Novel Diagnostic and Prognostic Modalities in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a heterogenous group of diseases that can be broadly classified into Crohn disease and ulcerative colitis (UC). The term IBD-unclassified (IBDU) applies to the subset of 10% to 15% of patients with IBD in whom this subcategorization is not possible. There is no gold standard single test that provides the diagnosis of IBD, so assigning a diagnosis of IBD is often not straightforward and involves integration of historical factors, physical examination findings, and evidence of inflammation on endoscopic, histologic, and radiologic evaluations. Consequently, there is significant uncertainty both in establishing the initial diagnosis and, importantly, in assessing for disease relapse after a period of remission. These challenges are compounded by the increased appreciation of the importance of an accurate and timely diagnosis. Delay in diagnosis can result in complications of stricturing or penetrating disease, whereas an incorrect diagnosis has emotional and insurance implications and can expose patients to the modest but nonetheless real risks of medical therapy. The uncertainty in diagnosing IBD and the need to get the diagnosis right has fueled improvements in techniques to assess bowel inflammation, including serologic and fecal markers and novel endoscopic and radiologic tools for imaging the entire bowel. These new techniques for evaluating patients with IBD are developed with the goals of improving early and accurate diagnosis, clarifying disease type and distribution in order to select optimal therapy, identifying patients at high risk
for aggressive disease, and detecting complications such as abscess or malignancy. The past decade has seen a dramatic advancement in diagnostic and prognostic modalities in IBD, and these novel instruments are reviewed in this article.

**SEROLOGIC BIOMARKERS**

Serologic biomarkers in IBD include an enlarging panel of antibodies directed against microbial and self-antigens and acute phase reactants. It is unclear whether these antimicrobial antibodies are mechanistically related to the pathogenesis of IBD. They may represent a loss of immune tolerance to commensal organisms, or they may simply be an indicator of increased bowel permeability with consequent exposure to luminal antigens. A variety of uses for these markers have been explored in IBD patients, including as potential diagnostic tools, follow-up parameters, prognostic indicators for phenotypic stratification, or subclinical disease markers in IBD patients or their family members. Serologic tests have several advantages in that they are easy to obtain, noninvasive, and objectively quantified.

**Antineutrophil Cytoplasmic Antibodies**

Antineutrophil cytoplasmic antibodies (ANCA) are detected on peripheral blood neutrophils by indirect immunofluorescence (IIF) techniques. Two major staining patterns have been described. A cytoplasmic (c-ANCA) staining pattern characterized by diffuse granularity of the cytoplasm is classically seen in patients with Wegener granulomatosis. These c-ANCA antibodies typically recognize proteinase-3 on enzyme-linked immunosorbent assay (ELISA) testing. By contrast, a thin homogeneous rim-like staining of the perinuclear cytoplasm (p-ANCA) is associated with microscopic polyangiitis and antibodies directed against myeloperoxidase. A third pattern exists, often referred to as atypical p-ANCA, that appears as a broad heterogeneous staining of the nuclear periphery, often with intranuclear inclusions to suggest that the antigen may be in the periphery of the nucleus rather than in the perinuclear cytoplasm. This atypical p-ANCA is associated with IBD and primary sclerosing cholangitis and autoimmune hepatitis type I. Atypical p-ANCA is present in 40% to 80% of patients with UC and 5% to 25% of patients with Crohn disease. The target antigens of atypical p-ANCA have not been identified, but several have been explored, including cathepsin G, elastase, β-glucuronidase, lactoferrin, and the natural antibiotic bactericidal permeability increasing protein. Myeloperoxidase and proteinase-3, the antigens recognized by typical p-ANCA and c-ANCA, respectively, are not autoantigens in IBD. Because the target antigens of atypical p-ANCA have not been identified, there is no ELISA test to distinguish these antibodies from typical p-ANCA. Rather one must rely on IIF, which has drawbacks, including differences in methodology and subjective interpretation of staining pattern that result in substantial variability of results among laboratories. Consequently, the numerous studies describing the performance characteristics of atypical p-ANCA in the detection of IBD have yielded heterogeneous and discrepant results. Given this variability, an alternative methodology was developed by Targan and colleagues to distinguish atypical p-ANCA from the typical vasculitis-associated p-ANCA by a 3-step process that involves IIF staining before and after treatment of neutrophils with deoxyribonuclease (DNase). Addition of DNase abolishes the fluorescent staining of atypical, UC-associated p-ANCA, allowing distinction from the typical p-ANCA pattern. In a large meta-analysis, the overall sensitivity of atypical p-ANCA for detecting UC was 55%, with a specificity of 89%, a positive likelihood ratio of 4.5, and a negative likelihood ratio of −0.5.
**Anti-Saccharomyces Cerevisiae Antibodies**

Anti-Saccharomyces cerevisiae antibodies (ASCA) are antibodies directed against a cell wall component of the baker’s and brewer’s yeast *S cerevisiae*. This antigen is not unique to the cell wall of *S cerevisiae*, however. Recently it has been discovered that *Candida albicans* is an immunogen for ASCAs. ASCAs are present in 50% to 60% of patients with Crohn disease, 10% to 15% of those with UC, 20% of healthy first-degree relatives of patients with Crohn disease, and 0% to 5% of healthy controls. IgG and IgA antibodies are produced, and the specificity for Crohn disease increases if both antibodies are positive. As with atypical p-ANCA, there is substantial heterogeneity of ASCA results among laboratories, attributable to a lack of standardization of the assay methodology and defined cutoff values.

