PO Box 2345, Beijing 100023, China
www.wjgnet.com
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EDITORIAL

Utility of serological markers in inflammatory bowel diseases: Gadget or magic?

Maria Papp, Gary L Norman, Istvan Altorjay, Peter Laszlo Lakatos

Maria Papp, Istvan Altorjay, Department of Gastroenterology, University of Debrecen, Debrecen, Hungary
Gary L Norman, INOVA Diagnostics, Inc. San Diego, United States
Peter Laszlo Lakatos, 1st Department of Medicine, Semmelweis University, Budapest, Hungary
Supported by Mecenatúra (11/2005) grant
Correspondence to: Peter Laszlo Lakatos, MD, PhD, 1st Department of Medicine, Semmelweis University, Koranyi str. 2/A H-1083, Hungary. kislakpet@bel1.sote.hu
Telephone: +36-1-2100278-1500 Fax: +36-1-3130250
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Abstract

The panel of serologic markers for inflammatory bowel diseases (IBD) is rapidly expanding. Although anti-*Saccharomyces cerevisiae* antibodies (ASCA) and atypical perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) remain the most widely investigated, an increasing amount of experimental data is available on newly discovered antibodies directed against various microbial antigens. The role of the assessment of various antibodies in the current IBD diagnostic algorithm is often questionable due to their limited sensitivity. In contrast, the association of serologic markers with disease behavior and phenotype is becoming increasingly well-established. An increasing number of observations confirms that patients with Crohn's disease expressing multiple serologic markers at high titers are more likely to have complicated small bowel disease (e.g. stricture and/or perforation) and are at higher risk for surgery than those without, or with low titers of antibodies. Creating homogenous disease sub-groups based on serologic response may help develop more standardized therapeutic approaches and may help in a better understanding of the pathomechanism of IBD. Further prospective clinical studies are needed to establish the clinical role of serologic tests in IBD.

Key words: Serologic markers; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Indeterminate colitis; Antineutrophil cytoplasmic antibodies; Anti-Saccharomyces cerevisiae mannan antibodies; Outer membrane porin

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INTRODUCTION

Serologic response to various microbial and autoantigens can develop in inflammatory bowel diseases (IBD). In addition to the well-established atypical perinuclear antineutrophil cytoplasmic antibodies (atypical P-ANCA) and anti-*Saccharomyces cerevisiae* mannan antibodies (ASCA), a number of new antibodies have recently been discovered and data on their clinical significance has been rapidly increasing.

The usefulness of different antibodies in Crohn's disease (CD) and ulcerative colitis (UC) as diagnostic markers, follow-up parameters, or as subclinical markers in affected families has been actively investigated. Another field of interest is the association of the serologic markers with the disease phenotype, disease course, and treatment stratification. The role of the antibodies in disease pathophysiology remains to be fully elucidated.

In this review we discuss current understanding of the clinical importance of various established and newly recognized serologic markers in IBD.

SEROLOGIC PANEL FOR INFLAMMATORY BOWEL DISEASE

Anti-neutrophil cytoplasmic antibody

The classic anti-neutrophil cytoplasmic antibody (ANCA) tests are used to diagnose and monitor the inflammatory activity in primary small vessel vasculitides. On the basis of an international consensus statement, ANCA testing is performed with serum samples by indirect immunofluorescence (IIF) on normal peripheral blood neutrophils. Two basic ANCA patterns are detectable: the cytoplasmic (C-ANCA) and the perinuclear (P-ANCA). The C-ANCA pattern appears as a granular, diffuse cytoplasmic fluorescence, often with accentuated fluorescence around the nuclear lobes. Typical P-ANCA reactivity results in homogeneous rim-like staining of the perinuclear cytoplasm. ANCA positive serum samples and also those with any other cytoplasmic fluorescence or an antinuclear antibody (ANA) that results in homogeneous or peripheral nuclear fluorescence should be tested in enzyme-linked immunosorbent assays (ELISA) for proteinase 3 (PR3) and myeloperoxidase (MPO)

