization for total parenteral nutrition (TPN) and/or intravenous fluids correction, among others. Blood transfusion and corticosteroids are recommended when considering surgery (RPC and IPAA)[[58](#_ENREF_58)]. As mentioned earlier, in UC, inflammation is typically confined to the mucosal layer and to the lesser degree to the submucosa. Children with UCoften have evidence of chronicity, rectal frugality, and little or no architectural warping. In otherwise usual cases of UC, these conditions may lead to a confusion with CC[59-61].

***Current advances in biomarker discovery to delineate the colitides***

To date, there has been significant interest in attempting to identify molecular biomarkers that can accurately delineate CC and UC phenotypes. These studies have been minimally successful at identifying such biomarkers. In serum these include: PLGF-1 (placenta growth factor-1), IL-7, TGFβ1, and IL-12P40[[62-](#_ENREF_62" \o "Kader, 2005 #664)[67](#_ENREF_67)]. In biopsies obtained from the mucosa, they are Rho GD1α, desmoglein, pleckstrin, VDAC **(**voltage-dependent anion channel**),** 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), and [C10orf76](http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&cmd=search&term=79591&RID=TYJ43SNX01S&log$=geneexplicitnucl&blast_rank=1)[[68](#_ENREF_68),[69](#_ENREF_69)]. In stool they are calprotectin, PMN-elastate, lactoferrin, and S100A12[[65](#_ENREF_65),[70](#_ENREF_70)-74]. Clearly these biomarbiometrics represent an advance in the field of colitides research and have been used for clinical prognostic trials but have not been shown to delineate UC from a CC phenotype[[62](#_ENREF_64),[64](#_ENREF_62),[73](#_ENREF_73),[74](#_ENREF_74)]. Thus far, the above mentioned features reflect colitides intestinal inflammation and do not discriminate UC from the CC phenotype[[65](#_ENREF_65" \o "Langhorst, 2008 #2009)].

***Histology-directed proteomic advances***

Histology-directed MALDI MS is the first attempt ever used to analyze and compare mined proteins of the colonic mucosal and submucosal tissue layers individually, in order to differentiate between UC and CC[[42](#_ENREF_42),[50](#_ENREF_50)]. The normal topography of the colon and the layers used in mining and extraction of analytical extracts are illustrated in Figure 1. The basic steps of the methodology of histology-directed mass spectral protein profiling are outlined in Figure 2. Specialized MALDI MS offers directly the possibility of direct proteomic assessment of the tissue itself. The histologic layers of colectomy samples from patients with histologically and clinically confirmed UC and CC, with no ambiguity, are analyzed individually using MALDI MS for proteomic profiling. The results have successfully identified highly significant MALDI MS mass-to-charge ratio (*m/z*) signals in colonic tissue layers that appear to be phenotype-specific and are likely to help distinguish UC and CC[[42](#_ENREF_42),[50](#_ENREF_50)]. Pre-sequencing and identification proteomic pattern peaks from colonic mucosal or/and submucosal tissue section are depicted in Figure 3[[50](#_ENREF_50)]. These signatures do not correlate to tissue of origin and thus represent disease-specific markers. Some of these are found in colonic mucosa, from which endoscopic biopsies could be subjected to proteomic analysis. Other signatures come from the submucosa and could be used for proteomic studies of serum. Other protein-signatures were found in both tissue layers. Identifying proteomic patterns characteristic of one specific colitis phenotype will significantly improve our understanding of the mechanistic events associated with IBD.

It is unlikely that a single protein or small cluster of proteins will have the necessary: (1) specificity; (2) sensitivity; (3) discrimination; and (4) predictive capacity, to differentiate the heterogeneity of IBD[[69](#_ENREF_69" \o "Shkoda, 2007 #2008)]. However, if it were possible, it would require a technology that can accommodate sampling large patient cohorts, while accounting for patient variability. MS is an important profiling and identification tool for such studies[[75](#_ENREF_75" \o "Norris, 2013 #4007)]. As necessary as the tool is, subsequent analysis and validation methods will determine the actual success of a detection system intended for non-invasive screening and evaluating treatment efficacy. The overall goal of delineating IBD by proteomics is to illuminate the pathobiology underlying the colitides. More specifically, it is to identify patterns differentiating the colonic IBDs that exhibit overlapping clinical and histologic signs, but require different approaches of care. The anticipation is that this approach will eventually provide molecular biometrics of interest that can tell UC from CC through endoscopic biopsies and eventually create a serum biomarker tool assay for the identified peptide, if the protein(s) is (are) secretory and transposable. Better understandings of the bio-pathophysiologic mechanisms may allow new therapeutic and preventive avenues for maintenance or remission in IBD.

***Matrix-assisted laser desorption/ionization MS***

Specialized matrix-assisted laser desorption/ionization (MALDI) MS offers the possibility of direct proteomic assessment of the tissue itself[[76](#_ENREF_76)]. The molecular specificity and sensitivity of MS can image and map biomolecules present in tissue sections. Applying complementary techniques of immunochemistry and fluorescence microscopy to MALDI MS data can improve the analysis of spatial arrangements of molecules within biological tissues. Accordingly, MALDI technology has become a popular in biology research. It combines two technologies, the MALDI “soft” ionization source and the TOF (Time of Flight) mass analyzer. The former volatilizes and ionizes molecules using a laser, a target, and an organic compound called a matrix, while the latter technology measures an ion’s mass-to-charge ratio (*m/z*) by measuring the time it takes to reach a detector. MALDI TOF mass spectrometers come in two basic types: MALDI TOF MS and MALDI TOF/TOF MS. The latter enables tandem mass spectrometry (MS/MS) studies[[69](#_ENREF_69" \o "Shkoda, 2007 #2008)]. Thus a combination of markers may improve the chances of achieving IBD proteomics goals.

MS in combination with laser capture microdissection (LCM) is another important profiling and identification tool for such studies. It allows direct tissue analysis of biomolecules and large organic molecules which are often too fragile for conventional ionization methods. These techniques may significantly enhance diagnostic accuracy and provide the basis for future bio-physiologic elucidations in IBD.

***MALDI IMS***

MALDI IMS stands out as a tool for imaging metabolites in the biological and medical fields, and as a new tool for pathology in the molecular age[[77](#_ENREF_77)]. There are several advantages in IMS technology. First, IMS does not require labeling or specific probes. Second, it is a non-targeted imaging method, meaning unexpected metabolites can easily be imaged. Finally, several kinds of metabolites can be imaged simultaneously. The technique effectively provides a better visualization of the underlying mechanisms of biological processes of endogenous, small metabolites[[78](#_ENREF_78),[79](#_ENREF_79)] and large proteins[[80](#_ENREF_80),[81](#_ENREF_81)] in cells and tissues[[82](#_ENREF_82),[83](#_ENREF_83)]. It can determine the distribution of hundreds of unknown compounds in a single measurement[[79](#_ENREF_79),[84](#_ENREF_84)-[86](#_ENREF_86)]. Further, IMS is capable of three-dimensional molecular images which can be combined with established imaging techniques like [magnetic resonance imaging](http://en.wikipedia.org/wiki/Magnetic_resonance_imaging) ([MRI](http://en.wikipedia.org/wiki/MRI))[[87](#_ENREF_87),[88](#_ENREF_88)].