**Newer Serologic Biomarkers**

Anti-OmpC antibody is an IgA antibody directed against the outer membrane porin C protein of *Escherichia coli* and is present in 55% of patients with Crohn disease but only in 5% to 11% of patients with UC and in 5% of healthy controls. In the pediatric population with Crohn disease, however, the prevalence of anti-OmpC antibodies is markedly reduced. Anti-OmpC antibodies are present in 5% to 15% of patients with Crohn disease who are ASCA negative, and they can help to identify a subset of patients that would otherwise be missed by conventional serologic testing.

Antibodies to I2, a bacterial sequence derived from *Pseudomonas fluorescens*, are associated with Crohn disease. Anti-I2 IgA antibodies are present in 30% to 50% of patients with Crohn disease, 10% of patients with UC, 19% of patients with other inflammatory conditions of the intestine, and 5% of healthy controls. The sensitivity and specificity of I2 antibodies for detecting Crohn disease are 42% and 76%, respectively. The low sensitivity and modest specificity limit the clinical utility of this antimicrobial marker.

Antipancreatic antibodies (PAB) are more common in Crohn disease (30%) than in UC (2%–6%) or healthy subjects (0%–2%). PAB antibodies are directed against an unidentified antigen on exocrine pancreatic tissue. Their utility in IBD diagnosis and management remains to be demonstrated.

CBir1 flagellin is a dominant antigen of the enteric microbial flora that induces an immune response in colitic mice. Approximately half of Crohn patients have anti-CBir1 antibodies compared with only 6% of patients with UC and 8% of control subjects. The presence of anti-CBir1 antibodies is independent of the presence of other antimicrobial or autoantigens in IBD patients. Among the subset of Crohn patients who are positive only for p-ANCA, 40% to 44% express anti-CBir1 antibodies compared with only 4% of p-ANCA–positive UC patients.

There has been increasing interest in exploring antibodies to carbohydrate (glycan)-based antigens because mucosal immune responses involve interaction with the glycosylated cell wall components of luminal fungi, yeast, and bacteria. Three novel antiglycan antibodies have been described in IBD patients. Antilaminaribioside carbohydrate IgG antibodies (ALCA), antichitobioside carbohydrate IgA antibodies (ACCA), and antimannobioside carbohydrate IgA antibodies (AMCA) are all associated with Crohn disease and are present in 44% to 50% of ASCA-negative patients. Although highly specific for Crohn disease, these carbohydrate antibodies demonstrate lackluster sensitivity (18%–28%) for distinguishing Crohn disease from UC. In a Hungarian cohort of patients, these antiglycan antibodies were shown to be associated with the *NOD2/CARD15* genotype in Crohn disease. Seow and colleagues demonstrated that antichitin IgA (anti-C) and antilaminarin IgA (anti-L) antibodies...
improve differentiation of Crohn disease from UC and are independently associated with complicated Crohn disease. These latter anticarbohydrate antibodies are related to, but distinct from, ALCA and ACCA.

Applications of Serologic Biomarkers

Diagnosis of IBD

The diagnostic precision of ASCA and p-ANCA profiles was evaluated in a meta-analysis of 60 studies, including a total of 4019 patients with Crohn disease, 3841 patients with UC, and 3748 controls.9 This analysis demonstrated that the serologic profile of positive ASCA and negative p-ANCA was associated with a 93% specificity and 55% sensitivity for detection of Crohn disease. A positive p-ANCA test, independent of ASCA result, was associated with a sensitivity of 55% and specificity of 89% for UC. In a subgroup of pediatric patients with a negative ASCA, the results improved to a sensitivity of 73% and specificity of 93% for atypical p-ANCA in detecting UC. Given the substantial heterogeneity in the results of individual studies, this pooled analysis may represent the most accurate estimate of the performance characteristics of these serologic biomarkers.

The suboptimal sensitivity of atypical p-ANCA and ASCA limits their clinical utility for excluding IBD with a negative test. Although the specificity of atypical p-ANCA and ASCA is high, a positive test must still be interpreted with caution and is reliable only in patients with a moderate-to-high pretest probability of having IBD. This point is underscored in an article by Austin and colleagues,30 in which the authors assumed a 94% specificity for serologic markers and applied the test to hypothetical populations with varying prevalence of disease. When applied to patients with a pretest probability of 5%, the positive predictive value was only 35%, indicating that most results are falsely positive. Consequently, the optimal use of serologic tests in the diagnostic workup of IBD remains unclear, but these tests should not supplant clinical judgment in assigning a diagnosis.

In the setting of established IBD, serologic profiles can assist in distinguishing Crohn disease from UC. The combination of positive ASCA in the absence of atypical p-ANCA is highly specific for Crohn disease, whereas the reverse serologic profile (ASCA-negative, atypical p-ANCA–positive) is strongly associated with UC.1 However, this does not hold true among Crohn patients with isolated colonic disease, many of whom have a serologic profile similar to that of UC with presence of atypical p-ANCA.

IBD-unclassified

Although most patients with IBD can be categorized as having either Crohn disease or UC using standard clinical, endoscopic, and radiologic techniques, this distinction is not possible in approximately 10% to 15% of patients, and a diagnosis of IBD-U is assigned. Serologic markers have been explored as a potential tool to aid in this distinction. A prospective study of patients with IBD-U analyzed the value of ASCA and atypical p-ANCA in clarifying disease subtype.31 After 1 year of follow-up, only 31 of the 97 patients could be categorized clinically as having definite UC or Crohn disease. The profile of positive ASCA with negative atypical p-ANCA predicted Crohn disease in 80% of these patients, whereas negative ASCA with positive atypical p-ANCA detected UC in 64% of patients. An important finding in this study was that nearly half of patients with IBD-U did not have either ASCA or atypical p-ANCA antibodies and they remained “seronegative” for a mean duration of 10 years. This highlights an important limitation to the utility of serologic tests in this context.

