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Journal
Gastroenterology, 149(5)

ISSN
0016-5085

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Publication Date
2015-10-01

DOI
10.1053/j.gastro.2015.08.006

Peer reviewed

HHS Public Access

Author manuscript

Gastroenterology. Author manuscript; available in PMC 2017 February 10.

Published in final edited form as:

Gastroenterology. 2015 October ; 149(5): 1265–1274.e3. doi:10.1053/j.gastro.2015.08.006.

DIAGNOSTIC AND PROGNOSTIC MICROBIAL BIOMARKERS IN IBD

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Abstract

The microbiome plays multi-faceted roles in the pathogenesis of inflammatory bowel diseases (IBD). Accordingly, the clinical challenge of patient heterogeneity in disease phenotype and response to treatment should in part be addressed by biomarkers that detect the host response to microbiota, and the levels of microbial taxa and products eliciting the host response in susceptible individuals. Molecular analysis has revealed much evidence for microbial taxonomic membership and microbial products in association with IBD, but their utility as clinical biomarkers is still in its infancy. A rich area of progress has been the development and validation of host serologic microbial biomarkers, which have achieved a distinctive position in the diagnosis and prognosis in IBD, and as a template for defining other categories of microbial biomarkers in disease state and phenotype.

Keywords

gastrointestinal disease; genetics; genomics; immunology

The microbiome in IBD

IBD arises from a combination of genetic susceptibility and environmental factors that trigger an inappropriate mucosal inflammatory response[1]. Most experimental colitis models demonstrate a requirement for the presence of intestinal microbiome in disease pathogenesis[2, 3]; there is also evidence of transmissibility of colitis by dysbiotic microbiota in mice with genetic defects in host-microbial interaction, which has begun to define

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Contributions: JB and MD equally contributed to all aspects of this manuscript

Disclosure of Competing Interests: Marla Dubinsky: Consultant, Prometheus Laboratories Inc. Jonathan Braun: Consultant, Janssen Pharmaceuticals, Inc.; Eli Lilly and Company, Inc.

organisms and their properties of candidate causal significance[3, 4]. In human IBD, many genetic loci involved in disease association target host-microbial interaction (REFER TO CHO REVIEW IN 14th ISSUE), and some may directly garden the composition and function of the microbial community[5, 6]. Classic surgical diversion and J-pouch experimentation has validated luminal content as a driver of disease-associated inflammation[7]. IBD patients have reduced bacterial diversity and altered bacterial composition compared to healthy controls[8–12], and some of these are related to founder effects and diet or environmental factors associated with IBD disease risk[8, 13]. Less studied, there is evidence too for an association and potential role for fungal microbiota[14] and viruses[15, 16] in IBD. Accordingly, microbial dysbiosis and its resultant host response are an important link between environmental and genetic factors giving rise to the spectrum of IBD and its phenotypes[8, 17]

Retrospective studies of patients with Crohn's disease (CD) or ulcerative colitis (UC) have identified a range of disease-associated bacterial taxa (reviewed in[8, 18–20]), mainly associated with IBD in general, but some more selective for CD or UC. Certain Firmicutes and Proteobacteracae are commonly associated with disease activity (e.g., Enterobacteriaceae, Ruminococcus gnavus, and Desulfovibrio), and a subset in patients during clinical quiescence[21]. Conversely, a subset of Firmicutes and Bacteroides are associated with health and often deficient even in quiescent patients (e.g., Faecalibacterium prausnitzii, Lachnospiraceae, and Akkermansia). Similar features are observed as inflammation is established in pouchitis (see Table 1).

Functional studies have further related these compositional changes with the functional genetic capacity of disease associated microbial communities, including elevated microbial oxidative stress pathways, decreased carbohydrate metabolism and amino acid biosynthesis, and elevated nutrient transport and uptake[11, 21–23]. At the level of measured fecal metabolites, IBD state and disease activity are associated with reduced secondary bile acids, and active IBD patients exhibited higher levels of 3-OH-sulphated BA.[24] These products reflect altered deconjugation, and desulphation activities of the microbiota, and in preclinical models are likely to directly promote the inflammatory mucosal host response[25]. Fecal levels of short chain fatty acids are significantly reduced in patients with IBD, and since these molecules are exclusively derived from enteric microbiota, this correlates with the reduction of organisms and metagenes associated with their production, but not by simple relationship to dietary modifiers[13, 26–29]. These metabolites are important for energy metabolism of epithelium, and butyrate is also notable as a powerful inducer of regulatory T cell differentiation[30–32]. While deficits in the level or activity of this cell type are heterogeneous in IBD, their protective role in IBD in model systems and humans is well-documented[33, 34].