Due to the fact that the enormous molecular diversity of metabolite species is unknown, IMS technology is seemingly appropriate for localizing metabolites, whether they are from the molecule of interest or not[[78](#_ENREF_78),[89](#_ENREF_89),[90](#_ENREF_90)]. The emerging technique of MALDI IMS has the capability to distinguish between parent and metabolites while maintaining spatial distribution in various tissues[[91](#_ENREF_91),[92](#_ENREF_92)]. In spite of the promising advances of MALDI IMS for visual-imaging tiny metabolites, substantial concerns remain regarding its spatial resolution. The primary limitation results from the size/volume of the organic matrix crystal and analyte migration during the matrix application. There is also a lack of efficient computational techniques for constructing, processing, and visualizing large and complex 3D data which prevents experimenters from tapping its full potential[[93](#_ENREF_93" \o "Trede, 2012 #4115)]. In attempting to solve these important issues, researchers have devised another sophisticated method: a nanoparticle (NP)-assisted laser desorption/ionization (nano-PALDI)-based IMS, in which the matrix crystallization process is eliminated[[94](#_ENREF_94),[95](#_ENREF_95)]. The use of novel nano-PALDI has enabled scientists to image compounds with spatial resolution at the cellular level (15 μM; approximating the diameter of a laser spot)[[96](#_ENREF_96)].

***Serologic test advances***

To date, a lack of validated information prevents recommending the use of serologic assays to screen general population patients for undiagnosed gastrointestinal symptoms in IBD-settings. As has been made clear, no unique biomarkers yet exist for the delineation between CC and UC. Serologic tests, ANCAs and anti-microbial antibodies are inadequately sensitive and specific to contribute much to the diagnosis of CC or to its differentiation from UC.

ANCAs are immunoglobulin G (IgG) antibodies directed against cytoplasmic components of neutrophils[[97](#_ENREF_97" \o "Quinton, 1998 #4025)]. The association with colitides of a subset of ANCA with a perinuclear staining pattern on immunofluorescence studies (pANCA) was first recognized for UC, where it was detected in 60%-70% of patients[[97](#_ENREF_97)]. The specificity of perinuclear staining for colitides can be validated and confirmed by its disappearance after DNase (deoxyribonuclease) digestion of neutrophils. pANCA is considered a marker of the immunologic disturbance that underlies the development of chronic colonic inflammation, and should not be positive in acute self-limited, presumably infectious colitis.

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs), the first anti-microbial antibodies to be described in CC, are IgG and IgA antibodies that recognize mannose sequences in the cell wall of S. *cerevisiae* strain Su1. ASCA is detected in 50%-70% of CC patients overall, 10%-15% of UC patients and in 5%–10% of controls with other gastrointestinal disorders[[97](#_ENREF_97" \o "Quinton, 1998 #4025)]. Newer anti-microbial antibodies (Abs), which include Abs against *Pseudomonas* *fluorescens-*associated sequence (anti-I2), anti-outer membrane protein C of *Escherichia coli* (anti-OmpC), anti-outer membrane protein of *Bacteroides caccae* (anti-OmpW), and anti-flagellin Abs (anti-CBir1), may result false positive and be detected in patients who otherwise have negative serology, but are nonspecific and can be detected in patients with other diseases[[98](#_ENREF_98),[99](#_ENREF_99)].

Differentiation of CC from UC is clinically problematic because inflammation is only confined to the colon. pANCA is positive in up to 35% of patients with CC; ASCA is less often detected in patients with CC. Hence, the utility of combined ANCA/ASCA testing is less in the setting where it is needed most. In the one published study clearly reporting sensitivity, specificity, and predictive values of combined serologic testing, the sensitivity of ASCA+pANCA–serology for CC *vs* UC was only 32%[[97](#_ENREF_97)]. In a long-term follow-up of patients with IC, Joossens *et al*[[100](#_ENREF_100" \o "Joossens, 2002 #4028)] observed 26 patients who were ASCA+/pANCA– at baseline. Eight were later diagnosed with CC and 2 with UC, while the other 16 patients remained IC. The ASCA–/pANCA+ profile was even less helpful for definitive diagnosis[[100](#_ENREF_100" \o "Joossens, 2002 #4028)].

When using upper gastrointestinal (GI) biopsies, the differentiation between UC and CC is relatively straightforward in most of patients. In appropriate clinical settings, granulomatous inflammation in GI biopsies validates CC. In pediatric CC, granulomas may only be found in biopsies from the upper GI. Without routine upper endoscopy, these cases will be missed. If granulomas are not found, a diagnosis of CC or UC can be derived from endoscopic findings with histology combined with clinical and imaging determinations[[101](#_ENREF_101)]. Determining cases of IBD as CC, UC, or IC is largely a matter of nomenclature. Supporting a determination with evidence from endoscopies, magnetic resonance enterography, or other techniques, improves clinical labelling of the condition. The colitides are a continuum between CC and UC, with a variety of inflammations between. Teasing out overlapping genetic profiles for UC and CC will be critical to applying correct treatment more accurately than using current nomenclature categories based on a current standard of histology[100]. Application and refinement of the above technologies and techniques will improve the possibility of approaching patients with individualized options reducing ineffective or unnecessary surgery. Usage of molecular biometrics to differentiate diseases of the same organ[[38](#_ENREF_38),[102](#_ENREF_102),[103](#_ENREF_103)] is becoming ground breaking in improving diagnostic challenges in colonic IBD settings[[42](#_ENREF_42),[50](#_ENREF_50),[104](#_ENREF_104)]. IBD has no permanent drug cure and results in significant morbidity and mortality[[9](#_ENREF_9),[104](#_ENREF_104),[105](#_ENREF_105)]. UC is absolute colonic disease while CC can involve any part of the GI system from the mouth to the anus, which may transmurally involve partial to a full-thickness of the intestinal wall[[43](#_ENREF_1)] and other organs through fistulization[[106](#_ENREF_106)-[108](#_ENREF_108)]. These diseases share several clinical biometric signatures but have different causes, mechanisms of tissue damage, and treatment options[[16](#_ENREF_16),[109](#_ENREF_109)]. Therefore, accurate diagnosis is paramount for provision of correct pharmacologic therapy[[110](#_ENREF_110),[111](#_ENREF_111)] and surgical care[[112](#_ENREF_112)-[114](#_ENREF_114)].

**CONCLUSION**

The term “colitides” characterizes colonic IBD and comprises ulcerative colitis and Crohn’s colitis (UC and CC). The etiopathogenesis of UC and CC remains enigmatic. Diagnostic accuracy for distinguishing these two pathologies is still a significant problem in GI medicine and is hindered by a growing overlap of histopathological interpretation. Despite all efforts, many patients continue to remain undetermined as UC or CC, and are said to have indeterminate colitis. Differentiations of UC and CC are concluded from imprecise clinical, histopathologic, and other examinations. This results in speculative colitis staging and severity which cannot be conclusively differentiated in up to 30% of patients with IBD. CC and UC diagnostic features often overlap[[115](#_ENREF_1" \o "M'Koma A, 2013 #3942)] even after a thorough histological assessment, the current gold-standard for distinguishing type of inflammation (for CC: lack of non-specific inflammation not confined beyond mucosa and diffused or focal granulomatous etc. For UC: inflammation limited to the mucosa, diffuse infiltration of acute and chronic inflammatory cells in the mucosa, continuous damage from the rectum to proximal colon, *etc.*).