In the meta-analysis by Reese and colleagues9 the authors reported that ASCA is less reliable for diagnosing Crohn disease in patients with exclusively colonic...
involvement, suggesting that ASCA and p-ANCA may be less useful in clarifying disease subtype in patients with IBD-U. Many of these patients have a "UC-like Crohn disease" with left-sided colonic inflammation and often have positive p-ANCA. However, anti-CBir1 antibody may assist in discriminating the "UC-like Crohn disease" from UC. Up to 44% of patients with Crohn disease who are ASCA negative and atypical p-ANCA positive will have anti-CBir1 antibodies as opposed to just 4% of patients with UC who have the identical ASCA/p-ANCA profile.25 The application of anti-CBir1 in the setting of IBD-U has not been specifically evaluated, however.

**Tool for disease monitoring**

There does not appear to be a relationship between disease activity and atypical p-ANCA titer in UC, and p-ANCA remains positive after colectomy.32 Likewise, ASCA levels do not correlate with disease activity in Crohn disease and do not decrease in the setting of clinical response to treatment.16 These data indicate that serial measurement of atypical p-ANCA and ASCA titers cannot be used to monitor disease activity or to predict impending disease exacerbations.

**Predicting disease behavior**

The capacity of serologic markers to identify IBD phenotypes and predict disease behavior is becoming increasingly well established. Among UC patients, atypical p-ANCA is associated with left-sided colitis, poor response to medical therapy, and higher rates of colectomy.33 In addition, several investigators have described an association between high titers of atypical p-ANCA and chronic pouchitis after ileal pouch-anal anastomosis surgery.34,35 Among patients with Crohn disease, a positive atypical p-ANCA characterizes a distinct phenotype of left-sided colonic inflammation and a generally favorable response to medical therapy.36

Several studies have shown that ASCA correlates with distinct clinical phenotypes in Crohn disease. Presence of ASCA is associated with ileal disease, young age at onset, fibrostenotic and penetrating behavior, and multiple bowel surgeries.12,37–39 In a cohort of children, ASCA was demonstrated to be associated with disease of the ileum and right colon and to correlate with increased likelihood of ileocecal resection.19 Among patients with UC undergoing ileal pouch-anal anastomosis, ASCA positivity predicts a greater likelihood of developing fistula complications or a change in disease diagnosis to Crohn disease.22,40,41

The presence of anti-OmpC antibodies in Crohn disease is associated with internal perforating disease and requirement for surgery in adults38 and with fibrostenotic and penetrating disease in children.42 Patients with Crohn disease with anti-I2 are more likely to have stenosing ileal disease and to require surgical resection.38,42 An increasing number of positive serologic markers portends a more aggressive disease behavior with a higher frequency of complications and surgery, such that a patient with 4 positive antimicrobial antibodies has an elevenfold increased risk of internal penetrating or stenosing disease when compared with seronegative patients with Crohn disease.38,42 Similar to the other antimicrobial antibodies, anti-CBir1 expression is associated independently with small bowel, internal penetrating, and fibrostenosing disease features.25 Presence of anti-CBir1 antibodies is associated with ileal disease, fibrostenotic and fistulizing complications.25 The combination of NOD2 and anti-CBir1 antibodies increases the strength of association with complicated Crohn disease.43,44 Among the newer antiglycan antibodies, ALCA is more common in perforating disease, whereas ACCA is associated with fibrostenotic complications.28 However, the magnitude of association with these antibodies is modest, and the
additive value to disease prognosis over the older antimicrobial antibodies is correspondingly small.

Subclinical markers
Some authors have reported that 16% to 30% of healthy first-degree relatives of patients with UC are positive for atypical p-ANCA.\textsuperscript{45,46} However, other authors were not able to confirm this finding.\textsuperscript{47,48} Among patients with Crohn disease, it has been consistently shown that 20% to 25% of healthy first-degree family members are ASCA positive.\textsuperscript{14–16} It remains to be determined whether this finding foreshadows an increased risk of future disease for these family members.

Among Israeli military recruits who eventually developed IBD, many were found to have positive serologic findings on blood specimens that were drawn as part of a biorepository, well before the onset of clinical disease.\textsuperscript{49} ASCAs were present in 31% of patients with Crohn disease (vs 0% in controls), and atypical p-ANCA was present in 25% of patients with UC (vs 0% of controls) at an average interval of 38 months before clinical diagnosis. These intriguing data highlight a potential role of these tests in the early identification of patients at risk for IBD and stress the need for further investigation on this topic.

C-Reactive Protein
C-reactive protein (CRP) is an acute phase protein, produced by hepatocytes under the stimulation of interleukin-6, interleukin-1β, and tumor necrosis factor-α\textsuperscript{50} and is well known as a marker of systemic inflammation. The rapid production of CRP in response to an acute-phase stimulus within hours and its short half-life make it well suited as a marker for monitoring of disease activity in IBD.\textsuperscript{50} However, CRP upregulation is not specific to IBD, and occurs in response to other inflammatory diseases, infectious stimuli, tissue necrosis, malignancy, and other conditions. Some patients have polymorphisms in the CRP gene that result in lower CRP levels,\textsuperscript{51} rendering CRP an unreliable measure of inflammatory activity in those patients.

CRP is elevated in 70% to 100% of patients with Crohn disease and 50% to 60% of patients with UC,\textsuperscript{52} and the level correlates with disease severity such that mean CRP concentration is higher in patients with severe Crohn disease than those with moderate disease and is even lower in patients with mild disease.\textsuperscript{53} The same trend is evident for UC, but the absolute levels of CRP are much lower than in Crohn disease.\textsuperscript{53} A study from the Mayo Clinic demonstrated a correlation between CRP levels and evidence of inflammation as assessed by endoscopy and histology in patients with Crohn disease.\textsuperscript{54} Among the patients with UC, elevated CRP was associated with clinical disease activity and endoscopic severity, but not histologic degree of inflammation.