Retrospective studies have identified microbial taxa associated with response to treatment[8]. The abundance of six groups of bacteria including those related to Eubacterium rectale and Bifidobacterium spp. predicted the response to anti-TNF-alpha medication.[11, 35]. Response to exclusive enteral nutrition in Crohn's disease has been associated with microbial compositional change (reviewed in[13]), and curiously not associated with Faecalibacterium prausnitzii[36, 37], a prominent enteric Clostridial species deficient in IBD and involved with

several aspects of mucosal homeostasis[38]. Steroid responsiveness is associated patients bearing microbiota closer to healthy individuals in terms of diversity and composition[11, 39]. In the recent Rossen study of fecal microbial transplant in UC, responders to donor fecal transplant were distinguished by a restoration of the health-associated Clostridium clusters IV, XIVa, and XVIII, and reduction in Bacteroidetes. As in enteral nutrition, Faecalibacterium prausnitzii was not associated with this response. Responders to autologous transplant also diverged from nonresponders, but in a distinct manner (changes in Bacilli, Proteobacteria, and Bacteriodetes).[40]

The technology to detect and quantitative microbiota taxa is presently next-generation sequencing of 16S amplicons. The production and taxonomic analysis of such data is well-defined and relatively inexpensive[41]. With the increasing focus on microbial function related to disease state, additional methodologies are coming into translational research, including transcriptional or metagenomic analysis of microbial samples[18], or the levels of metabolites or proteins of microbial origin in enteric biosamples[42–46]. The former are based on high-throughput nucleic acid sequencing methods, which are well-defined for primary data production, but are under rapid change with respect to bioinformatics, and suffer as biomarker endpoints because detection is presently limited to relative rather than absolute quantitation. The latter involve NMR or liquid-chromatography mass spectrometry methods, where both primary data production and bioinformatics are less developed, and while suitable for discovery research, lack the robustness required for later translational or clinical applications. In particular, annotation of output data from LC-MS is extremely limited, especially of microbial origin[47, 48]. Indeed, the need to overcome these gaps in the assessment of enteric microbial metabolic products is engendering innovative molecular microbiology strategies[49–51], and is the target of a current NIH initiative (www.hmp2.org).

Recently, an independent and important new strategy to detect IBD-associated microbiota was introduced by Palm and colleagues based on their coating with disease-associated IgA anti-microbial antibodies, a strategy termed IgA-SEQ[52] (reviewed in[53]). In this discovery method, in situ IgA-coated fecal bacteria from IBD patients are selected by stringent flow-cytometry-based cell sorting. Using 16S sequencing, the selected microbial taxa included both known and novel microbial species associated with colitis. Using the same strategy, an eloquent study by Kau and colleagues similarly uncovered microbiota that elicited or protected undernourished Malawian children from potentially lethal dietary enteropathy[54]. These studies open the promise that the patient's own intestinal IgA response can identify commensals essential to disease pathology; and, that targeted elimination or restoration of such bacteria may reverse or prevent disease development.

What is the near-term utility of microbial composition and products as IBD biomarkers? Some features are promising. First, microbiota from the mucosal (biopsy) versus fecal compartment are more predictive of disease state, and microbiota even in remote mucosal sites (e.g., rectal mucosa of patients with restricted ileal Crohn's disease), may be similar to disease-affected sites in predictiveness for disease state.[12] Small studies have also observed IBD-associated compositional change in the salivary[55] or lingual[56] microbiota. Second, nucleic-acid based 16S analysis has analytic robustness in data production amenable to clinical laboratory production. And, a number of algorithms are under development to

provide simple data representation for clinical use, such as dysbiosis index[12]. These observations suggest that a broader ecosystem change may exist in IBD that can be probed at mucosal sites via minimally-invasive sampling, with rectal mucosa sampling perhaps the best prospect based on present data.

However, the clinical applicability of such analysis is presently immature. First, there is exceptional diversity in microbial between individuals, and in single individuals over time[29, 57] Accordingly, there is great dispersion in microbial composition among patients with IBD, and between patients and healthy individuals.[12] Thus, the levels of candidate indicator organisms or microbial indices is far below the predictive value for utility in diagnosis, disease phenotype, or response to treatment. Nonetheless, with the accessibility of microbial compositional markers, and their increasing adoption in longitudinal and interventional human studies, the outlook for their refinement as useful biomarkers is bright. In the long-term, we are also intrigued by the prospect of disease-relevant metabolites of microbial origin that would permit clinical assessment of the microbial ecosystem in blood, saliva, or urine, that would be amenable to clinically relevant monitoring of microbiome-targeted therapy. Finally, as noted above, there is excitement that new generation screens of IgA-binding microbiota may uncover highly relevant microbial species for monitoring or targeting in IBD.

