REVIEW

Current and future role of biomarkers in Crohn's disease risk assessment and treatment

Cyrus P Tamboli[1]
David B Doman[2]
Amar Patel[3]

[1]Department of Internal Medicine, Division of Gastroenterology, University of Iowa, Iowa City, IA, USA; [2]George Washington School of Medicine, Silver Spring, MD, USA; [3]Peloton Advantage, LLC, Parsippany, NJ, USA

Background: Crohn's disease (CD), a chronic inflammatory bowel disease (IBD), occurs in genetically susceptible individuals who develop aberrant immune responses to endoluminal bacteria. Recurrent inflammation increases the risk of several complications. Despite use of a traditional "step-up" therapy with corticosteroids and immunomodulators, most CD patients eventually require surgery at some time in their disease course. Newer biologic agents have been remarkably effective in controlling severe disease. Thus, "top-down," early aggressive therapy has been proposed to yield better outcomes, especially in complicated disease. However, safety and cost issues mandate the need for careful patient selection. Identification of high-risk candidates who may benefit from aggressive therapy is becoming increasingly relevant. Serologic and genetic markers of CD have great potential in this regard. The aim of this review is to highlight the clinical relevance of these markers for diagnostics and prognostication.

Methods: A current PubMed literature search identified articles regarding the role of biomarkers in IBD diagnosis, severity prediction, and stratification. Studies were also reviewed on the presence of IBD markers in non-IBD diseases.

Results: Several IBD seromarkers and genetic markers appear to be associated with complex CD phenotypes. Qualitative and quantitative serum immune reactivity to microbial antigens may be predictive of disease progression and complications.

Conclusion: The cumulative evidence provided by serologic and genetic testing has the potential to enhance clinical decision-making when formulating individualized IBD therapeutic plans.

Keywords: Crohn's disease, serologic testing, inflammatory bowel disease, complicated disease, biomarkers

Introduction

Crohn's disease (CD) is a prevalent chronic inflammatory bowel disease (IBD) marked by heterogeneous symptoms indicative of an underlying inflammatory process. The hallmark pathology of CD is chronic transmural inflammation, but the phenotypic spectrum varies greatly both in location and behavior (ie, stricturing or penetrating phenotypes).[1] As the disease progresses, persisting inflammation may lead to penetration and strictures, perhaps culminating in medically refractory disease requiring multiple hospitalizations and surgical intervention.[2–4] The traditional treatment paradigm includes a "step-up" approach of corticosteroids and immunomodulators, with or without biologic agents as severity progresses or patients fail to respond.[5–7] Whereas this approach may be effective in the near term,[8–10] it may not prevent overall disease progression.[11–13] Within 10 years of diagnosis, more than half of CD patients still require surgical resection within 20 years,[14] approximately 50%–70% of CD patients develop

Correspondence: Cyrus P Tamboli
Department of Internal Medicine,
Division of Gastroenterology, University of Iowa, Iowa City, IA 52242-1081, USA
Tel +1 319 384 9841
Fax +1 319 353 6399
Email cyrus-tamboli@uiowa.edu

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a stricturing or penetrating intestinal complication,[2,15] and the cumulative risk of hospitalization rises to nearly 80%.[16] Risk of hospitalization is greatest within the first year after diagnosis of CD (32%–83% of patients), with the annual incidence of hospitalizations remaining steady at 20% over the next 5 years.[16,17]

"Top-down" therapy,[7,18] with the earlier introduction of biologic agents such as antitumor necrosis factor alpha (anti-TNF-α) antibodies, has demonstrated high rates of remission and mucosal healing.[19–23] However, the top-down approach is not appropriate for all patients, as not all of them will develop complicated disease.[2,12,16] Early use of immuno-suppressants or biologics soon after diagnosis may increase the risks, including malignancies and infections.[7,18] The high costs of these therapies[24,25] also prohibit top-down therapy as a universal approach.[7] Therefore, the ability to identify patients at risk for developing a complicated disease course is critical to the effective use of targeted top-down strategies.

Clinical and nonserologic predictors of disease course

Clinical features have some predictive value for prognosis in CD, but their interpretation remains problematic. Studies have shown that an initial requirement for steroids, young age at diagnosis, presence of small bowel, and/or perianal disease at diagnosis, and cigarette smoking[26–28] are associated with an adverse prognosis. However, factors such as referral bias,[29] varying definitions of adverse outcomes, and varying prior disease treatments in these studies complicate the prognosis and make predictions difficult for the individual CD patient. Clinical phenotyping issues remain complex; ongoing efforts are being made to standardize a clinical classification scheme for IBD.[30] Disease localization may be comparable only at the time of diagnosis, since CD behavior evolves over time. Vernier-Massouille et al[31] showed a convergence in rates of CD subtypes, with inflammatory (decreasing prevalence) and stricturing (increasing prevalence) disease over 10 years of follow-up after diagnosis (Figure 1). Most studies suggest that ileal disease is an independent predictor of adverse outcomes, particularly the need for early surgery.[29,32–34] While some clinical features do show associations with adverse prognosis, they are usually described retrospectively, and many features lack standardization. The resulting heterogeneity leads to significant difficulty in using these clinical data for creating therapeutic algorithms in CD.[35]

Inflammatory biomarkers such as C-reactive protein (CRP), fecal calprotectin, and fecal lactoferrin may be useful in differentiating active IBD from inactive IBD and other
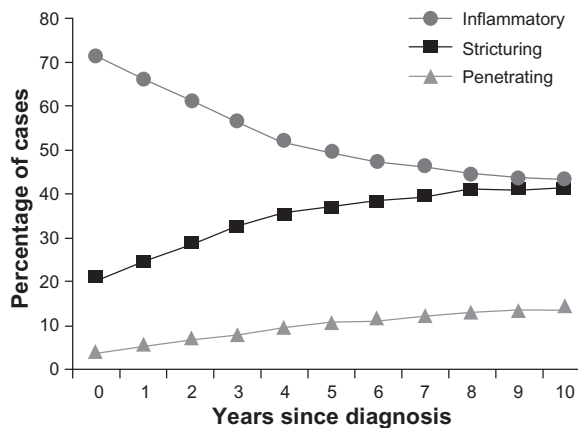