CRP may also be useful in prognosticating disease course. A prospective study followed 71 patients with Crohn disease in remission with serial measurements of CRP and other biomarkers every 6 weeks.\textsuperscript{55} Elevated CRP (>20 mg/L) forecasted clinical disease exacerbation within 6 weeks. In the setting of severe UC requiring parenteral steroids, a high CRP level has been demonstrated in several predictive models to portend a poor prognosis and higher rate of colectomy.\textsuperscript{56,57} A population-based study from Norway showed that elevated CRP at diagnosis predicted nearly a fivefold increase in the odds of subsequent surgery among patients with UC.\textsuperscript{58} A similar association between CRP level at diagnosis and surgery was demonstrated for patients with Crohn ileitis.

Although CRP has been used for decades, it has received increased attention lately due to the finding in randomized controlled studies that elevated CRP levels are
associated with lower placebo response and consequently increased treatment effect. This may be explained by the observation that CRP can aid in the distinction between inflammatory and irritable bowel symptoms, the latter being more likely to exhibit improvement with placebo. Other authors have confirmed the ability of CRP to predict treatment response. Louis and colleagues demonstrated that an elevated CRP level (>5 mg/mL) is associated with a favorable response to infliximab. However, in using CRP as an inclusion criterion or stratification in clinical trials, one must remember that some patients with active IBD may not produce CRP and therefore will be excluded in those studies.

**Summary and Recommendations**

Serologic biomarkers represent a novel and exciting tool for evaluation of IBD, but currently they have limited clinical application. Insufficient sensitivity and specificity of these antibodies renders them unreliable for establishing the diagnosis of IBD, particularly in patients with a low pretest probability of disease. Likewise, their use in clarifying disease diagnosis among patients with IBDU is hampered by the observation that patients with Crohn disease with isolated colonic involvement have a serologic profile similar to UC. Consequently, serologic panels cannot be routinely recommended for diagnosing IBD or distinguishing between Crohn disease and UC. The potential for serologic biomarkers to predict disease onset or behavior is intriguing, and prospective studies are underway to assess whether these markers also predict response to therapies and warrant a different treatment approach that results in changed short- and long-term outcomes. However, there is insufficient evidence that serologic profiles should override careful clinical evaluation in determining therapeutic management decisions.

CRP is a helpful adjunct tool in evaluating patients with IBD. Although the lack of specificity of CRP limits its utility for initial diagnosis, CRP can be valuable for assessing disease relapse in patients with established IBD and who have demonstrated an elevated CRP at the time of clearly active disease. Thereafter, use of CRP to confirm or predict relapse is helpful. Whether it warrants an immediate change in therapy has not been prospectively studied, but it certainly can assist in the assessment of adherence with current therapy and discussions with the patient.

**RADIOLOGIC IMAGING**

Most patients with Crohn disease have involvement of the distal ileum, a region of the bowel that is challenging to evaluate. Moreover, IBD patients have chronic disease, which may be progressive over time or alternate between periods of activity and quiescence, necessitating repeat evaluations throughout their life to assess disease distribution and activity. Objective assessment of bowel inflammation is essential to assign a diagnosis of IBD and is critical to inform management decisions in patients with established disease. Although direct endoscopic visualization of the intestinal mucosa along with histologic confirmation remains the gold standard for identifying active inflammation, this approach is invasive and ileoscopy is not always technically feasible. Noninvasive imaging tests are a valuable alternative to endoscopic evaluation of the small intestine for the purposes of diagnosis, determination of disease activity, and identification of complications of disease, such as obstruction or infection.

Fluoroscopic barium studies, including small bowel follow through (SBFT) and small bowel enteroclysis (SBE), have traditionally been the mainstay of small bowel imaging. These studies can provide details about mucosal ulceration or irregularities, luminal
narrowing or distention, and presence of fistulous communications. Sensitivity of 85% to 95% and specificity of 89% to 94% have been reported for SBFT in patients with ileal Crohn disease, although this is dependent on the experience of the radiologist.63,64 SBFT and SBE have comparable sensitivities, but SBFT is preferred by patients because it avoids the need for nasojejunal intubation.65 These conventional barium examinations serve as the benchmark for comparison in evaluating the performance of novel imaging studies. Advances in computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound have overcome barriers that previously limited their application to image the small bowel. With these advancements, there is now an expanded array of diagnostic imaging techniques for evaluation of IBD.

CT

Cross-sectional imaging with CT offers several distinct advantages over conventional small bowel radiography. Multiplanar imaging allows clear delineation of superimposed bowel loops, and visualization of extraintestinal structures permits identification of disease complications, such as abscesses, fistulae, or phlegmonous changes. Traditional CT evaluation of the abdomen uses a positive contrast agent such as iodine or barium that highlights intraluminal filling defects such as polyps or masses. By comparison, CT enterography (CTE) uses the combination of neutral (low-density) oral contrast and intravenous (IV) contrast to provide optimal distinction between the enhancing small intestinal wall and the adjacent low-attenuation intestinal lumen. This technique facilitates evaluation of the bowel wall and mucosa. Bowel distention is typically achieved with a low-concentration barium solution mixed with sorbitol and water. The amount and timing of oral contrast ingestion has not been standardized, but volumes of 900 to 1800 mL have been described.66

CT enteroclysis involves placement of a nasojejunal tube and rapid infusion of enteric contrast directly into the proximal small bowel. This technique improves bowel distention, but it is time consuming and uncomfortable for patients, and the incremental yield of this approach over CTE is modest at best. Findings on CTE or CT enteroclysis that indicate active inflammation include segmental mural enhancement, wall thickening, and mural stratification. Likewise, fibrofatty proliferation, engorgement of the vasa recta (sometimes called a “comb sign”), and reactive mesenteric adenopathy are extraluminal findings that support a diagnosis of active inflammation. Luminal narrowing, if present, can be because of inflammation with edema or chronic fibrostenotic disease, and the distinction between these 2 conditions is not always possible with CT.