Serologic biomarkers in IBD

In the generation prior to recent emergence of the technologies enabling IgA-SEQ, it was already well-established that specific adaptive immune responses are altered towards antigens of the luminal commensal microbiota in IBD patients. The possibility of a dietary antigen, akin to celiac disease, led to the discovery of immune responses directed against a specific oligomannose epitope present on the cell wall of the yeast *Saccharomyces cerevisiae* strongly increased in CD patients[58, 59]. Shortly thereafter ASCA was also found in in 20% of healthy first degree relatives of patients with Crohn's disease first suggesting that these antibodies might be a subclinical marker for CD in families[60]. The inheritability patterns of CD were relatively unknown at the time of these initial studies and the authors questioned whether ASCA reflected environmental or genetic factors or a combination thereof. The genetic discoveries since that time have afforded researchers the opportunity to examine the connection between IBD associated genotypes and serotypes. A logical story would be to establish a link between genes involved in bacterial sensing and autophagy (NOD2, TLR5, IRGM, ATG16L1) and the interleukin-23 signaling pathway (IL12B, IL23R, STAT3) with development of antimicrobial antibodies in CD patients. In a cohort over 600 CD patients they reported that variants in innate immune genes involved in pattern recognition and autophagy influenced antimicrobial seroreactivity in CD. No such association was seen with the IL-23 signaling pathways. The authors suggested that the additive effect of NOD2 3020insC and ATG16L1 T300A suggests a role for autophagy in development of ASCA[61]. Vasseur et al also determined an association between NOD2 positivity and ASCA[62]. The same group demonstrated that other disease associated genetic variants had the opposite influence in CD patients such that alteration of CARD8, a component of the inflammasome, was associated with lower levels of anti-mannans and anti-glycans antibodies[63]. This question still remains a very important focus of research however

gaining an understanding of the influence of the microbiome-on serologic expression may help answer this question.

Although the comparison to celiac was made, these antibodies do not link to disease causation and the expanded utility of ASCA and other antibodies targeted against multiple other microbial antigens have evolved into markers of disease state and disease behavior and their predictive value of pre-clinical disease is gaining momentum. These microbial antigens include *E. coli* outer-membrane porin C (anti-OmpC), the *Pseudomonas fluorescens*-related protein (anti-I2), a protein found in the flagella of bacteria, the CBir1 flagellin, and several other glycan epitopes or bacterial flagella as well as less well defined antigen-mixtures such as cell lysates or membrane-associated antigens of different *Bacteroides spp., Klebsiella pneumoniae, Enterococcus faecalis* and *Candida albicans*[64]. As compared to the certainty of the known microbial driven immune responses, the perinuclear antinuclear cytoplasmic antibody (pANCA) target remains unclear. Kohavy proposed that pANCA detects a structural domain recurrent among mycobacteria and cross-reactive with a DNA-binding domain of histone H1[65]. The antigen for IBD pANCA is distinct from pANCA associated with small vessel systemic vasculitides and most commonly associated with a more UC-like phenotype. Unlike ASCA there has not been a link to any genetic susceptibility state.

In order for seromarkers to be an effective marker for diagnosis, prognosis or the holy grail of a pre-clinical predictor they must at minimum demonstrate stability in an individual patient. The question is whether their mere presence is what needs to remain stable or is it the magnitude of sero-response that needs to be held to that gold standard. Reider et al found a significant fluctuation in ASCA levels but not negative vs. positive status[66]. Another study reported no difference between ASCA levels measured at diagnosis and 1–2 years after treatment among both adults and pediatric patients with CD[67]. Interestingly, ASCA levels were examined in a postoperative CD cohort and the group found that at baseline, 44/60 (73%) of patients were positive for ASCA IgG, 45/60 (75%) for ASCA IgA and 52/60 (87%) positive for at least one of both. ASCA serum levels remained stable during first year from resection[68].

There are no major studies testing whether disease activity is associated with levels of the serologic response. It has been proposed that the status of ASCA (positive or negative) in a particular patient is more so driven by the underlying genetic susceptibility of an IBD patient and that the actual titer level or height of the serologic response is tied more so to the degree of inflammation at the level of the intestine. This could explain the stability of the antibody status seen in some of these studies over time and the association of high ASCA levels with disease prognosis. The genetic tie in to serologies may help explain why they, ASCA in particular, can be found in patients with non IBD immune conditions. Given the shared intestinal target of both CD and celiac disease it was interesting to see the report demonstrating that seroreactivity to different microbial antigens was found in patients with early-stage celiac disease and that ASCA antibodies seem to be gluten-dependent. The authors concluded that microbial targets might play a role in the early development of celiac disease[69].

In systemic lupus erythematosus (SLE), a study found that the frequency of both ASCA IgG and ASCA IgA was higher in the SLE patients compared to the control group (29.3 vs. 3.1 %, $p < 10^{-6}$ and 12.1 vs. 0.6 %, $p = 10^{-4}$, respectively)[70]. Given a similar genetic background and the presence of subclinical intestinal inflammation in more than 50% of Ankylosing Spondylitis (AS) patients, Wallis et al examined the relevance of antibodies in patients with AS. They found that patients with AS alone demonstrated more frequent antibodies to CBir1 yet the AS-IBD patients demonstrated elevated responses when compared to AS alone for ASCA, anti-OmpC and anti-CBir1[71]. Even diseases far removed from the GI tract such as demyelinating central nervous system diseases has shown an association of ASCA to disease state[72]. It appears in addition to ASCA, other GI-related antibodies such as anti-gliadin (AGA) antibodies have been found in a spectrum of autoimmune diseases. In a study from 2012, IgA AGA was more frequently measured in antiphospholipid syndrome (APS) (7.1 %, P=0.012) and in pemphigus vulgaris (25%, P =0.008) patients, as compared with healthy controls. Interestingly IgG-AGA was more common among Crohn's disease (20.5%, P = 0.023) and rheumatoid arthritis (6.5%, P=0.027) patients. IgG anti tTG were frequently observed in APS (6.1%, P=0.012), in giant cell arteritis (11.5%, P=0.013) and in ulcerative colitis (11.1%, P=0.018) patients. ASCA was found more frequently in CD (IgA 19.3% and IgG 27.7%) and consistent with the other SLE studies, IgG ASCA were also found in (SLE) (4.5%, P=0.01), in Graves' disease (5.7%, P=0.018), in cryoglobulinemia (7.1%, P=0.006), and in patients with vasculitides (6.5%, P=0.002)[73]. This begs the question as to what these adaptive immune responses to microbial antigens really reflect or are in response to. Perhaps all of these disease states, whether GI or not, share a common microbial profile of dysbiosis and that is what more so determines the presence or absence as well as magnitude of these serologic responses. Research is currently underway to explore this possibility.