Figure 1 Crohn's disease phenotypic behavior over time, according to Montreal classification at diagnosis and follow-up in 404 pediatric patients. Dramatic changes occurred in the proportion of disease behavior subgroups – from inflammatory nonpenetrating, nonstricturing disease (B1) to stricturing (B2) or penetrating disease (B3) ($P < 0.01$).
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gastrointestinal disorders,[36] as well as measuring response to various treatments.[37] Pretreatment CRP levels have shown utility in predicting treatment response to anti-TNF-α agents in CD in some but not all studies.[20,38] The value of CRP as a pretreatment predictor of severe disease remains mostly unknown. Henriksen et al[39] found a CRP > 53 mg/L at diagnosis to be predictive of a high risk of surgery (82%) after 5 years in patients with ileal disease (odds ratio [OR] 6.0; 95% confidence interval [CI] 1.1–31.9), L1 according to the Vienna classification. Although the predictive value of an elevated CRP is suggested in this subset (~30% of those with L1 classification),[39] the sensitivity and specificity of CRP in CD are modest overall. Fecal calprotectin is a natural anti-biotic, cytoplasmic protein released into the colonic lumen by activated polymorphonuclear neutrophil cells and/or monocyte-macrophages during cell death. Fecal calprotectin levels are elevated in active IBD. Lactoferrin, similar to cal-protectin, is a glycoprotein component of polymorphonuclear neutrophil granules whose concentrations become elevated in feces during an acute mucosal inflammatory response. Four fecal markers of inflammation – calprotectin (PhiCal™ enzyme-linked immunosorbent assay [ELISA] test), lactoferrin (IBD-SCAN™ ELISA test), the Hexagon OBTI (immunochromatographic test for detection of human hemo-globin), and LEUKO-TEST (lactoferrin latex-agglutination test) – were evaluated to discriminate irritable bowel syn-drome (IBS) from IBD in a prospective study.[40] Accuracy was similar with both fecal lactoferrin and fecal calprotectin assays (~90%), but these tests do not differentiate between various types of inflammatory colitides (ie, diverticulitis, infectious or ischemic colitis). These findings have been

replicated.[36] Fecal calprotectin and lactoferrin outperform serum CRP or the clinical Crohn's disease activity index at correlating with endoscopic levels of inflammation (Spearman's $r = 0.729$ and 0.773, respectively; $P < 0.001$), especially colonic inflammation.[41] In clinical practice, these tests can be used to differentiate between IBD and IBS or to corroborate clinical flare-ups.

CD-specific serologic and genetic markers
Serologic markers in IBD: role of familial studies

Subsets of IBD patients may have abnormal immune responses to various microbial antigens.[42,43] Antibodies to *Saccharomyces cerevisiae* (ASCA) occur in 50%–70% of CD patients.[44] The pathophysiological associations of seromarkers with IBD subtypes are supported by familial studies. Atypical antineutrophilic cytoplasmic antibodies (ANCA) are associated with ulcerative colitis (UC) in approximately 70% of patients,[44] although familial studies do not suggest that ANCA has genetic underpinnings. Papo et al[45] and Folwaczny et al[46] both found no increase in ANCA prevalence among unaffected relatives of IBD patients (~3%–5%). Perinuclear ANCA (pANCA) was subsequently associated with Crohn's colitis.[47]

In contrast, ASCA has shown strong familial associations, suggesting its primary role as a stable biomarker in CD. Sendid et al[48] found 20% of unaffected relatives were ASCA-positive in CD families versus less than 1% of unaffected relatives in control families. A Belgian study also found similar results (21%)[49] but showed that ASCA is not associated with any alteration in intestinal permeability. An Italian study demonstrated elevated ASCA (~25%) in unaffected relatives of IBD patients, which included purely UC-affected families.[50] These investigators concluded there may be a primary genetic influence on ASCA status in IBD families. The possible genetic underpinnings of ASCA in CD are complex. An IBD twin study found only a 5% seroprevalence of ASCA among 20 unaffected (discordant) monozygotic twins with a CD sibling, versus 26% among 27 discordant dizygotic twins. This suggests the importance of shared environmental factors in familial CD.[51] However, there still may be a genetic component to ASCA. Seibold et al[52] showed that ASCA positivity is associated with mutations in the mannan-binding lectin (*MBL*) gene that result in *MBL* deficiency. The physiologic role of *MBL* includes immune recognition of yeasts and other mannose-expressing pathogens.[53] Hence, it may be that

ASCA seroreactivity occurs when such pathogens are able to penetrate a permeable intestinal barrier, especially in the setting of *MBL* deficiency.[53] Newer IBD markers (described below) have also shown increased familial expression,[54] particularly for CD.

A natural question that follows from familial ASCA is whether ASCA presence positively predisposes to future CD development. The literature on this issue is sparse. One study of 102 ASCA-positive first-degree relatives of IBD patients revealed a less than 2% cumulative incidence of IBD over 7 years.[55] In a nonfamilial study, Israeli et al[56] found 31% ASCA seropositivity before CD diagnosis in military recruits. An additional 23% of CD patients seroconverted after CD diagnosis, and none of the 95 non-IBD controls were ASCA-positive over the same 38-month median follow-up. Prospective studies would be most informative in this regard.