In a prospective study of 96 patients undergoing both ileoscopy and CTE, the findings of mural enhancement and wall thickness were able to detect active Crohn disease, with a sensitivity of 80% to 90%.67 CTE and ileoscopy were equally accurate at predicting histologic inflammation,67 and radiologic findings correlated with clinical disease activity. In comparison with conventional fluoroscopic imaging, at least 2 studies have found that CTE is more sensitive than SBFT for detecting active disease.68,69 Studies that compared CTE with capsule endoscopy report varying results, attributable in part to differences in study design. A prospective study comparing multiple small bowel imaging modalities described a sensitivity of 53% for CTE compared with capsule endoscopy (71%), ileoscopy (65%), and SBFT (24%).69 This study defined any positive small bowel finding consistent with Crohn disease as a true positive and calculated the diagnostic yield of each modality. This methodology does not allow calculation of specificity because each finding is by definition a true positive. A similar analysis by Solem and colleagues70 prospectively compared CTE with capsule endoscopy, ileoscopy, and SBFT, using a consensus clinical diagnosis of Crohn disease as the reference standard. This study
demonstrated equivalent sensitivities for CTE and capsule endoscopy (83%), but CTE was far more specific (89% vs 53%). The authors suggested an algorithm involving these modalities, in which ileocolonoscopy and biopsy is the first test, followed by CTE and, if necessary and if there are no obstructive symptoms or findings, capsule endoscopy.

CTE and CT enteroclysis offer several distinct advantages over conventional fluoroscopic barium studies. SBFT is prone to obscuration of findings because of superimposed bowel loops, but this can be overcome using multiplanar images on CT. In addition, CT can better detect extraintestinal processes that may be missed with SBFT or with endoscopic imaging. When compared with capsule endoscopy, CTE is similarly or slightly less sensitive but far more specific.

The benefits of CTE, however, must be weighed against the deleterious effects of cumulative ionizing radiation, particularly in patients who are young or pregnant. A recent study drew attention to the alarming dose of radiation exposure that many patients with Crohn disease receive. This radiation exposure was primarily attributable to the increased use of CT imaging. Factors associated with exposure to high levels of ionizing radiation included penetrating disease, upper gastrointestinal tract involvement, diagnosis at an early age, requirement for steroids or infliximab, and multiple surgeries.

**MRI**

Concerns about diagnostic radiation exposure with CT have fueled interest in developing alternative imaging modalities that avoid this risk. Historically, MRI of the bowel was hampered by long image acquisition times resulting in respiratory and peristaltic motion artifact that compromised image quality. However, technologic advances in image acquisition combined with administration of bowel relaxant medication during the procedure have led to substantial improvements in the quality of MR images of the intestine. Administration of large-volume oral contrast results in luminal distention, facilitating evaluation of bowel wall thickness and regularity. MR enteroclysis can also be performed by distending the small intestine with enteric contrast administered through a nasojejunal tube. Analogous to CT or conventional enteroclysis, MR enteroclysis results in superior distention of the bowel but at the sacrifice of patient comfort, and the increased distention may not be necessary in many cases. A prospective study of patients randomized to MR enterography or MR enteroclysis showed identical sensitivity (88%) in both groups.

Superior image quality requires adequate bowel distention with oral contrast, minimization of motion artifact with ultrafast sequences and bowel relaxants, and enhanced bowel wall visualization with IV contrast. Several enteric contrast agents have been investigated for MR enterography. Most studies in Crohn disease use biphasic contrast agents that demonstrate low signal intensity on T1-weighted images, allowing clear delineation of the dark lumen from the hyperenhancing inflammatory segments of the bowel wall after IV contrast. Large volumes of water or even milk have been used effectively, but these liquids can be rapidly absorbed, resulting in suboptimal distention of the distal bowel. Other centers have reported better success with osmotic and/or bulking agents that retain water in the lumen, including mannitol, sorbitol, and locust bean gum. Reduction of peristalsis with IV administration of hyoscine butylbromide (Buscopan) or glucagon is integral to preventing motion artifact and preserving image quality. Multiple pulse sequences and multiphase acquisitions are obtained, and each provides complementary information. Typical MRI findings that indicate active inflammatory disease include mural thickening; ulcerations; hyperenhancement; increased mesenteric vascularity (the “comb sign”);
mesenteric inflammation; reactive adenopathy; and complications such as obstruc-
tion, perforation, or abscess.74

Several investigators have reported that MRI is more sensitive than conventional
fluoroscopic barium studies for detecting transmural bowel inflammation and has
a sensitivity of as high as 96%.78–80 However, mild mucosal changes are better visu-
alized with traditional small bowel series radiographs.78 The multiphasic sequences
obtained with MRI can be useful to distinguish inflammatory from fibrostenotic stric-
tures by detecting the differential water content of edematous tissue.81 Diffusion-
weighted imaging has also been shown to be a feasible technique for the detection
of inflammation in Crohn disease.82 When compared with endoscopy, MR enterogra-
phy demonstrates a sensitivity of 84% to 96% and a specificity of 92% to 100% for
detection of IBD.80,83 In a prospective comparison of MR enterography and CTE,
the sensitivities were similar for detecting active Crohn disease (91% vs 95%,
respectively).84

MRI can also provide detailed and anatomically important information about peria-
nal fistulae, which guides operative management, and it has therefore become a domi-
nant imaging modality for assessment and staging of perianal complications in Crohn
disease.85–87 Although MR colonography has been investigated, its role in IBD diag-
nosis and management remains undetermined, but it may potentially be of use in
patients with Crohn colitis or UC in whom optical colonoscopy is incomplete, contra-
indicated, or declined by the patient.