Diagnosis and Phenotype

The recognition of IBD and subsequent diagnostic evaluation, in most cases, can be straightforward when the clinical presentation is unambiguous. However, a diagnostic challenge arises in patients who present with overlapping, non-specific and indolent symptoms that are characteristic of both organic and non-organic disorders. In the face of diagnostic uncertainly clinicians are often obligated to exclude IBD using invasive diagnostic testing, in particular cross sectional imaging and colonoscopy with biopsies. Suspicion of IBD commonly results in extensive diagnostic investigations of patients who are ultimately found to have a functional bowel disorder. In contrast, the diagnosis of IBD, particularly CD, can be missed or delayed due to the non-specific nature of both the intestinal and extra-intestinal symptoms at presentation. Given these clinical challenges, the search continues for an accurate non-invasive diagnostic marker to aid clinicians in the prompt recognition of IBD and the differentiation of these disorders from mimickers.

The ideal non-invasive diagnostic test must be both highly sensitive and specific. Moreover it should be as good as the gold standard. To date no such test has been developed however advances in testing strategies and the addition of novel markers has helped the characteristics of available tests. Numerous studies have examined the diagnostic value of these markers, ASCA and pANCA in particular, in IBD and non-IBD patients[74–78]. Most

studies do conclude that the specificity of serological markers for IBD is high, but low sensitivity making them less useful as diagnostic tests. A prospective study was performed in children with non alarm type symptoms undergoing a complete diagnostic evaluation to rule in or out IBD as the same time they underwent serologic testing[76]. Diagnosis of IBD vs. non-IBD was made based on gold standard and blinded to serological analyses. The results of this study showed that serologies had an overall accuracy of 84%. Antibodies against laminaribioside (ALCA), chitobioside (ACCA) and mannobioside (AMCA) are targeting glycans and found more frequently and in higher levels in CD patients than in subjects with UC and healthy controls. Like ASCA, these antibodies have been found to be associated with NOD2 mutations[79].

There is renewed interest in the association of anti-zymogen glycoprotein (GP2) antibodies with Crohn's disease and its ability to help further differentiate CD from UC and non IBD. It has been demonstrated that in addition to its expression on pancreatic acinar cells, GP2 is located on the microfold (M) cells of the follicle-associated epithelium (FAE) of intestinal Peyer's patches. Data has shown that ileal inflammation is required for the development of anti-GP2 antibodies in CD, and suggested that the intestine rather than the pancreatic juice was the antigenic source required for the initiation of anti-GP2 antibodies. Moreover there was no correlation between ASCA and anti-GP2 titers[80]. More recently a novel assay was developed to IgA and IgG anti-MZGP2 combined testing led to a sensitivity of 31% and a specificity of 96%. A total of 832 sera were studied, including 617 consecutive IBD patients from 323 CD and 294 UC follow-up in a tertiary center, and 112 pathological and 103 normal controls. Positivity for either ASCA (IgA or IgG) or anti-MZGP2 (IgA or IgG) showed a sensitivity of 75% (70, 80) and a specificity of 84% (79, 89). Like ASCA, anti-GP2 may be more important in impacting disease location and behavior[81].

The question of whether a defective intestinal barrier resulting in loss of tolerance to microbial antigens can be extrapolated to loss of immune tolerance to dietary antigens. There have been multiple studies seeking to prove the connection between diet and IBD pathogenesis. Antibodies to food derivatives, common dietary allergens, gliadin have been previously reported to be more prevalent in IBD patients as compared to controls. Frehn and colleagues showed that anti-dietary Abs (wheat and milk extracts, purified ovalbumin) showed no general alterations in IBD patients. This data renders testing for antibodies against dietary antigens less relevant as a diagnostic tool or as a marker of disease pathogenesis[82].

Although UC and CD share may epidemiologic, immunologic, therapeutic and clinical features, they are currently considered to be two distinct subtypes of IBD. This entity referred to as IBD unspecified (IBDU) occurs in approximately 10–15% of IBD patients. Indeterminate colitis (IC) is now applied to those patients whose diagnosis remained unknown after careful examination of the resected surgical specimens. There still remains however a lot of inconsistency in the literature when defining IBDU since it is generally based on imprecise clinical definitions and very small retrospective studies. It must be emphasized that surgical options often rely on a correct diagnosis.

