Recent Advances in the Diagnosis and Classification of Inflammatory Bowel Disease

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The availability of an increasing number of inflammatory bowel disease (IBD)–specific serologic antibodies, the discovery of disease-susceptibility genes, the introduction of pharmacogenetic markers, and the recent application of wireless capsule enteroscopy to the evaluation of patients with IBD are providing new types of information that must be integrated with more traditional IBD paradigms. The challenge facing researchers and clinicians is to determine how to incorporate these potentially clinically relevant insights into our understanding of disease pathogenesis and to define the spectrum of potential applications to the management of patients with IBD. The ultimate diagnostic and predictive value of these tests will likely be optimized when they are applied in combination, rather than individually. As the clinical relevance of this expanding diagnostic armamentarium is defined, it is hoped that these tests will enable clinicians not only to diagnose IBD accurately but also to determine disease patterns prospectively, suggest prognoses, and allow for individualization of therapeutic regimens.

Introduction
Early recognition of inflammatory bowel disease (IBD) may allow for therapeutic interventions at an earlier point in the inflammatory process, thereby potentially avoiding or minimizing subsequent disease- and therapy-related complications. Proper characterization of the location and specific subtype of IBD in patients with resistant symptoms may very well have an impact on further medical and surgical treatment decisions. The conventional gold standard for diagnosis and stratification of IBD has been based on a combination of established clinical, endoscopic, histopathologic, and radiologic criteria [1]. Approaches to stratification in IBD have not reflected specific pathogenic mechanisms, but rather have been largely descriptive, focusing on the clinical characteristics of anatomic distribution of inflammation and disease behavior patterns.

This article provides a brief overview of new individual diagnostic modalities and then focuses on their value and potential role in the evaluation of patients with IBD, which can be applied at several levels: diagnosis (initial delineation of IBD from non-IBD), differentiation of ulcerative colitis (UC) from Crohn’s disease (CD), stratification into phenotypic subgroups with specific patterns of disease distribution and behaviors, prognostication to better predict the clinical course of the individual’s disease and perhaps its likely outcomes, and characterization of potential therapeutic subgroups.

Overview of Serologic Tests
Serologic tests in patients with IBD detect the presence of abnormal antibodies directed against self or non-self proteins. These antibodies are not directly pathogenic to the host but rather serve as subclinical indicators of immune dysregulation. These antibodies can be used to stratify IBDs, and in a research setting they can serve as surrogate markers to trace the immunologic derangements and disease susceptibility [2].

Serum immune markers
Antineutrophil cytoplasmic antibodies
Serum antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against intracellular components of neutrophils. In contrast to the cANCA characteristic of Wegener’s granulomatosis, which is directed against specific antigens within the cytoplasmic granules, the two ANCA subtypes that predominate in patients with IBD are pANCA, which exhibits predominant perinuclear highlighting, and sANCA, a newly described ANCA subtype characterized by a diffuse “speckled” staining pattern over the entire neutrophil. The intestinal mucosa is the site of antigenic B-cell priming and pANCA production in IBD, suggesting that recognition of mucosal antigen(s) (self or bacterial) leads to local production of pANCA. Serum pANCA in IBD thus reflects mucosal pANCA production [3].
pANCA

The pANCA characteristic of patients with IBD is unique among ANCAs. Unlike the pANCAs of patients with vasculitides, when pANCA-containing sera from patients with IBD are incubated with neutrophils, the vast majority of pANCAs localize to the inner side of the nuclear membrane periphery, as demonstrated on confocal microscopy [4]. Investigators have shown that histone H1, a protein bound to nuclear heterochromatin DNA, is among the antigens toward which IBD-associated pANCA reacts [5]. Cross-reactivity with protein epitopes expressed by colonic bacteria has also been suggested [6]. Antibody levels are relatively stable over time and are not present in non-IBD colitis control subjects, implying that they are not nonspecific markers of colonic inflammation [7].