IBD diagnostics: serologic markers as a screening or diagnostic tool

If seromarkers such as ASCA do precede CD development in as many as one-third of individuals, the positive predictive value (PPV) of the test becomes a relevant issue. Several recent studies have shed light on the spectrum of non-IBD diseases demonstrating ASCA phenomena (Table 1).[57–65] Data for newer IBD markers are not yet available, and clinicians using serologic markers in the evaluation of IBD should be aware of this. ASCA was originally reported as an antibody to the nonpathogenic yeast *S. cerevisiae* in CD.[43,66] However, the clinically relevant yeast *Candida albicans* also expresses ASCA epitopes under conditions favoring their virulence; a study from the ASCA-pioneering group in Lille, France,[57] confirmed that 100% of patients with systemic candidiasis have acute ASCA titers above cutoff values considered significant in CD. However, this does not preclude the role of ASCA in CD. *Candida albicans* may be of greater relevance to CD than *S. cerevisiae*, which has never been considered pathogenic in CD. The same group[57] confirmed that *C. albicans* is an immunogen for ASCA in CD and is more prevalent in healthy relatives of patients with CD.[67] Bacterial infections and other chronic diseases may also generate ASCA positivity in some individuals (Table 1).[58] Rates of 21%–44% seropositivity have been reported in cystic fibrosis. Bacterial infection was suspected of playing a role in this context.[59,60] Intestinal tuberculosis, highly prevalent in many areas of the world, may be difficult to clinically or endoscopically distinguish from CD.[68] Makharia et al[61] reported seropositivity rates of 43% for immunoglobulin A (IgA) and 47% for ASCA

Table I Seroprevalence of ASCA positivity, IgA and IgG in non-IBD disease

Disease	ELISA assay	ASCA IgA	ASCA IgG	ASCA IgA or IgG	Study
Systemic candidiasis	Lille assay, inhouse	–	–	100%	Standaert-Vitse et al[57]
Various acute bacterial infections	Aesku Diagnostics, Germany	–	–	22%	Berlin et al[58]
Cystic fibrosis, pediatric	Quanta-Lite, Inova Diagnostics, USA	13%	11%	21%	Condino et al[59]
Cystic fibrosis, adult	Quanta-Lite, Inova Diagnostics, USA	44%	–	–	Lachenal et al[60]
Intestinal tuberculosis	Aesku Diagnostics, Germany	43%	47%	67%	Makharia et al[61]
Celiac disease (pre-treatment)	Quanta-Lite, Inova Diagnostics, USA	20%	54%	59%	Granito et al[62]
Type 1 diabetes mellitus	Orgentec, Germany	10%	21%	25%	Sakly et al[63]
Primary biliary cirrhosis	Quanta-Lite, Inova Diagnostics, USA	19%	11%	23%	Muratori et al[64]
Primary sclerosing cholangitis	Quanta-Lite, Inova Diagnostics, USA	32%	28%	44%	Muratori et al[64]
Autoimmune hepatitis	Quanta-Lite, Inova Diagnostics, USA	12%	16%	18%	Muratori et al[64]
Ankylosing spondylitis	Quanta-Lite, Inova Diagnostics, USA	23%	12%	–	Riente et al[65]
Rheumatoid arthritis	Quanta-Lite, Inova Diagnostics, USA	18%	10%	–	Riente et al[65]

Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; ELISA, enzyme-linked immunosorbent assay; IBD, inflammatory bowel disease; IgA, immunoglobulin A; IgG, immunoglobulin G.

in Indian patients with intestinal tuberculosis, rates that did not differ from those in CD patients. Noninfectious diseases considered in a differential diagnosis of IBD may also demonstrate ASCA phenomena. For example, ASCA titers may be elevated in untreated celiac disease and disappear completely after introducing a gluten-free diet. This suggests that abnormal intestinal permeability plays an important role in ASCA generation, as well as for other antibodies in celiac disease.[62] ASCA positivity may also reflect a phenotypic continuum between ulcerative jejunitis, celiac disease, and classical CD. Occasionally, clinicians will encounter patients with IBD or suspected IBD, or with associated diseases such as ankylosing spondylitis or rheumatoid arthritis. In these settings, ASCA has been shown to be nonpredictive of occult IBD.[69] In addition, pANCA has been associated with autoimmune hepatitis (AIH) type 1, particularly in men and in those AIH-1 patients with smooth muscle antibody of the anti-actin type.[70] Therefore, caution is required when interpreting positive tests in such patients, particularly those without gastrointestinal symptoms. Testing for ASCA alone may have limited usefulness in predicting CD. Furthermore, there is clinical overlap of ASCA in UC.

These limitations have led to the development of serologic marker combinations in panels to increase their predictive values (Table 2).[71–76] Sandborn and colleagues[77] reported a PPV of 86% for CD with the ASCA-positive/ANCA-negative combination. Similarly, Peeters et al[78] reported a PPV of 91% for this combination. The predictive value is increased by testing ASCA for both IgA and IgG (immunoglobulin G) subfractions.[79] However, a meta-analysis of more than 60 ASCA and ANCA studies in IBD[80] showed a modest overall sensitivity of the ASCA-positive/ANCA-negative combination for CD (55%) (Table 2).[44,78,80–86] Several novel

bacterial antigens in CD have been identified as potentially useful in serologic testing. Approximately 55% of CD patients test positive for antibodies to *Escherichia coli* outer membrane porin C (anti-OmpC)[87] and for antibodies to a bacterial sequence derived from *Pseudomonas fluorescens* (anti-I2). Reactivity to CBir1 flagellin, a colitogenic antigen of the enteric flora in C3H/HeJBir mice strain, is highly prevalent (50%) in CD.[88] In addition, anti-CBir1 is detected in patients who are nonreactive to the ASCA, OmpC, I2, and ANCA antigens.[89] Recently, a class of antibodies called antiglycans have been shown to be prevalent in CD.[90] These homogeneous antibodies are directed against carbohydrate moieties on cell surfaces of erythrocytes, immune cells, and microorganisms.[90]

Investigators have sought to define specific patterns of reactivity with serologic biomarkers. Such patterns may better distinguish CD from UC or further characterize patients with indeterminate colitis, possibly into a CD or UC diagnosis. Computer algorithm modeling of clinical pattern recognition has been developed to facilitate pattern recognition.[80,87,91] For example, the presence of anti-CBir1 and pANCA antibodies among CD patients can help to distinguish between UC and a UC-like CD phenotype.[89] In addition, when combined with ASCA and pANCA testing, anti-OmpC and anti-I2 antibodies can help identify up to 84% of patients with CD; this yield drops to 54% when ASCA is considered alone.[87]

IBD prognostics: individual serologic markers and CD disease behavior

While initially used for diagnostic purposes, serologic panels are more useful in clinical practice for their prognostic information. While each serologic marker is associated with some form(s) of complicated disease behavior, a qualitative