There is always a hesitation when offering IBDU patients pouch surgery because of concern of pouch failure, refractory pouchitis, and a postoperative diagnosis of CD. Yu et al compared the ten year outcome of IBDU and chronic UC patients undergoing ileal-pouch anal anastomosis (IPAA)[83]. Those patients going into surgery with a diagnosis of IBDU had significantly more episodes of pelvic sepsis (17 percent IBDU vs. 7 percent chronic UC; $P < 0.001$), pouch fistula (31 vs. 9 percent; $P < 0.001$), and pouch failure (27 vs. 11 percent; $P < 0.001$). Moreover, 15 percent of patients with IBDU, but only 2 percent of patients with chronic UC, had their original diagnosis changed to CD ($P < 0.001$).

Given the CD-specificity of ASCA and the UC-specificity of pANCA, these antibodies have been widely studied and have become with the addition of novel markers more widely accepted as useful discriminatory markers that help clinicians differentiate UC from CD colitis. However the discriminatory strength of these markers is amplified when they are evaluated in combination. A pANCA+/ASCA- serological profile was shown to be 19 times more likely to be present in the serum of a patient with UC than CD. Conversely, pANCA−/ASCA+ is 16 times more likely in CD than UC[84]. Quinton et al obtained serum samples from 100 patients with Crohn's disease, 101 patients with ulcerative colitis, 27 patients with other miscellaneous diarrheal illnesses, and 163 healthy controls[59]. The combination of a positive pANCA test and a negative ASCA test yielded a sensitivity, specificity, and positive predictive value of 57%, 97%, and 92.5% respectively for UC. The combination of a positive ASCA test and a negative pANCA test yielded a sensitivity, specificity, and positive predictive value of 49%, 97%, and 96% respectively for CD. It should be noted that in patients with pure colonic CD, the prevalence of ASCA positivity is relatively low. Ruemmele also studied ASCA and pANCA in cases of colitis among children with IBD[85]. IgA and IgG ASCA titers were significantly greater and highly specific for CD (95% for either, 100% if both positive). pANCA was 92% specific for UC and absent in all non-IBD controls. The majority of patients with CD positive for pANCA had a UC-like presentation. A meta-analysis was performed to examine the test characteristics of ASCA and pANCA[86]. Sensitivity, specificity, and likelihood ratios (LR+, LR−) were calculated for different test combinations for CD, UC, and for IBD compared with controls. A total of sixty studies comprising 3,841 UC and 4,019 CD patients were included. The ASCA+ with pANCA− test offered the best sensitivity for CD (54.6%) with 92.8% specificity and an area under the receiver operating characteristic (ROC) curve (AUC) of 0.85 (LR+ = 6.5, LR− = 0.5). Sensitivity and specificity of pANCA+ tests for UC were 55.3% and 88.5%, respectively (AUC of 0.82; LR+ = 4.5, LR− = 0.5). Sensitivity and specificity were improved to 70.3% and 93.4% in a pediatric subgroup when combined with an ASCA negative test. Meta-regression analysis showed decreased diagnostic precision of ASCA for isolated colonic CD (RDOR = 0.3). This study concluded that ASCA and pANCA testing are specific but not sensitive for CD and UC. It may be particularly useful for differentiating between CD and UC in the pediatric population. The first prospective study was conducted in IBDU patients and reported by Joosens et al in 2001[87]. They enrolled 97 predefined IBDU patients and followed them prospectively over time blinded to their ASCA and pANCA status. Over 6 years, 17 of 97 patients were diagnosed with CD, 66 of the 97 patients remained indeterminate, and 14 patients of the 97 declared as UC. Thus a definitive diagnosis was reached for 31 of 97 patients (32%). Their initial serum antibody characterization

demonstrated that 48% of the population was ASCA−/pANCA−, 27% were ASCA+/pANCA−, 21% were ASCA−/pANCA+, and 4% were ASCA+/pANCA+. ASCA+/pANCA − correlated with CD in 8 of 10 (80%) patients, whereas ASCA−/pANCA+ correlated with UC in 7 of 11 (63.6%) patients. The remaining 4 cases became CD, clinically behaving as UC-like CD. Thus 100% of UC or UC-like CD were pANCA positive. At time of last follow up, almost half of the patients (47 of 97 [48.5%]) were negative for ASCA and pANCA. Only 7 seronegative cases (14.9%) became CD or UC compared with 48% (24 of 50) of seropositive patients ($P < 0.001$). The conclusions of this study are that IBDU may represent a distinct form of IBD based on the lack of IBD-associated antibodies.