The reported prevalence of pANCA ranges from 50% to 80% in UC and from 10% to 40% in CD in most series [8]. Whereas a portion of the discrepancies between studies can be attributed to differences in patient populations, including ethnicity and referral center bias, methodologic differences are likely the biggest factor [8,9]. Although the antigen to which pANCA(s) in IBD react has not been definitively determined, it has been well-characterized, and several lines of evidence support the concept that the pANCAs of CD are the same as the pANCAs of UC [8]. The CD subpopulation expressing pANCA is clinically distinct, having a “UC-like” behavioral phenotype, with clinical features of left-sided colitis and endoscopic or histopathologic features that are typical of UC [7,10••]. This clinical, immunologic, and serologic commonality suggests that the presence of serum pANCA reflects a specific type of mucosal inflammation that may be common to the subgroups, both UC- and CD-expressing pANCA. An increased incidence of ANCA expression has been demonstrated in family members of ANCA-positive UC patients [11].

sANCA

This antibody is characterized less well, and the antigen toward which it reacts is unknown at this time. Enzyme-linked immunosorbent assay (ELISA) levels of sANCA in CD tend to be very low [8]. sANCA has been detected in approximately 14% to 20% of patients with CD, the incidence in UC has not been reported [8,12].

ASCA

Anti–Saccharomyces cerevisiae antibodies (ASCA) are present in 50% to 70% of the sera of patients with CD, and in 6% to 14% of patients with UC; they are rarely expressed in individuals who do not have IBD [8]. This marker is highly specific for CD, particularly for those who express high levels of the IgA and IgG immunoglobulin subtypes of ASCA. ASCA is more commonly expressed in patients with early-onset (up to 70%), compared with later-onset disease (25% of CD patients with onset after age 40) [10••]. Because self-antigens do not react with ASCA, this serum immune marker is not considered an autoantibody.

*S. cerevisiae* is the species of yeast commonly used in baking and brewing. Whereas yeast cell wall phosphopentamannans are the epitopes responsible for the antigenic reactivity in ASCA-positive CD sera, ASCA production may reflect antigenic cross-reactivity to epitopes coincidentally shared with a true causative agent of the disease [13]. The target antigen for ASCA is likely a luminal bacterial antigen that cross-reacts with saccharomyces. Mannans, believed to be the major antigenic component of yeast cell walls, are an important antigenic constituent of mycobacteria and other microorganisms [14]. The presence of serum ASCA in patients with IBD is not simply a matter of increased permeability. Elevated levels of antibodies to *Candida albicans*, another common yeast, and to other common antigens, such as gliadin, ovalbumin, and betalactoglobulin, have not been observed in CD [15]. Evidence suggests that, as with pANCA, expression of ASCA is not an epiphenomenon of intestinal insult, but rather a reflection of a specific mucosal immune-mediated response [8,16,17]. As with ANCA, familial associations have been observed in patients with ASCA [18,19].

Serologic markers to microbial antigens

Serologic responses to bacterial and mycobacterial antigens have been studied in IBD patients and may represent another family of serologic markers that are associated with specific subgroups of IBD. Loss of tolerance to normal commensal bacteria has been implicated as an initial step in the inflammatory cascade in some IBD patients [20]. The presence of such markers may identify the subgroup of CD patients whose inflammation is perpetuated by luminal bacteria and who may respond to treatment directed at altering the microbial flora.

**OMP-C**

Screening of colonic bacteria with recombinant DNA demonstrated that OMP-C reacts to an outer membrane protein of the cell wall *Escherichia coli* and other enterobacteriaceae that are responsible for controlling the flow of nutrients, metabolites, and small hydrophilic antibiotics in and outside of the cell. This function is predominantly controlled by outer membrane proteins called porins [8,21]. Thus, antibodies against OMP-C may represent aberrant bacterial reactivity or cross-reactivity with pathogenic organisms and may also be diagnostic markers in patients with CD. OMP-C has been reported to be expressed in 55% of patients with CD [22].

**Pseudomonas 12 peptide**

Sutton et al. [23] recently described this novel RNA-derived immunologically associated bacterial sequence of *Pseudomonas fluorescens*, which exhibits seroreactivity and specific lesional abundance in patients with CD. 12 is expressed in the majority of CD patients (54%) and less