Table 2 Summary of seromarker characteristics in IBD

Seromarker	Antigenic determinant	Disease indication	Sensitivity, specificity, PPV, NPV
ASCA	Mannose residue forms the phosphopeptidomannan of the cell wall of *Saccharomyces cerevisiae*; also expressed by *Candida albicans*	CD • Sensitivity for CD improved when used in combination with pANCA	Sensitivity: 53%[80] Specificity: 89%[80] PPV: 73%[a,81] PPV: 84% with (−) pANCA[81] NPV: 68%[78]
pANCA	Unidentified protein of the nuclear envelope of neutrophils	UC • May be (+) in CD with UC-like phenotype • May predict chronic pouchitis following IPAA	Sensitivity: 55%[80] Specificity: 89%[80] PPV: 82%[a,81] NPV: 89%[78]
Anti-OmpC	Outer membrane porin, originally isolated from *Escherichia coli*[87]	CD • May identify CD in up to 15% of ASCA (−) patients	Sensitivity: 20%–55%[44] Specificity: 89%[44] PPV: 83.4%[b,82] NPV: 25.3[b,82]
Anti-I2	Bacterial sequence derived from *Pseudomonas fluorescens*	CD	Sensitivity: 42%[44] Specificity: 76%[44] PPV: 96%[c,83] NPV: 26%[‡c,83]
Anti-CBir1	Flagellin, CBir (*Clostridium subphylum*)	CD • May help to differentiate CD from UC in pANCA (+) patients	Sensitivity: 50%[c,84] Specificity: 53%[c,84] PPV: 45%[c,85] NPV: not reported
Combination seromarker panel (Prometheus IBD Serology – 7)	ASCA (IgA, IgG), anti-OmpC, anti-CBir1, NSNA with IFA perinuclear pattern and DNAse sensitivity	Differentiating IBD from non-IBD, and CD from UC	Sensitivity: 80%[86] Specificity: 61.5%[86] PPV: 68%[86] NPV: 75%[86]

Notes: [a]Value reported for distinguishing CD from UC; [b]Using expanded-spectrum IgA antibody to multiple outer membrane porins (Omp); [c]Exclusive pediatric cohort.
Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; CD, Crohn's disease; IBD, inflammatory bowel disease; IFA, indirect fluorescent-antibody assay; IgA, immunoglobulin A; IPAA, ileal pouch-anal anastomosis; NPV, negative predictive value; NSNA, neutrophil-specific nuclear auto-antibodies; OmpC, outer membrane porin C; pANCA, perinuclear ANCA; PPV, positive predictive value; UC, ulcerative colitis.

response to multiple markers is more predictive of a severe course in CD. Studies in CD have correlated ASCA reactivity with increased risk of surgery within 3 years of diagnosis,[92] small bowel disease location,[93] early age at diagnosis, and a complicated disease course.[94] Additional CD studies have linked pANCA levels to UC-like disease behavior[47,94] and a lack of fibrostenosing/penetrating disease.[93,94] In UC patients undergoing ileal pouch anal anastomosis, high levels of pANCA before proctocolectomy are associated with development of chronic pouchitis.[95,96] The next generation of serologic markers after ASCA and pANCA have been associated with an aggressive disease course in CD (Table 3). Anti-OmpC and anti-I2 are associated with fibrostenosing and internal-perforating disease behavior as well as small bowel surgery.[50,71,73] Additionally, multivariate logistic regression analysis has shown that these two markers are independently associated with a complicated CD phenotype and/or surgery.[71] Patients who express anti-CBir1 are nearly twice as likely to develop small bowel disease and complicated phenotypes such as fibrostenosis and internal-perforating disease.[89] A recent study reported that anti-CBir1 can be predictive of the development of pouchitis after ileal pouch anal anastomosis in pANCA-positive patients.[96]

Serologic panels and disease behavior

An association between severe, complicated CD and high-level immune responses was confirmed in multiple studies analyzing quantitative antibody levels in panels of serologic markers (Table 3). In 2004, Mow et al[71] associated cumulative antibody responses to I2 and OmpC with distinct disease phenotypes. Sera from 303 CD patients were analyzed for anti-I2, anti-OmpC, and ASCA. Quartile scores of 1–4 were assigned to the individual antigens based on antibody levels measured; a quartile sum score (range 3–12) was derived for each patient to represent the cumulative quantitative immune response to all four antigens.[71] Patients with a qualitative antigen reactivity to I2, OmpC, and oligomannan (ASCA) were more likely to develop complicated disease (fibrostenosing and internal-perforating disease) and require small bowel surgery than patients expressing fewer than three antibodies ($P \leq 0.001$).[71] Quartile sum score analysis suggested that the magnitude of antibody responses to I2, OmpC,

Table 3 Studies of serologic panels in predicting disease phenotype in Crohn's disease