This same group reported that by adding anti-OmpC and anti-I2, the predictive capacity of serological tests only increases marginally and specificity drops significantly[88]. Despite another 1.5 years of follow up, there still remained a large group of IBDU patients who remained negative for serological markers and may represent a separate phenotype. The entity of a UC-like Crohn's phenotype was first introduced by Vasiliauskas EA et al in 1996[89]. pANCA-positive patients with CD were reported to are endoscopically and/or histopathologically documented left-sided colitis and symptoms of left-sided colonic inflammation, clinically reflected by rectal bleeding and mucus discharge, urgency, and treatment with topical agents. One hundred percent of patients with CD expressing pANCA had "UC-like" features. The presence of pANCA in up to 25% of CD patients however limits its ability to distinguish UC form CD on its own. Novel antibodies like anti-CBir1 may help to dissect the pANCA positive IBD group. Targan et al found CBir1 reactivity in 44% of pANCA positive CD patients vs. only 4% of pANCA positive UC patients[90]. This suggests that pANCA positive/antiCBir1 positive colonic CD patients may represent a unique UC-like phenotype. It is unclear as to whether the natural history of UC-like CD is different than chronic UC especially when it comes to therapeutic responses and post op outcomes. In a single center observational study, there was minimum benefit of serologies at predicting UC or CD despite most patients declaring themselves as one or the other[91]. The definitions of IC do vary across centers and studies thus rendering the predictive value of serologies alone difficult to interpret. Moreover, our genetic colleagues have most recently described the association of close to 200 Single Nucleotide Polymorphisms (SNP) with IBD with the majority overlapping between UC and CD[92]. Thus we would go as far as to say that any patient who presents with disease isolated to the colon and labelled as UC cannot with confidence be diagnosed with UC. The natural history of such a patient may never be defined until we have a better way to truly distinguish UC from CD beyond genotype or serotype or long after a colectomy and ileal pouch anal anastomosis (IPAA) has been performed and the patient never develops a CD like presentation over their lifetime. There has been many papers looking at the association and diagnostic value of serologies in helping to differentiate acute vs chronic pouch inflammation. For those patients with chronic pouch inflammation the question is whether these patients have chronic pouchitis which is typically responsive to antibiotics or could this inflammation be Crohn's disease which often require non-antibiotic based interventions. A recent meta-analysis reported on the association of ANCA and ASCA with pouchitis outcomes. This study showed that the odds of chronic pouchitis was 76% higher in ANCA-positive patients than ANCA-negative (OR: 1.76; 95% CI: 1.19–2.61; $P < 0.01$) but no such association was found for ASCA (OR: 0.89;

95% CI: 0.49–1.59; P = 0.68). Neither ANCA or ASCA-positivity was associated with the risk of acute pouchitis[93]. Fleshner et al suggested that high pANCA levels (> 100 ELISA U/ml) were seen more often in patients with chronic pouchitis as compared to acute pouchitis patients. Moreover the same group then showed that patients with high pANCA levels in combination with CBir1 positivity have the highest risk of rapidly progressing to chronic pouchitis[94].

Perhaps the more important distinction is between chronic pouchitis and actual Crohn's disease as the management strategies are quite different. Patients often ask what the risk of developing Crohn's after undergoing IPAA surgery. It is estimated that between 5–10% of UC patients are at risk of developing Crohn's and that risk is increased if they undergo IPAA after given a diagnosis of IBD unspecified (IBD-U)[95]. Knowing the risk prior to surgery could help patients manage expectations and may impact the type of surgery a patient may undergo. Melmed et al reported that ASCA positivity collected pre colectomy in the face of a family history of Crohn's disease was the most predictive of Crohn's disease post IPAA[96]. The majority of studies acquire the specimens postoperatively which renders the markers less reliable as true predictors of the natural history of the pouch. The group from Cleveland Clinic examined both serum and fecal ASCA levels. They reported a significant difference in the mean values of fecal ASCA between inflammatory and fistulizing Crohn's disease like (CDL) of the pouch[97]. Interestingly, in addition to its predictive value for chronic pouchitis in the face of pANCA positivity, CBir1 has been shown to be associated with Crohn's disease and pouch fistulae following IPAA[98, 99].

Prognosis

It has become increasingly clear that both the quantitative and qualitative expression of these immune responses serve as an immunologic risk marker for IBD phenotypes. The notion that the utility of serologies goes beyond differentiation of IBD subtypes was first introduced by Vasiliauskas et al when they reported that high ASCA levels were found to be associated with fibrostenosing (FS) and internal-penetrating (IP) disease as well as the need for small bowel surgery[100]. Moreover, pANCA was shown to be associated with a more benign, UC-like disease course in CD and less small bowel complicating disease. Multiple studies confirmed this association for ASCA as well as other anti-mannan antibodies[101]. The other anti-microbial responses OmpC and Cbir1 have also been shown to be associated with a more aggressive disease phenotype and rapid progression to disease complications[102]. A recent meta-analysis evaluated the stratification powers of ASCA, antibodies to OmpC, Cbir1 and I2 in characterizing progression of Crohn's disease (CD). Anti-OmpC was associated with the highest risk of both complications and surgery in CD patients, and the power became stronger when antibodies were assessed in combination[103].