Treatment options for UC and CC differ significantly. Thus appropriate individualized prognosis and treatment requires accurate diagnosis. An estimated 90% of patients with IC undergo pouch surgery (RPC and IPAA) for fulminant colitis[[36](#_ENREF_36),[48](#_ENREF_48),[49](#_ENREF_49),[115](#_ENREF_115),[116](#_ENREF_116)] contrasting with 30% of patients in whom UC or CC was a correct diagnosis. Additionally, failure to recognize specific indicators of CC (*e.g.*, granulomas and transmural inflammation) often leads to mistakes in pathological interpretation[[24](#_ENREF_24),[36](#_ENREF_36)] This results in a reciprocal misdiagnosis rate of 15% (CC as UC: UC as CC). Adding = the 15% of cases labeled as IC accounts for nearly a third of the all IBD patients. Those undergoing surgery for a presumably confirmed diagnosis of UC subsequently are diagnosed postoperatively with recurrent CC in the ileal pouch[[36](#_ENREF_36)]. This is critical because functional failure and higher complication rates are estimated at up to 60%[[35](#_ENREF_35),[117](#_ENREF_117)-[123](#_ENREF_123)] and often require excision of the pouch with a permanent end ileostomy[[35](#_ENREF_35),[121](#_ENREF_121)-[124](#_ENREF_124)]. At this stage, patient health quality of life is significantly jeopardized for life.

There has been wide ranging interest in attempting to identify molecular biomarkers that can consistently delineate these diseases. These studies have been minimally successful at identifying quiescent or active IBD in serum[[62](#_ENREF_62)-[67](#_ENREF_67)], in mucosal biopsies[[68](#_ENREF_68),[69](#_ENREF_69)], and in fecal matter[[65](#_ENREF_65),70-[74](#_ENREF_74)]. Clearly these features represent an intriguing advance in the science of IBD for clinical disease prognostic purposes. However, these markers have not been shown to distinguish UC from CC phenotype[[62](#_ENREF_62),[64](#_ENREF_64),[73](#_ENREF_73),[74](#_ENREF_74)]. A serology panel including anti-neutrophil cytoplasmic antibodies (ANCA), perinuclear antineutrophil cytoplasmic autoantibodies (pANCA), anti-saccharomyces cerevisae IgG and IgA antibodies (ASCA), calgranulin (S100A12), anti-OmpC antibodies, fecal lactoferrin, calprotectin, and polymorphonuclear neutrophil elastase (PMN-e)[[65](#_ENREF_65)] is marketed as a promising approach to monitor disease activity and prognosis and may prove to be beneficial in the management of IBD. The specificity, sensitivity and diagnostic accuracy of these parameters with reference to clinical disease indices and/or endoscopically measured inflammation in IBD setting remain unclear. What we have learned to date is that: (1) Although not yet commercially available as tests, patients with CC are more likely than healthy control and/or IBD patients to be positive for a range of biomarkers such as S100A12 (calgranulin), ASCA, OmpC, CBir1, pseudomonas fluorescens protein, and pANCA[[125](#_ENREF_125),[126](#_ENREF_126)]. Significant increases of these proteins are noted during active intestinal inflammation. The greater the number of positive serologies and the higher the titer, the more aggressive the course. These biomarkers are also seen in an active UC[[127](#_ENREF_127" \o "Foell, 2003 #4100)]; (2) A combination of these biomarkers and a disease-specific activity index could promote the diagnostic accuracy in clinical medicine with reference to endoscopic inflammation but at present none are superior in the ability to reflect endoscopic inflammation[[70](#_ENREF_70)]; (3) These molecular biometrics significantly assist in predicting relapses in patients with confirmed IBD (active or quiescent)[[2](#_ENREF_2)-[5](#_ENREF_5),[17](#_ENREF_17),[21](#_ENREF_21),[128](#_ENREF_128)] but are not discriminatory between UC/CC; (3) Patients who are pANCA+ and ASCA- are more likely to have UC than CC, while in pANCA- and ASCA+ patients the reverse may be true[[67](#_ENREF_67)]. However, these biomarkers have not demonstrated clinical utility as predictors or monitoring tools of IBD activity[67].

At the present time there is insufficient biometric information to recommend use of serologic assays in screening for IBD in patients from the general population who have undiagnosed gastrointestinal symptoms. Further, no efficacy for the delineation of CC and UC clearly exist.

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**REFERENCES**

1 **M'Koma AE**. Inflammatory bowel disease: an expanding global health problem. *Clin Med Insights Gastroenterol* 2013; **6**: 33-47 [PMID: 24833941]

2 **Farrokhyar F**, Swarbrick ET, Irvine EJ. A critical review of epidemiological studies in inflammatory bowel disease. *Scand J Gastroenterol* 2001; **36**: 2-15 [PMID: 11218235 DOI: 10.1080/00365520150218002]

3 **Bernstein CN**, Wajda A, Svenson LW, MacKenzie A, Koehoorn M, Jackson M, Fedorak R, Israel D, Blanchard JF. The epidemiology of inflammatory bowel disease in Canada: a population-based study. *Am J Gastroenterol* 2006; **101**: 1559-1568 [PMID: 16863561 DOI: 10.1111/j.1572-0241.2006.00603.x]

4 **Heyman MB**, Kirschner BS, Gold BD, Ferry G, Baldassano R, Cohen SA, Winter HS, Fain P, King C, Smith T, El-Serag HB. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. *J Pediatr* 2005; **146**: 35-40 [PMID: 15644819 DOI: 10.1016/j.jpeds.2004.08.043]

5 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517 [PMID: 15168363 DOI: 10.1053/j.gastro.2004.01.063]

6 **Bewtra M**, Su C, Lewis JD. Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clin Gastroenterol Hepatol* 2007; **5**: 597-601 [PMID: 17382602 DOI: 10.1016/j.cgh.2007.01.015]

7 **Sandler RS**, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The burden of selected digestive diseases in the United States. *Gastroenterology* 2002; **122**: 1500-1511 [PMID: 11984534 DOI: 10.1053/gast.2002.32978]

8 **Baldassano RN**, Piccoli DA. Inflammatory bowel disease in pediatric and adolescent patients. *Gastroenterol Clin North Am* 1999; **28**: 445-458 [PMID: 10372276 DOI: 10.1016/S0889-8553(05)70064-9]

9 **Blumberg R**, Cho J, Lewis J, Wu G. Inflammatory bowel disease: an update on the fundamental biology and clinical management. *Gastroenterology* 2011; **140**: 1701-1703 [PMID: 21530735 DOI: 10.1053/j.gastro.2011.03.013]

10 **Burisch J**, Munkholm P. Inflammatory bowel disease epidemiology. *Curr Opin Gastroenterol* 2013; **29**: 357-362 [PMID: 23695429 DOI: 10.1097/MOG.0b013e32836229fb]

11 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864]

12 **Bernstein CN**, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut* 2008; **57**: 1185-1191 [PMID: 18515412 DOI: 10.1136/gut.2007.122143]

13 **Shen B**, Remzi FH, Brzezinski A, Lopez R, Bennett AE, Lavery IC, Queener E, Fazio VW. Risk factors for pouch failure in patients with different phenotypes of Crohn's disease of the pouch. *Inflamm Bowel Dis* 2008; **14**: 942-948 [PMID: 18300279 DOI: 10.1002/ibd.20409]

14 **M'Koma AE**, Wise PE, Muldoon RL, Schwartz DA, Washington MK, Herline AJ. Evolution of the restorative proctocolectomy and its effects on gastrointestinal hormones. *Int J Colorectal Dis* 2007; **22**: 1143-1163 [PMID: 17576578 DOI: 10.1007/s00384-007-0331-x]

15 **Shen B**. Crohn's disease of the ileal pouch: reality, diagnosis, and management. *Inflamm Bowel Dis* 2009; **15**: 284-294 [PMID: 18816633 DOI: 10.1002/ibd.20661]

16 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]

17 **Cobrin GM**, Abreu MT. Defects in mucosal immunity leading to Crohn's disease. *Immunol Rev* 2005; **206**: 277-295 [PMID: 16048555 DOI: 10.1111/j.0105-2896.2005.00293.x]