MR enterography is a safe and accurate method of evaluating the small bowel and
extraluminal structures in patients with Crohn disease. The advantages of this tech-
nique over CT include a lack of ionizing radiation, greater safety in renal insufficiency
and in pregnancy, and superior evaluation of the pelvic soft tissues and perianal
fistulae. The downside of MRI includes a lack of appropriately trained radiologists
outside of specialized centers and a considerable cost to perform the examination.
Some authors have proposed in a cost-utility analysis that MR imaging may be superior
to SBFTbecause of improved diagnostic accuracy that outweighs the increased cost.88
This assertion remains to be substantiated with prospective data (Figs. 1 and 2).

Ultrasound

Abdominal ultrasound is an evolving imaging modality with a variety of potential appli-
cations in patients with established or suspected IBD. There has been renewed
interest in exploring ultrasound as a tool in IBD because of its lower cost, excellent
safety profile, and ability to be used in pregnant patients. Improvements in ultrasound
equipment allow high-resolution images with improved depth of penetration, permit-
ting detailed visualization of the bowel wall and adjacent mesenteric structures.

Inflammation is identified by increased bowel wall diameter, alteration of the normal
sonographic pattern of mural stratification, and increased blood flow as assessed by
Doppler evaluation. In a meta-analysis, the sensitivity of ultrasound for the initial diag-
nosis of Crohn disease ranges from 75% to 94%, with a specificity of 67% to 100%,
depending on the cutoff value for defining mural thickening.89 Ultrasound is most
sensitive for detecting disease of the ileum (95%), with decreased sensitivity for de-
tecting disease in the left colon (88%), transverse colon (82%), or jejunum (72%).90
Ultrasound can accurately identify bowel wall thickening and can distinguish fibrosis
from acute edema.91 In a thickened bowel segment, loss of mural stratification is asso-
ciated with inflammation, whereas retained stratification suggests fibrosis.92 Introduct-
ion of a new microbubble contrast agent that persists in the bloodstream for several
minutes has facilitated development of contrast-enhanced ultrasound (CE-US) for
imaging parenchymal organs. By evaluating the presence and distribution of blood
flow within the bowel wall, CE-US can assess disease activity with high sensitivity (93%) and specificity (93%) and has a strong correlation with clinical disease activity indices.91

Some challenges remain, however, that limit widespread application of abdominal ultrasound. Although ultrasound findings are sensitive for the bowel loop being examined, intraluminal gas can obscure visualization of some bowel loops altogether, and obese patients can be challenging to evaluate because of poor depth of penetration. Furthermore, an experienced operator and equipment with enhanced resolution are essential to obtaining high-quality images, and expertise in abdominal ultrasound is

Fig. 1. Ileal stricture seen on magnetic resonance enterography, coronal view.

Fig. 2. Ileal inflammation seen on magnetic resonance enterography, axial view.
not pervasive. Nonetheless, these intriguing data suggest a developing role for ultrasound imaging in patients with IBD that warrants continued investigation.

**Positron Emission Tomography**

Positron emission tomography (PET) scanning is a nuclear medicine technique that uses $[^{18}F]$ fluoro-2-deoxy-D-glucose to identify areas of increased metabolic activity, and it has been used to evaluate multiple infectious, inflammatory, and malignant diseases. Several authors have demonstrated in prospective studies that PET, alone or in combination with CT, can successfully identify active inflammation in both children and adults with established or suspected IBD. A study by Meisner and colleagues reported that PET alone was sufficient to detect inflammation in UC, whereas PET/CT was more useful in Crohn disease. Although data are quite limited, PET appears to have excellent sensitivity and may even be able to detect subclinical inflammation in patients with UC. However, questions remain about its specificity, and the practical application of PET in IBD management has not been established.

**Summary and Recommendations**

Novel imaging techniques represent a valuable noninvasive alternative to endoscopy for evaluation of the small bowel. CTE is preferable to SBFT as the initial radiologic imaging study in patients with ileal Crohn disease because of the capacity for multiplanar imaging and the ability to detect mural thickening and extraluminal findings. In addition, the utility of SBFT is declining, as the latest generation of radiologists has less experience with conventional barium techniques and interpretation. As the technology and experience with MR enterography imaging improves, MRE is favored over CTE not only due to the ability to distinguish active inflammation from fibrosis but also due to the avoidance of ionizing radiation. This is especially true for the evaluation of perianal Crohn disease, where MRI is clearly superior to other radiologic techniques. Abdominal ultrasound is an attractive and cost-effective option, but it remains substantially limiting by the absence of experience. PET scanning, although intriguing, remains investigational for the evaluation of patients with IBD.

**ENDOSCOPIC EXAMINATION**

**Small Bowel Capsule Endoscopy**

Small bowel capsule endoscopy (SBCE) was first introduced in 2001 and has emerged as a highly sensitive modality for the detection of small intestinal pathology, including Crohn disease. The principal advantage of SBCE over conventional endoscopy is the ability to visualize the entire small bowel. SBCE also has an easier preparation and is less invasive and better tolerated. In comparison to small bowel radiologic procedures, SBCE is very sensitive for detection of subtle mucosal lesions, but it provides no information about extraluminal processes.