Study	Year	Design	Population	N	Serologic markers[a]	Key findings
Mow et al[71]	2004	Retrospective	Adult	303	ASCA, anti-OmpC, anti-I2	• Anti-I2, anti-OmpC, and ASCA were each individually associated with IP/S disease or small bowel surgery • Patients seropositive for anti-I2, anti-OmpC, and ASCA were more likely to develop complicated disease behavior than those with reactivity to 2, 1, or 0 markers ($P \leq 0.001$) • Percentage of patients with complicated disease increased with increasing magnitude of antibody response
Arnott et al[72]	2004	Retrospective	Adult	142	ASCA, anti-OmpC, anti-I2	• Presence and magnitude of responses associated with small bowel disease ($P = 0.02$), disease progression ($P < 0.001$), perforating disease ($P = 0.008$), and surgery ($P < 0.001$)
Papadakis et al[73]	2007	Retrospective	Adult	731	ASCA, anti-OmpC, anti-I2, anti-CBir1	• Confirmed that anti-CBir1 is independently associated with complicated CD phenotype ($P = 0.0004$) • Proportion of patients with IP/S disease increased with increasing reactivity to all 4 antigens • Addition of anti-CBir1 reactivity enhanced discrimination of CD phenotypes, particularly complicated disease (IP/S), small bowel involvement, and UC-like disease
Dubinsky et al[74]	2006	Prospective	Pediatric	196	ASCA, anti-OmpC, anti-I2, anti-CBir1	• Anti-OmpC ($P = 0.0006$) and anti-I2 ($P = 0.0034$) were associated with IP/S disease • Frequency of IP/S disease increased with increasing number of immune responses ($P = 0.002$) • OR of developing IP/S disease was highest among children with all 4 immune markers (OR 11.0; 95% CI 1.5–80.4; $P = 0.03$)
Dubinsky et al[75]	2008	Prospective, longitudinal	Pediatric	796	ASCA, anti-OmpC, anti-CBir1	• Frequency of IP/S disease and surgery increased significantly with both the number of immune responses ($P < 0.0001$) and magnitude of responses ($P < 0.0001$) • Immune reactivity to OmpC and CBir1 and presence of ASCA were associated with faster progression to complicated disease and surgery, significantly faster than reactivity to 1 or 2 antigens ($P < 0.0001$) • The magnitude of immune response also influences faster progression to complicated disease and surgery ($P < 0.0001$)
Ippoliti et al[b,76]	2009	Retrospective	Adolescent and adult	731	ASCA, CBir1, OmpC, I2, *NOD2*	• *NOD2* was associated with small bowel disease involvement ($P < 0.001$), fibrostenosing phenotype ($P < 0.0001$), history of small bowel surgery ($P < 0.05$), and inversely with UC-like phenotype ($P < 0.01$) • The prevalence of fibrostenosis was significantly associated with the number of positive antibodies as well as QSS • With combined serologic reactivity and *NOD2* status, ORs for developing fibrostenotic disease were greater with presence of *NOD2* variants and also increased with higher QSS

Notes: [a]pANCA was measured in some studies but not calculated with the antibodies to determine cumulative association with aggressive disease; [b]Ippoliti et al studied CD patients' seroreactivity and their *NOD2* status.
Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; CD, Crohn's disease; CI, confidence interval; IP/S, internal-penetrating/stricturing disease; *NOD2*, nucleotide oligomerization domain 2; OmpC, outer membrane porin C; OR, odds ratio; QSS, quartile sum score; UC, ulcerative colitis.

and oligomannan was also associated with complicated small bowel disease (Figure 2).[71] In a similar study, Arnott et al also found that the presence and magnitude of anti-OmpC, anti-I2, and ASCA were significantly associated with complicated disease (Table 3).[72] Papadakis et al[73] examined a serologic panel that included anti-CBir1 in addition to ASCA, anti-I2, and anti-OmpC to predict disease severity in 731 patients with CD. Quartile sum scores for this cohort revealed that increasing levels of reactivity to all four antigens were associated with fibrostenosing and internal-perforating disease.[73]
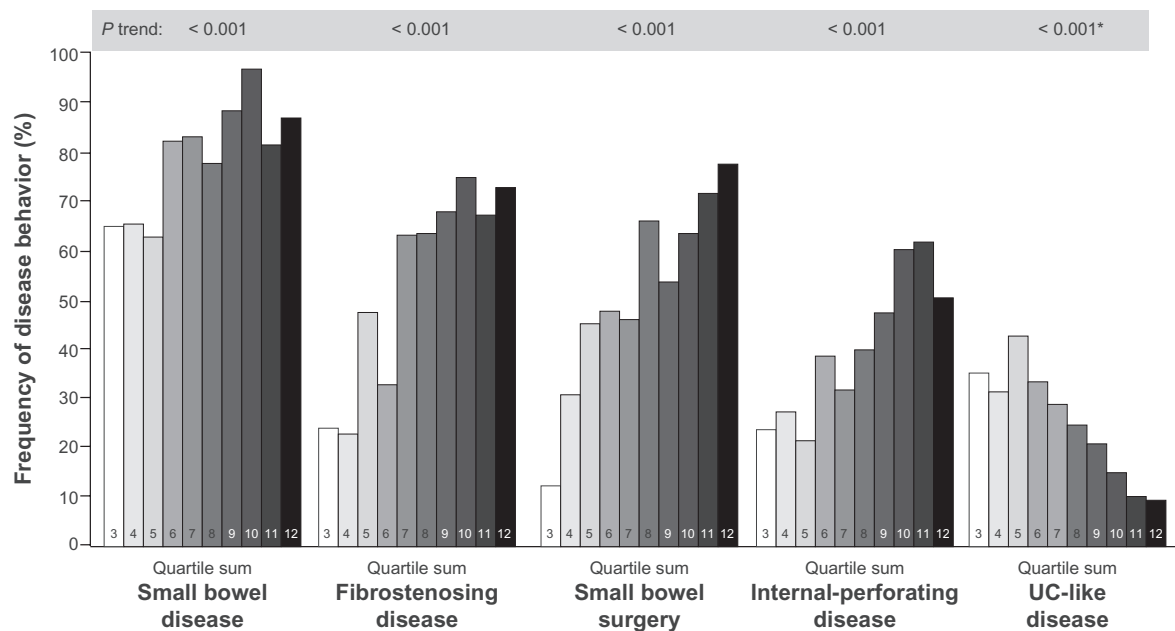
Figure 2 Frequency of complicated small bowel disease, as represented by antibody quartile sum score. A summation score of 3–12 for antibody titers toward I2, OmpC, and ASCA was calculated and categorized into quartiles (QSS). Patients with higher sum scores had greater frequency of complicated course (fibrostenosing, penetrating, or small bowel disease, or need for surgery).

Note: *Denotes negative P trend.

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Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; OmpC, outer membrane porin C; QSS, quartile sum score; UC, ulcerative colitis.