18 **Marx G**, Seidman EG, Martin SR, Deslandres C. Outcome of Crohn's disease diagnosed before two years of age. *J Pediatr* 2002; **140**: 470-473 [PMID: 12006965 DOI: 10.1067/mpd.2002.123281]

19 **Targan SR**, Karp LC. Defects in mucosal immunity leading to ulcerative colitis. *Immunol Rev* 2005; **206**: 296-305 [PMID: 16048556 DOI: 10.1111/j.0105-2896.2005.00286.x]

20 **Hyams JS**. Crohn's disease in children. *Pediatr Clin North Am* 1996; **43**: 255-277 [PMID: 8596683 DOI: 10.1016/S0031-3955(05)70405-3]

21 **Hyams JS**, Davis P, Grancher K, Lerer T, Justinich CJ, Markowitz J. Clinical outcome of ulcerative colitis in children. *J Pediatr* 1996; **129**: 81-88 [PMID: 8757566 DOI: 10.1016/S0022-3476(96)70193-2]

22 **Nosti PA**, Stahl TJ, Sokol AI. Surgical repair of rectovaginal fistulas in patients with Crohn's disease. *Eur J Obstet Gynecol Reprod Biol* 2013; **171**: 166-170 [PMID: 24011379 DOI: 10.1016/j.ejogrb.2013.08.011]

23 **Nielsen OH**, Rogler G, Hahnloser D, Thomsen OØ. Diagnosis and management of fistulizing Crohn's disease. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 92-106 [PMID: 19153563 DOI: 10.1038/ncpgasthep1340]

24 **Farmer M**, Petras RE, Hunt LE, Janosky JE, Galandiuk S. The importance of diagnostic accuracy in colonic inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 3184-3188 [PMID: 11095339 DOI: 10.1111/j.1572-0241.2000.03199.x]

25 **Cotran RSKV**, Collins T. Robbins pathologic basis of disease. 6th ed. Philadelphia: Saunders Co., 1999

26 **Pallone F**, Blanco Gdel V, Vavassori P, Monteleone I, Fina D, Monteleone G. Genetic and pathogenetic insights into inflammatory bowel disease. *Curr Gastroenterol Rep* 2003; **5**: 487-492 [PMID: 14602058 DOI: 10.1007/s11894-003-0038-2]

27 **Heller F**, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 2002; **17**: 629-638 [PMID: 12433369 DOI: 10.1016/S1074-7613(02)00453-3]

28 **Krishnan A**, Korzenik JR. Inflammatory bowel disease and environmental influences. *Gastroenterol Clin North Am* 2002; **31**: 21-39 [PMID: 12122733 DOI: 10.1016/S0889-8553(01)00003-6]

29 **Seldenrijk CA**, Morson BC, Meuwissen SG, Schipper NW, Lindeman J, Meijer CJ. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease: diagnostic implications. *Gut* 1991; **32**: 1514-1520 [PMID: 1773958 DOI: 10.1136/gut.32.12.1514]

30 **Theodossi A**, Spiegelhalter DJ, Jass J, Firth J, Dixon M, Leader M, Levison DA, Lindley R, Filipe I, Price A. Observer variation and discriminatory value of biopsy features in inflammatory bowel disease. *Gut* 1994; **35**: 961-968 [PMID: 8063225 DOI: 10.1136/gut.35.7.961]

31 **Rizzardi AE**, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz AP, Metzger GJ, Schmechel SC. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. *Diagn Pathol* 2012; **7**: 42 [PMID: 22515559 DOI: 10.1186/1746-1596-7-42]

32 **Gavrielides MA**, Gallas BD, Lenz P, Badano A, Hewitt SM. Observer variability in the interpretation of HER2/neu immunohistochemical expression with unaided and computer-aided digital microscopy. *Arch Pathol Lab Med* 2011; **135**: 233-242 [PMID: 21284444]

33 In: College of American Pathologists. Northfiield, IL, 2013 Available from: <http://www.cap.org>

34 **Staradub VL**, Messenger KA, Hao N, Wiley EL, Morrow M. Changes in breast cancer therapy because of pathology second opinions. *Ann Surg Oncol* 2002; **9**: 982-987 [PMID: 12464590 DOI: 10.1007/BF02574516]

35 **Keighley MR**. The final diagnosis in pouch patients for presumed ulcerative colitis may change to Crohn's disease: patients should be warned of the consequences. *Acta Chir Iugosl* 2000; **47**: 27-31 [PMID: 11432239]

36 **Wagner-Bartak NA**, Levine MS, Rubesin SE, Laufer I, Rombeau JL, Lichtenstein GR. Crohn's disease in the ileal pouch after total colectomy for ulcerative colitis: findings on pouch enemas in six patients. *AJR Am J Roentgenol* 2005; **184**: 1843-1847 [PMID: 15908540 DOI: 10.2214/ajr.184.6.01841843]

37 **Loginov AS**, Parfenov AI, Sivash ES, Tsvetkov VF, Zinov'ev OI. [Crohn's disease. The problem of early diagnosis]. *Ter Arkh* 1992; **64**: 82-85 [PMID: 1440317]

38 **Griffiths AM**. Challenging question: can we diagnose Crohn's disease without histology? *Dig Dis* 2013; **31**: 202-206 [PMID: 24030226 DOI: 10.1159/000353368]

39 **Baumgart DC**, Sandborn WJ. Crohn's disease. *Lancet* 2012; **380**: 1590-1605 [PMID: 22914295 DOI: 10.1016/S0140-6736(12)60026-9]

40 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.12.003]

41 **Stange EF**, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, Feakins R, Fléjou JF, Herfarth H, Hommes DW, Kupcinskas L, Lakatos PL, Mantzaris GJ, Schreiber S, Villanacci V, Warren BF. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *J Crohns Colitis* 2008; **2**: 1-23 [PMID: 21172194 DOI: 10.1016/j.crohns.2007.11.001]

42 **M'Koma AE**, Seeley EH, Washington MK, Schwartz DA, Muldoon RL, Herline AJ, Wise PE, Caprioli RM. Proteomic profiling of mucosal and submucosal colonic tissues yields protein signatures that differentiate the inflammatory colitides. *Inflamm Bowel Dis* 2011; **17**: 875-883 [PMID: 20806340 DOI: 10.1002/ibd.21442]

43 **Bousvaros A**, Antonioli DA, Colletti RB, Dubinsky MC, Glickman JN, Gold BD, Griffiths AM, Jevon GP, Higuchi LM, Hyams JS, Kirschner BS, Kugathasan S, Baldassano RN, Russo PA. Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. *J Pediatr Gastroenterol Nutr* 2007; **44**: 653-674 [PMID: 17460505 DOI: 10.1097/MPG.0b013e31805563f3]

44 **Geboes K**, Van Eyken P. Inflammatory bowel disease unclassified and indeterminate colitis: the role of the pathologist. *J Clin Pathol* 2009; **62**: 201-205 [PMID: 18952692 DOI: 10.1136/jcp.2008.059311]

45 **Burakoff R**. Indeterminate colitis: clinical spectrum of disease. *J Clin Gastroenterol* 2004; **38**: S41-S43 [PMID: 15115931 DOI: 10.1097/01.mcg.0000123991.13937.7e]

46 **Tremaine WJ**. Is indeterminate colitis determinable? *Curr Gastroenterol Rep* 2012; **14**: 162-165 [PMID: 22314810 DOI: 10.1007/s11894-012-0244-x]

47 **Mitchell PJ**, Rabau MY, Haboubi NY. Indeterminate colitis. *Tech Coloproctol* 2007; **11**: 91-96 [PMID: 17510748 DOI: 10.1007/s10151-007-0337-y]

