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REVIEW

Diagnosis of inflammatory bowel disease: Potential role of molecular biometrics

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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 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.

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

Core tip: Pouch surgery (the restorative proctocolectomy and ileal 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

Colonic 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[1,2]. 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]. 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], and global rates seem to be increasing[1,10-12], attributable to the rapid modernization and Westernization of the population[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. 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]. Although the etiology of IBD is at present not delineated, histopathologic and clinical assessments demonstrate that CD 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. CD 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, 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]. 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]. 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]. 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] 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]. Despite these extremely thorough standards, inevitable situations arise in which objectivity cannot be guaranteed and where significant disagreement occurs between specialists[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]. The consensus statement on the diagnosis, management and surveillance of both CC[40] and UC[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.

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

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

Reproduced by permission of the publisher from ref. [38].

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[42,43]. In most cases the diagnostic uncertainty arises from the overlap of clinical and histologic features, making CC appear like UC[44]. 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]; 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].

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 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%) ($P < 0.05$)[46,47,49]. 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[42,50-52].

Clinico-histopathologic findings in Crohn's colitis

Crohn's colitis is recognized to encompass a heterogeneous group of disorders[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] and the Paris pediatric modification[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].

Histologic features useful for the diagnosis of CC have been reviewed by Griffiths[38], (Table 1) but, according to Van Assche *et al*[40] presented at The second European evidence-based Consensus on the diagnosis and management of Crohn's colitis, there are no data available as to how many of these features must be present to allow a firm diagnosis[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]. 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,

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 gastrointestinal involvement
Granulomas	No	Granulomas

and the definition of a granuloma. Granulomas occur more commonly in the submucosa than the mucosa[55]. 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]. Moreover, according to Griffiths[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 Odze[56], and are depicted in Table 2. Clinically, the hallmark of UC is hematochezia[57,58]. Additional clinical presentations include rectal tenesmus and incontinence, abdominal pain, severe inflammation of the rectum (proctitis), leukocytosis, hospitalization for total parenteral nutrition and/or intravenous fluids correction, among others. Blood transfusion and corticosteroids are recommended when considering surgery (RPC and IPAA)[58]. As mentioned earlier, in UC, inflammation is typically confined to the mucosal layer and to the lesser degree to the submucosa. Children with UC often 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: placenta growth factor-1 (PLGF-1), IL-7, TGFβ1, and IL-12P40[62-67]. 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[68,69]. In stool they are calprotectin, PMN-elastate, lactoferrin, and S100A12[65,70-74]. 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[62,64,73,74]. Thus far, the above mentioned features reflect colitides intestinal inflammation and do not discriminate UC from the CC phenotype[65].

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[42,50]. 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[42,50]. Pre-sequencing and identification proteomic pattern peaks from colonic mucosal or/and submucosal tissue section are depicted in Figure 3[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]. 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]. 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