When added to the quantitative responses to the other three antigens, anti-CBir1 reactivity enhanced the discrimination of complicated disease phenotypes (fibrostenosing or internal penetrating), small bowel involvement, and UC-like behavior.[73] Dubinsky et al[74,75] conducted the first two prospective studies in pediatric CD patients that demonstrated a relationship between serologic responses and aggressive disease behavior. In the first study in 196 patients tested for anti-I2, anti-OmpC, ASCA, and anti-CBir1, the frequency of complicated disease behavior increased as the number of immune responses increased; the presence of four positive markers was associated with the highest likelihood of aggressive disease (Table 3).[74] These initial findings were confirmed in another, larger study of 796 pediatric CD patients using ASCA, anti-OmpC, and anti-CBir1.[75] The frequency of internal-penetrating disease, stricturing disease, and surgery increased substantially with both the number and magnitude of immune responses.[75] Kaplan–Meier estimates for time to development of internal-penetrating/stricturing disease and CD-related surgery by quartile sum scores are presented in Figure 3.[75] In both instances, time to adverse outcome (complex disease, surgery) is generally shorter in those patients with the highest quartile scores, whereas those in the lowest quartile have a very high probability of remaining free of adverse outcomes over long periods. The prospective

design of these studies supports the use of serologic testing to predict future disease behavior.[74,75]

Future directions
Genetic markers in assessing aggressive disease behavior

The identification of genetic markers in CD is an active area of research.[7,71,97–101] The nucleotide oligomerization domain 2 (*NOD2*), also known as caspase-activating recruitment domain 15 (*CARD15*) at the IBD1 locus is the first major susceptibility gene described for CD.[102–104] Three major-effect *NOD2/CARD15* variants have been found to account for the majority (81%) of over 30 such allelic mutations in CD; the mutations R702W, G908R, and 1007fs being designated as single nucleotide polymorphisms (SNPs) 8, 12, and 13, respectively.[100,102] Data from across the general population suggest a low penetrance for *NOD2/CARD15* mutations. However, among CD patients, each of the three SNPs has been shown to be independently associated with development of symptoms, with the greatest risk conferred by the SNP13 mutant allele and in those with multiple mutations.[104–106] The *NOD2/CARD15* variant genotypes have been associated with severe CD phenotypes.[97,98,100,101,107,108] Abreu et al[97] compared *NOD2/CARD15* genotypes to serum immune markers, disease behavior, and disease location in two consecutive cohorts of
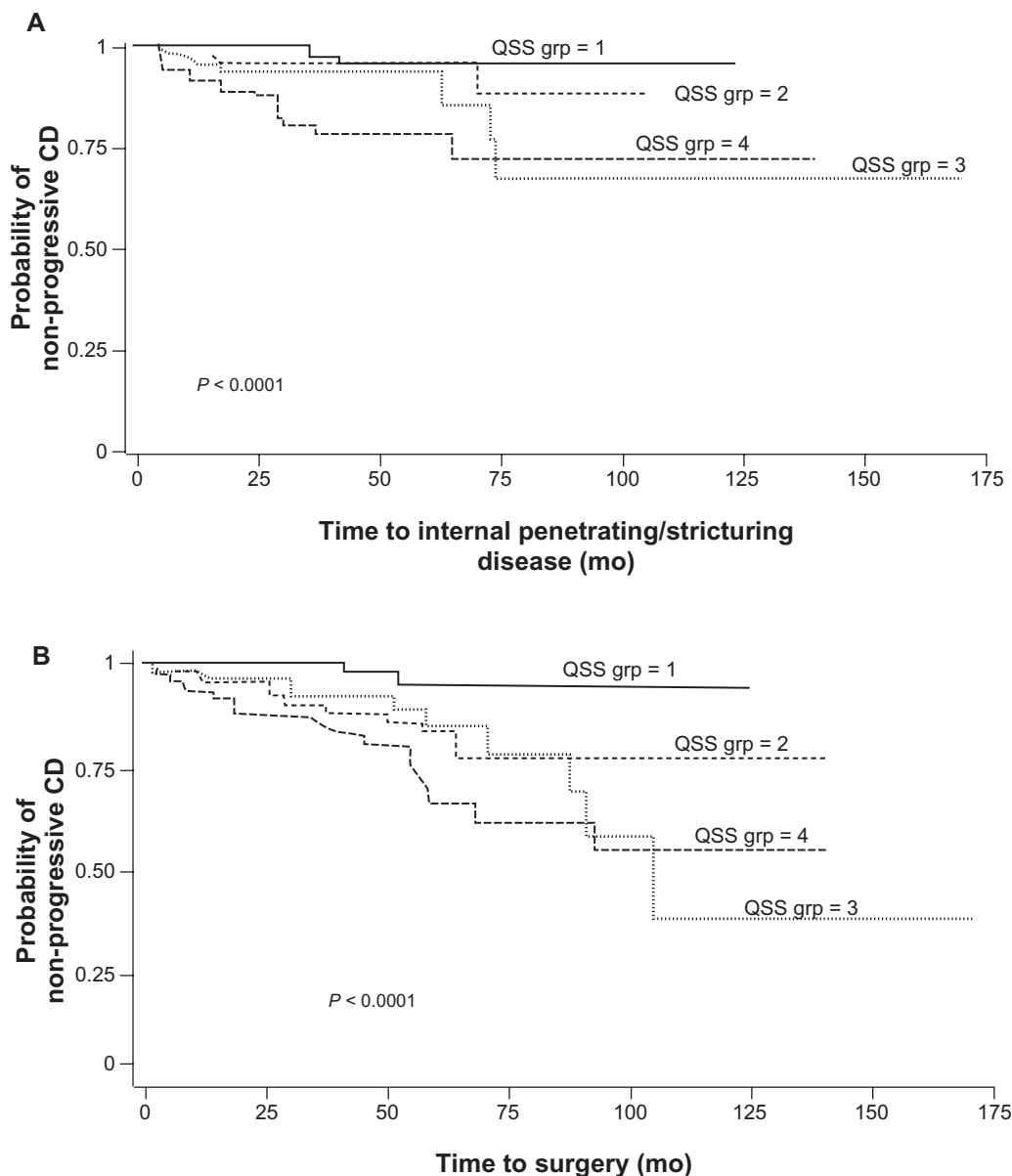
A

B

Figure 3 Kaplan–Meier plot estimates for internal-penetrating/stricturing disease and need for bowel surgery by QSS of antibody titers toward I2, OmpC, and ASCA among CD patients. The figure depicts a higher probability of maintaining a simple, noncomplicated disease course during prospective follow-up, among those patients who fall into the lower QSS groups (QSS groups 1 and 2) (**A**). Conversely, half of the patients in QSS group 3 required surgery within 100 months of follow-up (**B**).
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Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; CD, Crohn's disease; OmpC, outer membrane porin C; QSS, quartile sum score.