48 **Marcello PW**, Schoetz DJ, Roberts PL, Murray JJ, Coller JA, Rusin LC, Veidenheimer MC. Evolutionary changes in the pathologic diagnosis after the ileoanal pouch procedure. *Dis Colon Rectum* 1997; **40**: 263-269 [PMID: 9118738 DOI: 10.1007/BF02050413]

49 **Brown CJ**, Maclean AR, Cohen Z, Macrae HM, O'Connor BI, McLeod RS. Crohn's disease and indeterminate colitis and the ileal pouch-anal anastomosis: outcomes and patterns of failure. *Dis Colon Rectum* 2005; **48**: 1542-1549 [PMID: 15937625 DOI: 10.1007/s10350-005-0059-z]

50 **Seeley EH**, Washington MK, Caprioli RM, M'Koma AE. Proteomic patterns of colonic mucosal tissues delineate Crohn's colitis and ulcerative colitis. *Proteomics Clin Appl* 2013; **7**: 541-549 [PMID: 23382084 DOI: 10.1002/prca.201200107]

51 **M'Koma A**, Wise PE, Schwartz DA, Washington MK, Muldoon RL, El-Rifai WM, Herline AJ. Gene Expression of Colonic Submucosa Differs Between the Inflammatory Colitides. *Cancer Research* 2011; **71** (8) [DOI: 10.1158/1538-7445.AM2011-LB-450]

52 **M'Koma AE**, Seeley EH, Washington MK, Schwartz DA, Muldoon RL, Herline AJ, Wise PE, Caprioli RM. Proteomic profiling of mucosal and submucosal colonic tissues yields protein signatures that differentiate the inflammatory colitides. *Inflamm Bowel Dis* 2011; **17**: 875-883 [PMID: 20806340 DOI: 10.1016/S0016-5085(09)61599-7]

53 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** SupplA: 5A-36A [PMID: 16151544]

54 **Levine A**, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; **17**: 1314-1321 [PMID: 21560194 DOI: 10.1002/ibd.21493]

55 **Rubio CA**, Orrego A, Nesi G, Finkel Y. Frequency of epithelioid granulomas in colonoscopic biopsy specimens from paediatric and adult patients with Crohn's colitis. *J Clin Pathol* 2007; **60**: 1268-1272 [PMID: 17293387 DOI: 10.1136/jcp.2006.045336]

56 **Odze R**. Diagnostic problems and advances in inflammatory bowel disease. *Mod Pathol* 2003; **16**: 347-358 [PMID: 12692200 DOI: 10.1097/01.MP.0000064746.82024.D1]

57 85 FS. Inflammatory Bowel Disease. In: GNJ Tytgat JBaSvD, editor. Proceedings of the Falk Symposium No 85; Den Haag, Netrhelands: Kluwer Academic Publishers, 1995

58 **M'Koma AE**. Follow-up results of hematology data before and after restorative proctocolectomy. Clinical outcome. *Dis Colon Rectum* 1994; **37**: 932-937 [PMID: 8076494 DOI: 10.1007/BF02052601]

59 **Glickman JN**, Bousvaros A, Farraye FA, Zholudev A, Friedman S, Wang HH, Leichtner AM, Odze RD. Pediatric patients with untreated ulcerative colitis may present initially with unusual morphologic findings. *Am J Surg Pathol* 2004; **28**: 190-197 [PMID: 15043308 DOI: 10.1097/00000478-200402000-00006]

60 **Holmquist L**, Ahrén C, Fällström SP. Clinical disease activity and inflammatory activity in the rectum in relation to mucosal inflammation assessed by colonoscopy. A study of children and adolescents with chronic inflammatory bowel disease. *Acta Paediatr Scand* 1990; **79**: 527-534 [PMID: 2386043 DOI: 10.1111/j.1651-2227.1990.tb11507.x]

61 **Finkelstein SD**, Sasatomi E, Regueiro M. Pathologic features of early inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; **31**: 133-145 [PMID: 12122728 DOI: 10.1016/S0889-8553(01)00009-7]

62 **Kader HA**, Tchernev VT, Satyaraj E, Lejnine S, Kotler G, Kingsmore SF, Patel DD. Protein microarray analysis of disease activity in pediatric inflammatory bowel disease demonstrates elevated serum PLGF, IL-7, TGF-beta1, and IL-12p40 levels in Crohn's disease and ulcerative colitis patients in remission versus active disease. *Am J Gastroenterol* 2005; **100**: 414-423 [PMID: 15667502 DOI: 10.1111/j.1572-0241.2005.40819.x]

63 **Shinzaki S**, Iijima H, Nakagawa T, Egawa S, Nakajima S, Ishii S, Irie T, Kakiuchi Y, Nishida T, Yasumaru M, Kanto T, Tsujii M, Tsuji S, Mizushima T, Yoshihara H, Kondo A, Miyoshi E, Hayashi N. IgG oligosaccharide alterations are a novel diagnostic marker for disease activity and the clinical course of inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 1173-1181 [PMID: 18177457 DOI: 10.1111/j.1572-0241.2007.01699.x]

64 **Burczynski ME**, Peterson RL, Twine NC, Zuberek KA, Brodeur BJ, Casciotti L, Maganti V, Reddy PS, Strahs A, Immermann F, Spinelli W, Schwertschlag U, Slager AM, Cotreau MM, Dorner AJ. Molecular classification of Crohn's disease and ulcerative colitis patients using transcriptional profiles in peripheral blood mononuclear cells. *J Mol Diagn* 2006; **8**: 51-61 [PMID: 16436634 DOI: 10.2353/jmoldx.2006.050079]

65 **Langhorst J**, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**: 162-169 [PMID: 17916108 DOI: 10.1111/j.1572-0241.2007.01556.x]

66 **Anand V**, Russell AS, Tsuyuki R, Fedorak R. Perinuclear antineutrophil cytoplasmic autoantibodies and anti-Saccharomyces cerevisiae antibodies as serological markers are not specific in the identification of Crohn's disease and ulcerative colitis. *Can J Gastroenterol* 2008; **22**: 33-36 [PMID: 18209778]

67 **Sandborn WJ**, Loftus EV, Colombel JF, Fleming KA, Seibold F, Homburger HA, Sendid B, Chapman RW, Tremaine WJ, Kaul DK, Wallace J, Harmsen WS, Zinsmeister AR, Targan SR. Evaluation of serologic disease markers in a population-based cohort of patients with ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 192-201 [PMID: 11515844 DOI: 10.1097/00054725-200108000-00003]

68 **Fukushima K**, Yonezawa H, Fiocchi C. Inflammatory bowel disease-associated gene expression in intestinal epithelial cells by differential cDNA screening and mRNA display. *Inflamm Bowel Dis* 2003; **9**: 290-301 [PMID: 14555912 DOI: 10.1097/00054725-200309000-00002]

69 **Shkoda A**, Werner T, Daniel H, Gunckel M, Rogler G, Haller D. Differential protein expression profile in the intestinal epithelium from patients with inflammatory bowel disease. *J Proteome Res* 2007; **6**: 1114-1125 [PMID: 17330946 DOI: 10.1021/pr060433m]

70 **Walkiewicz D**, Werlin SL, Fish D, Scanlon M, Hanaway P, Kugathasan S. Fecal calprotectin is useful in predicting disease relapse in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2008; **14**: 669-673 [PMID: 18240279 DOI: 10.1002/ibd.20376]

71 **Costa F**, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; **54**: 364-368 [PMID: 15710984 DOI: 10.1136/gut.2004.043406]

72 **Sidler MA**, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. *Inflamm Bowel Dis* 2008; **14**: 359-366 [PMID: 18050298 DOI: 10.1002/ibd.20336]