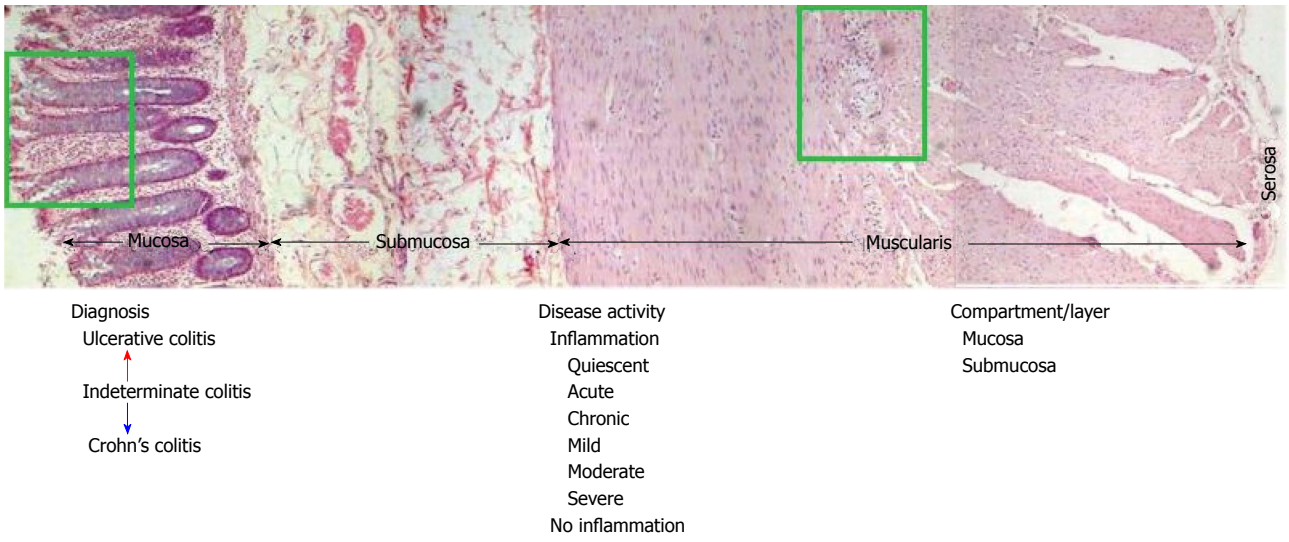

Figure 1 Human colon cross section depicts layers for mining proteomic patterns that delineates untreated ulcerative and Crohn's colitis phenotype. The colon is comprised of four distinct layers: (1) the mucosa; (2) the submucosa; (3) the muscularis (two thick bands of muscle); and (4) the serosa. Comparable 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.

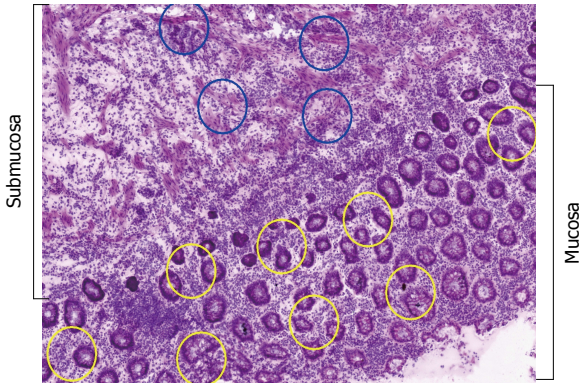

Figure 2 Histology-directed tissue layer profiling for matrix-assisted-laser desorption/ionization mass spectrometry. Digital photomicrographs acquired from histology and matrix-assisted-laser desorption/ionization sections were used to identify and designate sites of interest for profiling. Comparisons 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).

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]. 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]. Thus a combination of markers may improve the chances of achieving IBD proteomics goals.

MS in combination with laser capture microdissection 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]. 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