Despite its many favorable attributes, SBCE also has some drawbacks. Biopsy or intervention is not possible, and there is no way to control the capsule; so significant pathology may be missed as a result of orientation of the camera away from a lesion, obscured visualization due to luminal bubbles or debris, or delayed intestinal transit resulting in an incomplete study. SBCE is contraindicated in patients with strictures because of the risk of capsule retention. However, even patients without symptoms or radiographic evidence of bowel obstruction are susceptible to capsule retention and the consequent need for surgical or advanced endoscopic intervention to retrieve the device.
The performance of capsule endoscopy has been evaluated in multiple studies. Triester and colleagues\textsuperscript{97} performed a meta-analysis of the yield of capsule compared to other diagnostic modalities in patients with nonstricturing small bowel Crohn disease. Pooled results of the 9 prospective studies comparing SBCE with barium radiography demonstrated a superior diagnostic yield of 69\% for capsule endoscopy versus 30\% for SBFT. Capsule endoscopy also outperformed ileocolonoscopy, push enteroscopy, CTE, and MR enterography. The diagnostic yield of SBCE was particularly high among patients with established disease who were being evaluated for disease recurrence or activity. These results may be exaggerated as a result of the “incremental yield” study design, which is biased to favor the most sensitive modality and does not account for false positives that may erode both the sensitivity and specificity of capsule endoscopy.\textsuperscript{98} Underscoring this assertion is that false positives are known to be common with SBCE, as 14\% of placebo patients and up to 75\% of patients on nonsteroidal antiinflammatory drugs (NSAIDs) have mucosal lesions on SBCE.\textsuperscript{99,100} Furthermore, there are no established diagnostic criteria for Crohn disease using SBCE, resulting in increased subjectivity and decreased diagnostic precision. Although most studies have defined the presence of more than 3 ulcerations in the absence of NSAID ingestion as a diagnostic criterion, as proposed by Mow and colleagues,\textsuperscript{38} this has not been prospectively validated. Given the concerns about the specificity of SBCE, but recognizing that it has excellent sensitivity, some experts have proposed that it be used primarily for monitoring of patients with established IBD rather than for initial diagnosis.\textsuperscript{98} Two studies have examined the potential of SBCE to aid in classification of disease among patients with IBD-U and suggest some modest utility in this context (\textbf{Fig. 3}).\textsuperscript{101,102}

\textbf{Balloon Enteroscopy}

Double-balloon enteroscopy (DBE) was first described in 2001 as a technique that allows deep intubation of the small intestine with an endoscope.\textsuperscript{103} A single-balloon device has also been developed with a similar intention. In contrast to push

\textbf{Fig. 3.} Stricture in ileum seen with SBCE.
enteroscopy, in which the scope is advanced through a redundant and floppy small bowel, the balloon technique allows the endoscopist to pull and pleat the bowel over the scope using an overtube. The enteroscope can be inserted via the oral or anal route and, using the combination of these approaches, complete examination of the entire small bowel can be achieved in many patients.\textsuperscript{104} Diagnostic sampling and therapeutic interventions are both possible with the double-balloon enteroscope. A study by Mensink and colleagues\textsuperscript{105} explored the utility of DBE in established Crohn disease. Of the 40 patients in the study, 24 patients (60\%) had findings indicative of active inflammation, leading to a change in therapy in 18 patients (75\%). Additional studies also support the utility of DBE in established Crohn disease as a complementary diagnostic modality.\textsuperscript{106,107} Oshitani and colleagues\textsuperscript{106} reported their outcomes in 40 patients with established Crohn who underwent DBE for evaluation of active disease. DBE was superior to radiologic evaluation for the detection of aphthae, erosions, and small ulcers in the ileum. The authors concluded that DBE is a useful complementary diagnostic modality in patients with Crohn disease. An international consensus statement on the role of small bowel endoscopy in the management of IBD concluded that there are not enough data to recommend DBE unless conventional diagnostic modalities have been inconclusive and histologic diagnosis would alter disease management.\textsuperscript{108} This was primarily due to a paucity of data available about the application of this novel endoscopic modality in IBD patients. Nonetheless, the committee endorsed DBE and other device-assisted enteroscopy techniques as valid modalities for diagnosis of Crohn disease because histologic corroboration is available.\textsuperscript{108} Balloon enteroscopy has the potential to affect management in select settings, but its exact role in the diagnosis and management of IBD remains to be established.

**Summary and Recommendations**

Capsule endoscopy is a highly sensitive modality for detecting small bowel erosions and ulcerations and is therefore useful to exclude active small bowel involvement in patients with known IBD. SBCE is currently reserved for patients with established IBD, in whom suspicion for small bowel inflammation remains despite negative findings on ileocolonoscopy and radiologic imaging. Balloon enteroscopy is most useful for obtaining mucosal biopsies of lesions detected with SBCE.