CD patients (Table 4). Multivariate analysis showed a significant association between the *NOD2/CARD15* variants and fibrostenosing disease (OR 2.8; 95% CI 1.3–6.0; $P = 0.011$).[97] In addition, the risk of developing fibrostenosing disease was greatest among carriers of two mutations (OR 7.4; 95% CI 1.9–28.9; $P = 0.004$). Similar findings have been observed in large European[100,107] and American[71,98] cohorts.

Genome-wide association studies have identified approximately 71 CD-associated gene susceptibility loci, with potentially many more genes.[104] Some of these have

been assessed for their relationship to CD phenotype and disease course. Weersma et al[109] examined genetic variants, including *NOD2/CARD15, Drosophilia* discs homolog 5 (*DRG5*), autophagy-related 16-like 1 gene (*ATG16L1*), and the interleukin 23 receptor gene (*IL23R*). Results showed that an increase in the number of allelic variants or genotypes was associated with an increased risk of developing CD and having a complicated disease course.[109] These findings suggest that it is possible to assess a given patient's genetic profile to determine risk of complicated disease.

Table 4 Significant individual associations of antibody responses and *NOD2/CARD15* genotype with Crohn's disease phenotypes[71,89,97]

Marker	Significant individual associations			
	Small bowel involvement	Complicated CD phenotype[a]	Small bowel surgery	UC-like behavior
ASCA	Yes	Yes	Yes	Yes (negative)
pANCA	Yes (negative)	Yes (negative)	Yes (negative)	Yes
Anti-I2	No	Yes	Yes	No
Anti-OmpC	No	Yes	Yes	No
Anti-CBir1	Yes	Yes	No	No
NOD2[b]	Yes	Yes	No	Yes (negative)

Notes: [a]Complicated CD phenotypes include fibrostenosing or internal-perforating disease; [b]NOD2 is a CD susceptibility gene.
Abbreviations: ASCA, antibodies to *Saccharomyces cerevisiae*; CARD15, Caspase-activating recruitment domain 15; CD, Crohn's disease; NOD2, nucleotide oligomerization domain 2; OmpC, outer membrane porin C; pANCA, perinuclear ANCA; UC, ulcerative colitis.

Synergism between serologic phenotypes and genetic variants

Emerging data from studies of familial expression of ASCA, anti-OmpC, and other IBD serologic markers suggest that genetic mutations lead to alterations in the expression of antibodies to microbial antigens.[49,54,99,101,110–112] Anti-CBir1 and ASCA expression were linked to *NFKB1* haplotypes and subsequently to reductions in *NF-kB* activation, thus describing another link between innate and adaptive immunity in IBD.[113] Studies have not always concurred regarding the association between *NOD2/CARD15* polymorphisms and seromarkers in IBD.[99,110,114] However, *NOD2/CARD15* variants seem to be more common in patients testing positive for multiple serologic markers, including those with high antibody levels (elevated quartile sum scores) (Figure 4).[101,111] Ippoliti et al[76] determined that a combination of altered innate and adaptive immune responses act synergistically to increase the development of complicated CD, particularly fibrostenosing disease. After grouping patients by serologic quartile sum scores of 4–6, 7–9, 10–13, and 14–16 and subdividing by the presence or absence of *NOD2/CARD15*, they calculated ORs for developing fibrostenotic disease (Table 5). The ORs were significantly greater among patients with the presence of *NOD2* variants than those without. The ORs were also increased with higher quartile sum scores.

Future diagnostic tests may quantitatively assign a risk probability for severe disease by using algorithms that analyze these serologic and genetic biomarkers. A new CD prognostic test was recently made available. This serogenetic panel is composed of seven assays for nine markers, including six serologic biomarkers, specifically ASCA-IgA, ASCA-IgG, anti-OmpC, anti-I2, anti-CBir1, and pANCA. In addition, the test recognizes three *NOD2* gene variants (*SNP8*, *SNP12*, and *SNP13*). The prognostic panel calculates
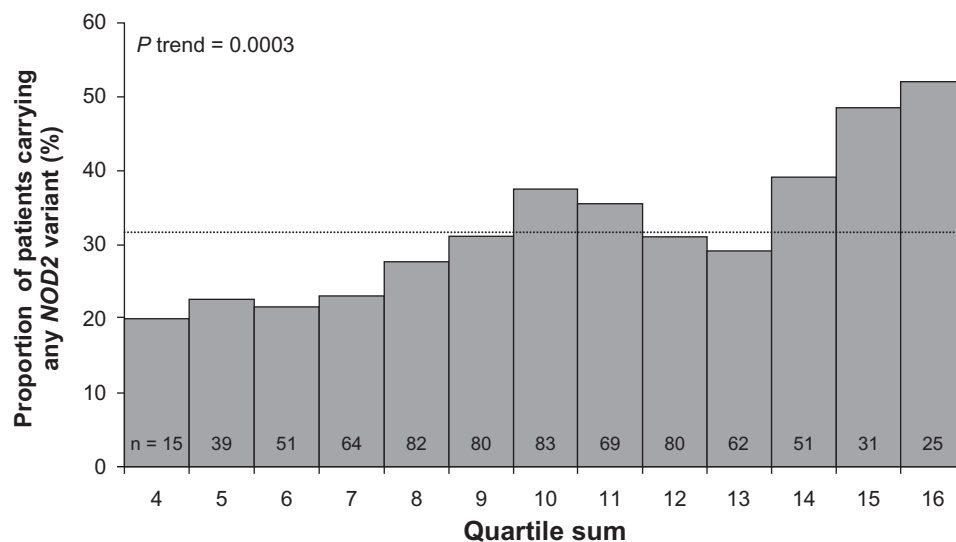

Figure 4 Frequency of carriage of *NOD2/CARD15* variant relative to semiquantitative antibody reactivity, as represented by quartile sum (range 4–16). The dotted line represents the mean 31.8% frequency of carriage of at least one *NOD2/CARD15* variant, across the entire cohort.
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Abbreviations: NOD2, nucleotide oligomerization domain 2; CARD15, caspase-activating recruitment domain 15.