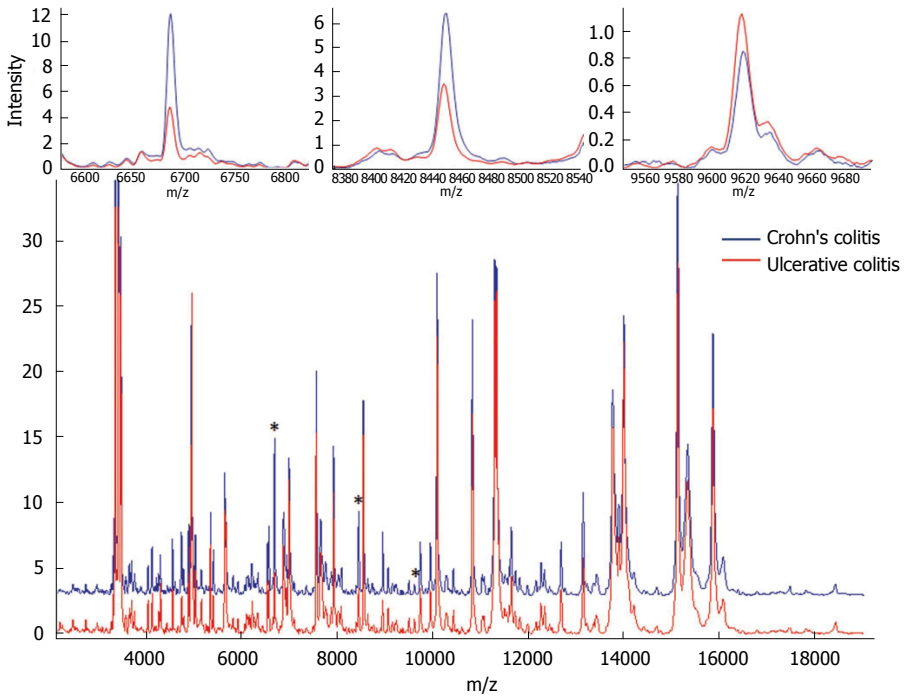

Figure 3 Show averaged mass spectrum proteomic pattern spectra from Crohn's colitis (blue) and ulcerative colitis (red). Differential distribution of three selected proteomic pattern peaks (m/z) obtained from colonic mucosal and/or submucosal tissue sections that were part of the Support Vector Machine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

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[78,79] and large proteins[80,81] in cells and tissues[82,83]. It can determine the distribution of hundreds of unknown compounds in a single measurement[79,84-86]. Further, IMS is capable of three-dimensional molecular images which can be combined with established imaging techniques like magnetic resonance imaging[87,88].

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[78,89,90]. The emerging technique of MALDI IMS has the capability to distinguish between parent and metabolites while maintaining spatial distribution in various tissues[91,92]. 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]. In attempting to solve these important issues, researchers have devised another sophisticated method: a nanoparticle-assisted laser desorption/ionization (nano-PALDI)-based IMS, in which the matrix crystallization process is eliminated[94,95]. The use of novel nano-PALDI has enabled scientists to image compounds with spatial resolution at the cellular level (15 μmol/L; approximating the diameter of a laser spot)[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, antineutrophil cytoplasmic antibodies (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]. The association with colitides of a subset of ANCA with a perinuclear staining pattern on immunofluorescence studies [perinuclear antineutrophil cytoplasmic autoantibodies (pANCA)] was first recognized for UC, where it was detected in 60%-70% of patients[97]. The specificity of perinuclear staining for colitides can be validated and confirmed by its disappearance after deoxyribonuclease (DNase) 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]. 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[98,99].

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]. In a long-term follow-up of patients with IC, Joossens *et al*[100] 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].

When using upper 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]. 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[38,102,103] is becoming ground breaking in improving diagnostic challenges in colonic IBD settings[42,50,104]. IBD has no permanent drug cure and results in significant morbidity and mortality[9,104,105]. 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] and other organs through fistulization[106-108]. These diseases share several clinical biometric signatures but have different causes, mechanisms of tissue damage, and treatment options[16,109]. Therefore, accurate diagnosis is paramount for provision of correct pharmacologic therapy[110,111] and surgical care[112-114].

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] 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[36,48,49,115,116] 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[24,36]. 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]. This is critical because functional failure and higher complication rates are estimated at up to 60%[35,117-123] and often require excision of the pouch with a permanent end ileostomy[35,121-124]. 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[62-67], in mucosal biopsies[68,69], and in fecal matter[65,70-74]. 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[62,64,73,74]. A serology panel including ANCA, pANCA, anti-saccharomyces cerevisae IgG and IgA antibodies (ASCA), calgranulin (S100A12), anti-OmpC antibodies, fecal lactoferrin, calprotectin, and polymorphonuclear neutrophil

elastase (PMN-e)[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[125,126]. 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]; (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]; (3) These molecular biometrics significantly assist in predicting relapses in patients with confirmed IBD (active or quiescent)[2-5,17,21,128] but are not discriminatory between UC/CC; (4) 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]. 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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