73 **Felley-Bosco E**, André M. Proteomics and chronic inflammatory bowel diseases. *Pathol Res Pract* 2004; **200**: 129-133 [PMID: 15237921 DOI: 10.1016/j.prp.2004.02.002]

74 **Bossuyt X**. Serologic markers in inflammatory bowel disease. *Clin Chem* 2006; **52**: 171-181 [PMID: 16339302 DOI: 10.1373/clinchem.2005.058560]

75 **Norris JL**, Caprioli RM. Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research. *Chem Rev* 2013; **113**: 2309-2342 [PMID: 23394164 DOI: 10.1021/cr3004295]

76 [**Norris JL**](http://www.ncbi.nlm.nih.gov/pubmed?term=Norris%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=17541451)**,** [Cornett DS](http://www.ncbi.nlm.nih.gov/pubmed?term=Cornett%20DS%5BAuthor%5D&cauthor=true&cauthor_uid=17541451), [Mobley JA](http://www.ncbi.nlm.nih.gov/pubmed?term=Mobley%20JA%5BAuthor%5D&cauthor=true&cauthor_uid=17541451), [Andersson M](http://www.ncbi.nlm.nih.gov/pubmed?term=Andersson%20M%5BAuthor%5D&cauthor=true&cauthor_uid=17541451), [Seeley EH](http://www.ncbi.nlm.nih.gov/pubmed?term=Seeley%20EH%5BAuthor%5D&cauthor=true&cauthor_uid=17541451), [Chaurand P](http://www.ncbi.nlm.nih.gov/pubmed?term=Chaurand%20P%5BAuthor%5D&cauthor=true&cauthor_uid=17541451), [Caprioli RM](http://www.ncbi.nlm.nih.gov/pubmed?term=Caprioli%20RM%5BAuthor%5D&cauthor=true&cauthor_uid=17541451). Processing MALDI Mass Spectra to Improve Mass Spectral Direct Tissue Analysis. *Int J Mass Spectrom* 2007; **260**: 212-221 [PMID: 17541451 DOI: 10.1016/j.ijms.2006.10.005]

77 **Norris JL**, Caprioli RM. Imaging mass spectrometry: a new tool for pathology in a molecular age. *Proteomics Clin Appl* 2013; **7**: 733-738 [PMID: 24178781 DOI: 10.1002/prca.201300055]

78 **Garrett TJ**, Yost RA. Analysis of intact tissue by intermediate-pressure MALDI on a linear ion trap mass spectrometer. *Anal Chem* 2006; **78**: 2465-2469 [PMID: 16579637 DOI: 10.1021/ac0522761]

79 **Khatib-Shahidi S**, Andersson M, Herman JL, Gillespie TA, Caprioli RM. Direct molecular analysis of whole-body animal tissue sections by imaging MALDI mass spectrometry. *Anal Chem* 2006; **78**: 6448-6456 [PMID: 16970320 DOI: 10.1021/ac060788p]

80 **Stoeckli M**, Staab D, Staufenbiel M, Wiederhold KH, Signor L. Molecular imaging of amyloid beta peptides in mouse brain sections using mass spectrometry. *Anal Biochem* 2002; **311**: 33-39 [PMID: 12441150 DOI: 10.1016/S0003-2697(02)00386-X]

81 **Chaurand P**, Norris JL, Cornett DS, Mobley JA, Caprioli RM. New developments in profiling and imaging of proteins from tissue sections by MALDI mass spectrometry. *J Proteome Res* 2006; **5**: 2889-2900 [PMID: 17081040 DOI: 10.1021/pr060346u]

82 **Cornett DS**, Reyzer ML, Chaurand P, Caprioli RM. MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. *Nat Methods* 2007; **4**: 828-833 [PMID: 17901873 DOI: 10.1038/nmeth1094]

83 **Grüner BM**, Hahne H, Mazur PK, Trajkovic-Arsic M, Maier S, Esposito I, Kalideris E, Michalski CW, Kleeff J, Rauser S, Schmid RM, Küster B, Walch A, Siveke JT. MALDI imaging mass spectrometry for in situ proteomic analysis of preneoplastic lesions in pancreatic cancer. *PLoS One* 2012; **7**: e39424 [PMID: 22761793 DOI: 10.1371/journal.pone.0039424]

84 **Caldwell RL**, Caprioli RM. Tissue profiling by mass spectrometry: a review of methodology and applications. *Mol Cell Proteomics* 2005; **4**: 394-401 [PMID: 15677390 DOI: 10.1074/mcp.R500006-MCP200]

85 **Reyzer ML**, Caprioli RM. MALDI-MS-based imaging of small molecules and proteins in tissues. *Curr Opin Chem Biol* 2007; **11**: 29-35 [PMID: 17185024 DOI: 10.1016/j.cbpa.2006.11.035]

86 **Woods AS**, Jackson SN. Brain tissue lipidomics: direct probing using matrix-assisted laser desorption/ionization mass spectrometry. *AAPS J* 2006; **8**: E391-E395 [PMID: 16796390 DOI: 10.1208/aapsj080244]

87 **Sinha TK**, Khatib-Shahidi S, Yankeelov TE, Mapara K, Ehtesham M, Cornett DS, Dawant BM, Caprioli RM, Gore JC. Integrating spatially resolved three-dimensional MALDI IMS with in vivo magnetic resonance imaging. *Nat Methods* 2008; **5**: 57-59 [PMID: 18084298 DOI: 10.1038/nmeth1147]

88 **Andersson M**, Groseclose MR, Deutch AY, Caprioli RM. Imaging mass spectrometry of proteins and peptides: 3D volume reconstruction. *Nat Methods* 2008; **5**: 101-108 [PMID: 18165806 DOI: 10.1038/nmeth1145]

89 **Shimma S**, Sugiura Y, Hayasaka T, Zaima N, Matsumoto M, Setou M. Mass imaging and identification of biomolecules with MALDI-QIT-TOF-based system. *Anal Chem* 2008; **80**: 878-885 [PMID: 18166020 DOI: 10.1021/ac071301v]

90 **Sugiura Y**, Shimma S, Konishi Y, Yamada MK, Setou M. Imaging mass spectrometry technology and application on ganglioside study; visualization of age-dependent accumulation of C20-ganglioside molecular species in the mouse hippocampus. *PLoS One* 2008; **3**: e3232 [PMID: 18800170 DOI: 10.1371/journal.pone.0003232]

91 **McEwen AB**, Henson CM, Wood SG. Quantitative whole-body autoradiography, LC-MS/MS and MALDI for drug-distribution studies in biological samples: the ultimate matrix trilogy. *Bioanalysis* 2014; **6**: 377-391 [PMID: 24471957 DOI: 10.4155/bio.13.336]

92 **Castellino S**, Groseclose MR, Wagner D. MALDI imaging mass spectrometry: bridging biology and chemistry in drug development. *Bioanalysis* 2011; **3**: 2427-2441 [PMID: 22074284 DOI: 10.4155/bio.11.232]

93 **Trede D**, Schiffler S, Becker M, Wirtz S, Steinhorst K, Strehlow J, Aichler M, Kobarg JH, Oetjen J, Dyatlov A, Heldmann S, Walch A, Thiele H, Maass P, Alexandrov T. Exploring three-dimensional matrix-assisted laser desorption/ionization imaging mass spectrometry data: three-dimensional spatial segmentation of mouse kidney. *Anal Chem* 2012; **84**: 6079-6087 [PMID: 22720760 DOI: 10.1021/ac300673y]