**FECAL MARKERS OF INFLAMMATION**

Endoscopy with biopsy and histologic evaluation remain the gold standard for identifying and quantifying bowel inflammation. However, endoscopy is expensive and invasive and comes with a small but real risk of complications. In IBD patients, especially, who require repeated evaluation throughout their lives, it would be helpful to have a noninvasive indicator of inflammation. This has driven investigation into surrogate markers of intestinal inflammation. The ideal marker would be simple to perform, noninvasive, inexpensive, rapid, and acceptable to patients and providers. It should also be accurate at identifying inflammation and should correlate well with disease state and prognosis. Fecal markers have a theoretical advantage over serologic markers in that they are more specific for intestinal processes and may reflect the entire intestinal tract. A variety of inflammatory diseases of the gastrointestinal tract, including IBD, are characterized by shedding of leukocytes in the feces. This observation led to the exploration of molecular methods to detect neutrophil-derived proteins in the stool as markers of bowel inflammation.
Calprotectin

Fecal calprotectin represents a promising noninvasive surrogate marker of intestinal inflammation in IBD. Calprotectin is a calcium-binding protein derived predominantly from neutrophils and to a lesser extent from monocytes and reactive macrophages. It is excreted in the feces and can be readily measured using a commercially available ELISA immunoassay. The protein remains stable in stool specimens for up to a week at room temperature, and only 1 to 2 g of stool are needed to reliably perform the analysis. These attributes add to the convenience of the test, allowing patients to collect a specimen at home and send it to a reference laboratory for analysis.

Calprotectin is not specific for the cause of intestinal inflammation and may be elevated after the use of NSAIDs or in the setting of enteric infection or intestinal malignancy. Intestinal bleeding can also elevate calprotectin levels, although a significant amount of bleeding is necessary to cause a false-positive result. The level of calprotectin fluctuates from one stool specimen to another in a single patient, and this fluctuation may be related to changes in diet or physical activity.

Results of a meta-analysis showed that calprotectin has a sensitivity of 95% and specificity of 91% for diagnosis in adults and children with suspected IBD. The 9 studies included in this meta-analysis were all prospective and used histologic diagnosis of IBD as the criterion standard. Calprotectin outperformed both CRP and erythrocyte sedimentation rate among the 4 studies in this meta-analysis that included these serum markers, and it is also more sensitive and specific than ASCA or atypical p-ANCA in this context. One of the most promising attributes of calprotectin is its capacity to predict disease exacerbation. At least 2 studies have assessed the diagnostic accuracy of calprotectin in predicting relapse among IBD patients in remission. Despite using different cutoff values, the results were remarkably similar, with a sensitivity of 89% to 90% and specificity of 82% to 83% for predicting relapse during a 12-month period. These findings were more robust for patients with UC as opposed to those with Crohn disease. Fecal excretion of calprotectin correlates closely with radiolabeled leukocyte excretion, a validated technique used to detect active inflammation in Crohn disease. Calprotectin levels fluctuate correspondingly with disease activity and correlate with validated clinical, endoscopic, and histologic assessments of disease activity, more so in UC than Crohn disease. Normalization of calprotectin has been shown to correspond to clinical improvement and mucosal healing in patients with UC. This observation suggests that calprotectin could potentially play a role as a surrogate marker of response to therapy. Recent data suggest that dramatically elevated calprotectin levels in patients with severe UC predict medically refractory disease and colectomy.

Lactoferrin

Lactoferrin is an iron-binding glycoprotein that is present in many body tissues, including human milk, tears, synovial fluid, and serum. It composes a major component of secondary granules in neutrophils. Leukocyte infiltration into the intestinal mucosa results in detectable increases in fecal lactoferrin concentration, establishing its utility as a biomarker of intestinal inflammation. Much like calprotectin, lactoferrin remains stable in fecal specimens over days and can be detected using a simple and inexpensive ELISA assay.

Lactoferrin has a sensitivity of 80% and a specificity of 82% for the diagnosis of IBD. Although these numbers suggest a slightly lower diagnostic accuracy of lactoferrin compared to calprotectin, direct comparison has not shown one to be consistently superior to the other. In addition to its utility in initial diagnosis of IBD, lactoferrin
also has a role in monitoring and management of established IBD patients. A correlation has been confirmed between fecal lactoferrin levels and disease activity, as assessed by standard clinical endoscopic and histologic instruments. In a prospective multicenter study of 89 patients with Crohn disease and 74 patients with UC in remission, elevated lactoferrin was able to predict disease relapse (25% vs 10%; \( P < .05 \)) within 12 months. Among postoperative patients with Crohn disease, those with symptomatic recurrence have higher lactoferrin levels than patients without recurrence. Even patients without symptomatic recurrence have persistently elevated lactoferrin levels at long-term follow-up. Two studies have shown a rapid and dramatic decline in fecal lactoferrin levels that paralleled clinical improvement after initiation of infliximab therapy in patients with Crohn disease. These data highlight a potential role for lactoferrin as a tool for monitoring response to therapy.

**Summary and Recommendations**

The neutrophil-derived fecal markers hold enormous promise as noninvasive tools for detection and monitoring of bowel inflammation in IBD. However, despite this potential, their exact role in the diagnostic and management algorithm has not been clarified and they are not routinely used in clinical practice, with the exception of the patient with persistent symptoms and an otherwise negative work-up, in which case they may suggest occult inflammation.

**SUMMARY**

Advances in technology have ushered in a variety of novel diagnostic and prognostic modalities for evaluating IBD. Clinicians now have an expanded arsenal of strategies to assist with timely and accurate diagnosis, to assess disease distribution and activity, and to identify disease complications. Although serologic biomarkers are not reliable for disease diagnosis, they have a promising role in predicting disease behavior and the possibility of stratifying treatments. Improvements in imaging techniques offer complementary strategies for noninvasive evaluation of the bowel and extraluminal structures while avoiding ionizing radiation. Endoscopic advancements with capsule and balloon enteroscopy now permit detailed visualization, diagnostic sampling, and therapeutic intervention throughout the entire intestinal tract. Neutrophil-derived fecal markers hold promise as convenient and rapid tests to diagnose and monitor disease, but their exact place in the IBD management algorithm is not yet established. The future will undoubtedly deliver more exciting and innovative approaches to determining diagnosis and prognosis in patients with IBD.

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