Antibody sum is indeed an important concept when staging the risk of disease progression for CD patients. It appears that having at least 2 antibodies (antibody sum of 2 or above) is associated with increased risk of disease complications[102]. Multiple models have been developed to try and determine whether there are other predictors of disease phenotype such as genotype. Interestingly, serotype remains an independent risk factor for CD complications and in some studies have been shown to have the highest prognostic power[104]. That being

said NOD2 genotype has been shown to be associated with stricturing small bowel disease and was shown to be additive to the risk of complication when combined with serotype (AUC = 0.801; 95% confidence interval: 0.757–0.846). The rapidity of disease progression was higher in patients positive for NOD2 variants and multiple high levels of serologies as compared to those with serologies alone[105]. In general classic statistical methods are used in the majority of prognostic studies. System Dynamics Analysis (SDA) is a valuable alternative to classic regression models as it addresses the inherent dynamic complexity of interaction of all of these variables and provides real time individualized prediction of patient outcomes. Such technique has been employed on both a pediatric and adult CD cohort. In both cohorts, serologies were strongly predictive of a poor prognosis[106, 107].

Neutralizing autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF Ab) reduce neutrophil antimicrobial function in patients with primary alveolar proteinosis (PAP). Subsequently these antibodies have been found in CD patients with ileal location and stricturing behavior. Other studies are needed to determine the predictive power of anti-GMCSF and how it is differentiated from the anti-glycans and other anti-microbial antibodies. That being said, this antibody may be important in identifying risk in the preclinical disease state such that CD patients with increased GM-CSF antibody had reduced neutrophil phagocytic capacity and increased accumulation of pSTAT3+ neutrophils in the affected ileum[108, 109]. The definitive phase 3 trial did not reach its primary outcome but the concept of giving GM-CSF to those with antibodies merits further exploration[109].

Predicting disease: a preclinical marker

Increases in intestinal permeability are a common finding of IBD, reflecting several changes in epithelial physiology in response to altered inflammatory and microbial mucosal ecosystem even in periods of remission[110]. The landmark paper of Hollander and colleagues established that increased intestinal permeability to ingested polyethylene glycol-400 is a trait in at-risk family members of IBD patients[111, 112]. This finding has been confirmed by others[113], and extended to healthy subjects bearing disease-variant alleles of NOD2, which likely reflects the deficient control of enteric microbiota due to such hypofunctional alleles of this microbial-sensing protein.[114] Since non-invasive permeability assays selective for the colon are now in common use, intestinal permeability should be reconsidered as one phenotype to include in studies ahead assessing at-risk individuals for IBD.

There are multiple reports showing that unaffected relatives of patients with CD have elevations in ASCA as compared to spouses and healthy controls. ASCA has also been shown to be high in twins concordant for CD but not when discordant, suggesting an environmental component[115]. Unaffected family members positive for NOD2 have been found to have higher levels of serologies as compared to NOD 2 negative patients suggesting a link between the innate and adaptive immune responses[116].

In addition to ASCA, anti-OmpC was also measured in unaffected family members. Interestingly, there was increased expression in the unaffected family members of CD patients[117]. The familial nature of these serologies is well established. The question remains as to whether these markers can be predictive of disease. van Schaik FD et al set out to

explore whether serologies predicted a diagnosis of IBD by studying individuals enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC) study[118]. A total of 77 subjects were diagnosed with CD and 167 with UC after a mean follow-up of 4.5 and 4.4 years following blood collection, respectively. Combinations of pANCA, ASCA, anti-CBir1 and anti-OmpC predicted incident CD and UC (area under curve 0.679 and 0.657, respectively). As with the Israeli study, the predictive value of the combination of markers increased when time to diagnosis of CD or UC decreased. Thus ASCA may be an early genetic marker of CD susceptibility. This study however did show that ASCA positivity was higher in patients whose specimen was drawn closest to data of diagnosis[119].

The Future of Serologic Immune Responses

Shared decision making and patient centered care is critical to optimize the quality of care provided to IBD patients. By demonstrating the impact of the various therapies on the baseline risk of disease progression, patients can make decisions based on their individual prognosis and not on the general CD population. Web based interfaces are being developed to allow for real time decision tools to be available in the clinic (Figure 1). Based on both the adult and pediatric Siegel et al. studies employing system dynamics analysis to construct risk prediction tool, the **P**ersonalized **R**isk and **O**utcome **P**rediction **T**ool (PROSPECT) has been developed to translate complex clinical information into a simplified graphical patient friendly representation on an individual's disease course so to inform decision making as it pertains to treatment options[106,107]. The PROSPECT tool combined with a decision aid is currently being tested in a randomized trial to determine whether treatment decisions as well as long term outcomes are different as compared to patients whose decisions are based on their interaction with their physician only. The widespread availability of the PROSPECT tool is currently being considered.

There is an abundance of evidence to support the diagnostic and prognostic utility of antimicrobial antigens. The generalized use of these tools in clinical practice is often limited by cost and access as well as the complexities of managing the IBD patient. An area that holds much promise is linking serotype and enterotype. More work is needed to determine whether the serologies are truly a surrogate for the interaction between a genetic innate immune defect and an environmental trigger that characterizes the heterogeneity of IBD patients or is it a marker of the various microbial profiles also reported to characterize IBD patients. The question remains as to whether patients in genetically defined high risk families should have serologies measured and monitored differently suggesting these are markers of preclinical disease. Research in "life before IBD" merits further consideration.