94 **Sugiura Y**, Setou M. Matrix-assisted laser desorption/ionization and nanoparticle-based imaging mass spectrometry for small metabolites: a practical protocol. *Methods Mol Biol* 2010; **656**: 173-195 [PMID: 20680591 DOI: 10.1007/978-1-60761-746-4\_10]

95 **Taira S**, Sugiura Y, Moritake S, Shimma S, Ichiyanagi Y, Setou M. Nanoparticle-assisted laser desorption/ionization based mass imaging with cellular resolution. *Anal Chem* 2008; **80**: 4761-4766 [PMID: 18476721 DOI: 10.1021/ac800081z]

96 **Moritake S**, Taira S, Sugiura Y, Setou M, Ichiyanagi Y. Magnetic nanoparticle-based mass spectrometry for the detection of biomolecules in cultured cells. *J Nanosci Nanotechnol* 2009; **9**: 169-176 [PMID: 19441292 DOI: 10.1166/jnn.2009.J012]

97 **Quinton JF**, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastien B, Charrier G, Targan SR, Colombel JF, Poulain D. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998; **42**: 788-791 [PMID: 9691915 DOI: 10.1136/gut.42.6.788]

98 **Davis MK**, Andres JM, Jolley CD, Novak DA, Haafiz AB, González-Peralta RP. Antibodies to Escherichia coli outer membrane porin C in the absence of anti-Saccharomyces cerevisiae antibodies and anti-neutrophil cytoplasmic antibodies are an unreliable marker of Crohn disease and ulcerative colitis. *J Pediatr Gastroenterol Nutr* 2007; **45**: 409-413 [PMID: 18030205 DOI: 10.1097/MPG.0b013e31812f7f6e]

99 **Ashorn S**, Honkanen T, Kolho KL, Ashorn M, Välineva T, Wei B, Braun J, Rantala I, Luukkaala T, Iltanen S. Fecal calprotectin levels and serological responses to microbial antigens among children and adolescents with inflammatory bowel disease. *Inflamm Bowel Dis* 2009; **15**: 199-205 [PMID: 18618670 DOI: 10.1002/ibd.20535]

100 **Joossens S**, Reinisch W, Vermeire S, Sendid B, Poulain D, Peeters M, Geboes K, Bossuyt X, Vandewalle P, Oberhuber G, Vogelsang H, Rutgeerts P, Colombel JF. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology* 2002; **122**: 1242-1247 [PMID: 11984510 DOI: 10.1053/gast.2002.32980]

101 **Jevon GP**, Madhur R. Endoscopic and histologic findings in pediatric inflammatory bowel disease. *Gastroenterol Hepatol (N Y)* 2010; **6**: 174-180 [PMID: 20567564]

102 **M'Koma AE**, Blum DL, Norris JL, Koyama T, Billheimer D, Motley S, Ghiassi M, Ferdowsi N, Bhowmick I, Chang SS, Fowke JH, Caprioli RM, Bhowmick NA. Detection of pre-neoplastic and neoplastic prostate disease by MALDI profiling of urine. *Biochem Biophys Res Commun* 2007; **353**: 829-834 [PMID: 17194448 DOI: 10.1016/j.bbrc.2006.12.111]

103 **Blum DL**, Koyama T, M'Koma AE, Iturregui JM, Martinez-Ferrer M, Uwamariya C, Smith JA, Clark PE, Bhowmick NA. Chemokine markers predict biochemical recurrence of prostate cancer following prostatectomy. *Clin Cancer Res* 2008; **14**: 7790-7797 [PMID: 19047106 DOI: 10.1158/1078-0432.CCR-08-1716]

104 **M'Koma AE**, Moses HL, Adunyah SE. Inflammatory bowel disease-associated colorectal cancer: proctocolectomy and mucosectomy do not necessarily eliminate pouch-related cancer incidences. *Int J Colorectal Dis* 2011; **26**: 533-552 [PMID: 21311893 DOI: 10.1007/s00384-011-1137-4]

105 **Ullman TA**, Itzkowitz SH. Intestinal inflammation and cancer. *Gastroenterology* 2011; **140**: 1807-1816 [PMID: 21530747 DOI: 10.1053/j.gastro.2011.01.057]

106 **Cullis P**, Mullassery D, Baillie C, Corbett H. Crohn's disease presenting as enterovesical fistula. *BMJ Case Rep* 2013; **2013** [PMID: 24248323]

107 **Rieder F**, Fiocchi C. Mechanisms of tissue remodeling in inflammatory bowel disease. *Dig Dis* 2013; **31**: 186-193 [PMID: 24030223 DOI: 10.1159/000353364]

108 **Zhang FM**, Wang HG, Wang M, Cui BT, Fan ZN, Ji GZ. Fecal microbiota transplantation for severe enterocolonic fistulizing Crohn's disease. *World J Gastroenterol* 2013; **19**: 7213-7216 [PMID: 24222969 DOI: 10.3748/wjg.v19.i41.7213]

109 **Podolsky DK**, Fournier DA. Alterations in mucosal content of colonic glycoconjugates in inflammatory bowel disease defined by monoclonal antibodies. *Gastroenterology* 1988; **95**: 379-387 [PMID: 3292335]

110 **Lautenschläger C**, Schmidt C, Fischer D, Stallmach A. Drug delivery strategies in the therapy of inflammatory bowel disease. *Adv Drug Deliv Rev* 2014; **71**: 58-76 [PMID: 24157534]

111 **Danese S**, Peyrin-Biroulet L. New mechanisms and targets for IBD Therapy: translational gastroenterology comes of age. *Curr Drug Targets* 2013; **14**: 1377-1378 [PMID: 24060146 DOI: 10.2174/13894501113146660220]

112 **Jan S**, Slap G, Dai D, Rubin DM. Variation in surgical outcomes for adolescents and young adults with inflammatory bowel disease. *Pediatrics* 2013; **131** Suppl 1: S81-S89 [PMID: 23457154 DOI: 10.1542/peds.2012-1427j]

113 **Sica GS**, Biancone L. Surgery for inflammatory bowel disease in the era of laparoscopy. *World J Gastroenterol* 2013; **19**: 2445-2448 [PMID: 23674844 DOI: 10.3748/wjg.v19.i16.2445]

114 **Buckley JP**, Kappelman MD, Allen JK, Van Meter SA, Cook SF. The burden of comedication among patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 2725-2736 [PMID: 24216689 DOI: 10.1097/01.MIB.0000435442.07237.a4]

115 **Price AB**. Overlap in the spectrum of non-specific inflammatory bowel disease--'colitis indeterminate'. *J Clin Pathol* 1978; **31**: 567-577 [PMID: 670413 DOI: 10.1136/jcp.31.6.567]

116 **Delaney CP**, Remzi FH, Gramlich T, Dadvand B, Fazio VW. Equivalent function, quality of life and pouch survival rates after ileal pouch-anal anastomosis for indeterminate and ulcerative colitis. *Ann Surg* 2002; **236**: 43-48 [PMID: 12131084 DOI: 10.1097/00000658-200207000-00008]

117 **Reese GE**, Lovegrove RE, Tilney HS, Yamamoto T, Heriot AG, Fazio VW, Tekkis PP. The effect of Crohn's disease on outcomes after restorative proctocolectomy. *Dis Colon Rectum* 2007; **50**: 239-250 [PMID: 17180251 DOI: 10.1007/s10350-006-0777-x]

118 **Neilly P**, Neill ME, Hill GL. Restorative proctocolectomy with ileal pouch-anal anastomosis in 203 patients: the Auckland experience. *Aust N Z J Surg* 1999; **69**: 22-27 [PMID: 9932915 DOI: 10.1046/j.1440-1622.1999.01464.x]