Table 5 Demonstration of synergism between *NOD2* variants and antibody levels in fibrostenosis

Quartile	Presence of *NOD2* variant?	Odds ratio (confidence limits)	*P* value
4–6	No	Reference	Reference
	Yes	0.2 (0–1.2)	
7–9	No	1.2 (0.6–2.2)	0.004
	Yes	2.7 (1.3–5.5)	
10–13	No	3.3 (1.8–6.0)	0.003
	Yes	7.3 (3.7–14.4)	
14–16	No	4.8 (2.3–10.1)	0.01
	Yes	9.6 (4.2–21.8)	

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Abbreviation: *NOD2*, nucleotide oligomerization domain 2.

probability of complications curve based on antibody quartile sum scores and *NOD2/CARD15* mutation status. The results are then analyzed by a logistic regression algorithm to quantify the likelihood that a patient will progress to a complicated CD phenotype. The test output is a probability score reflecting the likelihood of disease progression to complications.[115]

Current research in identifying predictors of treatment response

Another area of growing interest that has potential to contribute to a personalized approach in CD is the prediction of response to medical therapies, particularly biologic agents.[116,117] Some clinical features have been shown to influence response to infliximab. In a prospective study in 74 CD patients, Arnott et al[116] found that smoking significantly influences response to infliximab, with smokers less likely to respond at 4 weeks (OR 0.24; 95% CI 0.06–0.91; $P = 0.035$) and more likely to relapse at 1 year (relative risk 3.2; $P = 0.0026$) than nonsmokers. Other factors that had predictive value were colonic disease, which increased the likelihood of response at 4 weeks nearly five-fold, and concomitant immunosuppression, which was associated with reduced risk of relapse at 1 year.[116] In addition, detectable trough serum concentrations of infliximab (irrespective of antibody formation) have been shown to be associated with higher rates of clinical and endoscopic remission.[117] Investigators have begun to explore the relationship of various serologic markers with response to medical therapies. Sandborn et al[118] found an increased frequency of pANCA positivity in patients with left-sided UC that was resistant to oral and rectal 5-aminosalicylates and corticosteroids. In 2004, Mow et al[119] reported the results of a small pilot study that suggested serum reactivity to microbial antigens, particularly to OmpC and I2, would help to predict response to combination

antibiotic therapy. Finally, the utility of serologic markers in predicting response to biologic agents was explored, with one study demonstrating an insignificant trend toward lower response rates to infliximab with the pANCA-positive/ASCA-negative combination in CD[120] and another associating the same combination with suboptimal early clinical response to infliximab in UC (OR 0.40; 95% CI 0.16–1.00; $P = 0.049$).[121] Investigators developed an algorithm to predict response to infliximab using a previous cohort of 287 patients with inflammatory or fistulizing CD and combining key clinical predictors (ie, age <40 years, concurrent use of immunosuppressants, disease location, and CRP levels) and pharmacogenetic data of three apoptotic SNPs (Fas ligand-843 C/T, Fas-670 G/A, and Caspase9 93 C/T).[122] The algorithm for inflammatory disease enabled prediction of response rates of 21.4%–100% and remission rates of 15.8%–85.7%, while the algorithm for fistulizing disease enabled prediction of response rates of 46.6%–100% and remission rates of 20%–57.6%.[122] Recently, Dubinsky et al[123] indicated that a combination of a phenotype, serotype, and genotype is the best predictive model of nonresponse to anti-TNF-α agents in pediatric patients. Specifically, the most predictive model included the presence of three novel "pharmacogenetic" loci, the IBD-associated loci BRWD1, pANCA, and a UC diagnosis ($P < 0.05$ for all). The relative risk of nonresponse increased 15 times as the number of risk factors increased from 0–2 to ≥3 ($P < 0.0001$).[123]

Impact of predictive factors

Current evidence suggests that a combination of clinical findings (eg, smoking) and the measurement of immune responses with serologic testing – in combination with genetic testing – can help to predict disease behavior.[124] Moreover, evidence shows that these tools may be used to stratify patients at the time of diagnosis on the basis of their risk of developing aggressive disease.[124] Screening for *NOD2/CARD15* genetic variants early in the patient's disease course may also provide additional evidence to suggest a patient's likelihood of disease progression and allow clinicians to tailor therapeutic strategies based on the aggressiveness of IBD subtype.[124] Early aggressive intervention would then be delivered to high-risk patients and less intensive therapies to those more likely to have a benign disease course. While serogenetic testing for diagnosing disease, predicting disease course, or determining treatment options[5] is not routinely used, clinical practice guidelines may ultimately evolve to include a therapeutic algorithm recommending use of top-down therapy in patients with or

at risk for complicated disease behavior, as assessed by the combination of clinical characteristics and serologic and genetic findings.[11] Furthermore, the identification of new pathogenetic treatments, including cytokines (eg, IL-23, IL-17), diapedesis inhibitors (eg, natalizumab, vedolizamab), and chemokine receptor antagonists (eg, CCX282-B), offer the promise of targeted biologic therapies. Future generations of IBD serologic profiles/genetic testing can be anticipated to play a role in identifying optimal biologic family therapeutic options.

Conclusion

Given the evidence to support the use of a top-down treatment approach, it is imperative to identify patients who are most likely to benefit from this strategy. Although clinical characteristics alone can help to predict a complicated disease course, these features lack the accuracy to effectively influence therapeutic decisions. Information gained from serologic testing, both qualitatively and quantitatively, can assist in determining the likelihood of a complicated CD. This personalized approach may be further improved with the incorporation of knowledge regarding *NOD2/CARD15* and other novel CD-associated genetic polymorphisms. Growing evidence suggests that the aberrant IBD innate immunity reflects underlying genetic determinants in CD patients. The subsequent maladaptive autoimmune response is in turn reflected by the presence of IBD serologic markers. Taken together, the patient's clinical and serogenetic profile may be used to inform clinicians regarding a patient's prognostic risk and help guide treatment decisions to alter the future natural history of CD now.

Disclosure

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