Acknowledgments

This work was supported by United States Public Health Service grants P01 DK46763 and UL1 TR000124.

Abbreviations used

CD	Crohn's disease
GI	gastrointestinal

IBD	inflammatory bowel disease
UC	ulcerative colitis

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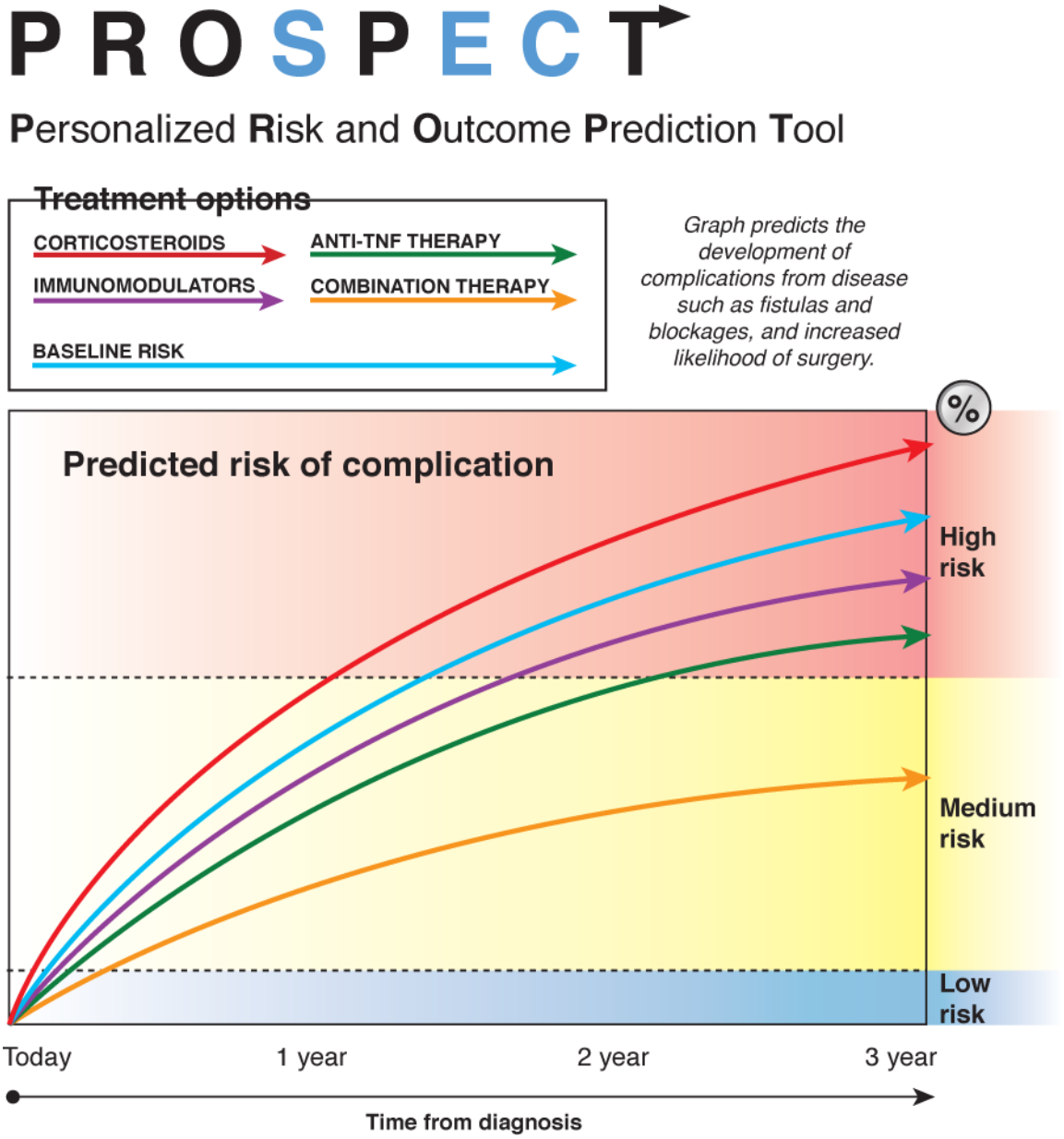

Figure 1.
Web-based tool to show patients their individualized probability of developing a complication over a 3-year period.

Table 1

Bacterial microbiota associated with IBD

Population subset	Associated bacterial microbiota
Low Risk	Bacteroidales, Ruminococcacae, Clostridiales, Erysipelotrichaceae, Bifidobacteriaceae
Increased Risk	Enterobacteriacae, Enterococcacae, Lachnospiracae (Blautia, Dorea), Prevotella
Active Disease	Enterobacteriaceae, Gemellaceae, Neisseriaceae, Pasteurellaceae, Fusobacteriaceae, Veillonellaceae