119 **Tekkis PP**, Heriot AG, Smith O, Smith JJ, Windsor AC, Nicholls RJ. Long-term outcomes of restorative proctocolectomy for Crohn's disease and indeterminate colitis. *Colorectal Dis* 2005; **7**: 218-223 [PMID: 15859957 DOI: 10.1111/j.1463-1318.2005.00800.x]

120 **McLaughlin SD**, Clark SK, Tekkis PP, Ciclitira PJ, Nicholls RJ. Review article: restorative proctocolectomy, indications, management of complications and follow-up--a guide for gastroenterologists. *Aliment Pharmacol Ther* 2008; **27**: 895-909 [PMID: 18266993 DOI: 10.1111/j.1365-2036.2008.03643.x]

121 **Deutsch AA**, McLeod RS, Cullen J, Cohen Z. Results of the pelvic-pouch procedure in patients with Crohn's disease. *Dis Colon Rectum* 1991; **34**: 475-477 [PMID: 2036927 DOI: 10.1007/BF02049932]

122 **Hyman NH**, Fazio VW, Tuckson WB, Lavery IC. Consequences of ileal pouch-anal anastomosis for Crohn's colitis. *Dis Colon Rectum* 1991; **34**: 653-657 [PMID: 1855421 DOI: 10.1007/BF02050345]

123 **Grobler SP**, Hosie KB, Affie E, Thompson H, Keighley MR. Outcome of restorative proctocolectomy when the diagnosis is suggestive of Crohn's disease. *Gut* 1993; **34**: 1384-1388 [PMID: 8244106 DOI: 10.1136/gut.34.10.1384]

124 **Mylonakis E**, Allan RN, Keighley MR. How does pouch construction for a final diagnosis of Crohn's disease compare with ileoproctostomy for established Crohn's proctocolitis? *Dis Colon Rectum* 2001; **44**: 1137-142; discussion 1137-142; [PMID: 11535853 DOI: 10.1007/BF02234634]

125 **Landers CJ**, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002; **123**: 689-699 [PMID: 12198693 DOI: 10.1053/gast.2002.35379]

126 **Kaiser T**, Langhorst J, Wittkowski H, Becker K, Friedrich AW, Rueffer A, Dobos GJ, Roth J, Foell D. Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut* 2007; **56**: 1706-1713 [PMID: 17675327 DOI: 10.1136/gut.2006.113431]

127 **Foell D**, Kucharzik T, Kraft M, Vogl T, Sorg C, Domschke W, Roth J. Neutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease. *Gut* 2003; **52**: 847-853 [PMID: 12740341 DOI: 10.1136/gut.52.6.847]

128 **Pardi DS**, Sandborn WJ. Predicting relapse in patients with inflammatory bowel disease: what is the role of biomarkers? *Gut* 2005; **54**: 321-322 [PMID: 15710974 DOI: 10.1136/gut.2004.048850]

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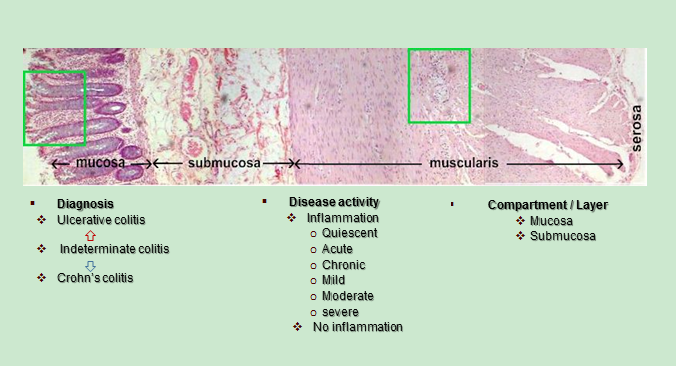
**Table 1 Microscopic features used for the diagnosis of Crohn’s colitis**

|  |  |
| --- | --- |
| Colon  Architecture  Crypt architectural irregularity  Reduces crypt numbers/mucosal atrophy  Irregular surface | Focal  Diffuse |
| Chronic inflammation  Distribution I  Distribution II  Granulomas  Mucin granulomas | Focal increased in intensity  Patchy increase  Diffuse increase  Superficial  Transmucosal  Basal plasma cells |
| Polymorph inflammation  Lamina propria  Crypt epithelial polymorphs  Polymorph exudates | Focal  Diffuse |
| Epithelial changes  Erosion/ulceration  Mucin  Paneth cells distal to hepatic flexure | Depletion  Preservation |
| Epithelial-associated changes  Increased intraepithelial lymphocytes > 15 |  |
| Terminal ileum/Ileocecal /Cecum  Architecture  Epithelial changes | Villus irregularity  Crypt architecture  Irregularity  Pseudopyloric gland  Metaplasia |

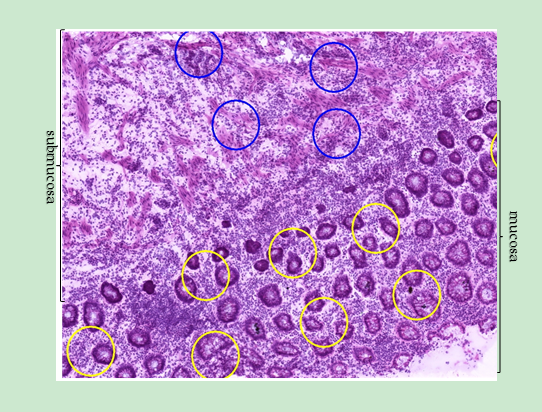
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**Table 2 Classic microscopic features in untreated ulcerative colitis (comparable Crohn’s colitis, hard criteria)**

|  |  |  |
| --- | --- | --- |
| **Feature** | **Ulcerative colitis** | **Crohn’s colitis** |
| Diffuse | Continuous disease | Segmental disease |
| Rectal | Involvement | Variable rectal involvement |
| Disease | Worse distally | Variable disease severity |
| Fissures | No | Fissures, sinus, fistula |
| Transmural aggregates | No | Transmural lymphoid aggregates |
| Ileal involvement | No, exception during backwash ileitis | Ileal involvement |
|  |  | Upper gastrointestional involvement |
| Granulomas | No | Granulomas |



**Figure 1** Human **colon** cross section **depicts layers for mining proteomic patterns that delineates untreated ulcerative and Crohn’s colitis phenotype.** The colon is comprised of four distinct layers: (1) the mucosa; (2) the submucosa; (3) the muscularis (two thick bands of muscle); and (4) the serosa. Comparable proteomic patterns that are mined from these layers are analyzed, based on the diagnosis [untreated ulcerative and Crohn’s colitis, (with no ambiguity)], disease activity and tissue layer.



**Figure 2 Histology-directed tissue layer profiling for matrix-assisted-laser desorption/ionization mass spectrometry.** Digital photomicrographs acquired from histology and matrix-assisted-laser desorption/ionization (MALDI) sections were used to identify and designate sites of interest for profiling. Comparisons were performed in both the training and independent test set samples between inflamed mucosa Crohn’s colitis (CC) *vs* ulcerative colitis (UC) and inflamed submucosa CC *vs* UC. Tissue section showing marked areas of pathological interest. Rings demonstrate matrix spots in mucosal and sub-mucosal layers (unpublished figure).

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**Figure 3 Show averaged mass spectrum proteomic pattern spectra from Crohn’s colitis (blue) and ulcerative colitis (red).** Differential distribution of three selected proteomic pattern peaks (m/z) obtained from colonic mucosal and/or submucosal tissue sections that were part of the Support Vector Machine (SVM) model. They are denoted by “a” symbol in the full spectra. Reproduced with permission from the publisher: Seeley *et al*[50].