antibodies, because these are the most common targets of C-ANCA and P-ANCA antibodies respectively (Minimum recommendation of consensus group). Ideally, ELISAs should be performed on all serum samples, since IIF alone detects only 90% to 95% of all ANCA positive serum samples in patients[1]. A third ANCA pattern of clinical importance is the so-called atypical P-ANCA staining. It has been suggested that since the target antigens of atypical P-ANCA are nuclear rather than cytoplasmic, this pattern would be more properly named anti-neutrophil nuclear antigen (ANNA). Until the target of atypical P-ANCA reactivity is identified however, it is likely that the atypical nomenclature will remain in common use. Atypical P-ANCA is recognized as a broad inhomogeneous rim-like staining of the nuclear periphery often with multiple intranuclear foci[2]. The antigen specificity of these atypical ANCAs are different from the classic C- and P-ANCAs, being localized in the nuclear periphery, in contrast to the cytoplasmic location of the classic C- and P-ANCAs. Atypical P-ANCAs are most commonly seen in patients with IBD, especially ulcerative colitis, and some autoimmune liver diseases such as autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC).

Atypical P-ANCA is present in the sera of 40% to 80% of patients with UC[3,4] and to a lesser extent in CD (5%-25%)[5]. The prevalence of the antibody is also high in patients with PSC (88%)[6] and AIH type I (81%)[7], but is detected in only 1%-3% of healthy control subjects. Some sera with atypical ANCA reactivity are positive for antibodies to elastase, lactoferrin, cathepsin G, lysosyme or bactericidal permeability-increasing protein (BPI), but since they are only detected in a few atypical P-ANCA positive sera, these antigens do not appear to be the primary targets of atypical P-ANCA reactivity.

The target antigen(s) of atypical P-ANCA have not been definitively identified. What is in agreement is that target antigen(s) are associated with inner side of the neutrophil nuclear membrane. A 50-kilodalton myeloid-specific protein has been identified by Tejung and appears to be the best current candidate as the primary target of atypical P-ANCA. Histone H1, which binds to the DNA linking nucleosomes, has been suggested as a target antigen of atypical P-ANCA[8]. However, histone H1 is found in all cells with nucleus and is not specific to neutrophils. There has been little independent support of this idea.

Differentiation of the atypical P-ANCA from the typical vasculitis P-ANCA pattern is difficult on ethanol-fixed neutrophils. The patterns are most reliably differentiated by testing sera on both ethanol and formalin-fixed neutrophil slides. The typical P-ANCA pattern converts to a cytoplasmic C-ANCA pattern when tested on formalin-fixed neutrophils, while atypical P-ANCA is mostly destroyed by the formalin treatment or presents weak homogeneous staining[9]. Despite the above-mentioned differences, IBD (and autoimmune liver disease)-associated atypical P-ANCA is still often only referred to as P-ANCA. This failure to clearly distinguish P-ANCA reactivity from atypical P-ANCA reactivity can lead to considerable confusion and should be highly discouraged because of the different clinical implications of each result.

Since the exact target antigen(s) of atypical P-ANCA has not been identified, there are currently no sensitive and specific solid-phase assays available to screen for these antibodies. IIF is the only widespread method to detect the antibodies; however it is technically demanding, subjective, and requires experienced observers for good interpretation. Joossens *et al*[10] evaluated the interassay and interobserver variability in the detection of UC-associated atypical P-ANCA comparing four different commercially available assays. Their results confirmed an earlier observation reporting inter-laboratory variability[11]. Not all ANCA assays are suitable for the detection of the anti-neutrophil cytoplasmic antibodies in IBD patients. The differences among the commercially available substrates are remarkable and probably result from differences in cell preparation, fixation methodologies, and conjugates. In addition to substrate differences, ANCA determinations and titer assignment are subjective and are highly dependent on the expertise of the observer. Furthermore, unlike the Consensus Recommendations for the vasculitis-associated ANCAs, there are no clear guidelines for immunofluorescence detection and interpretation of atypical P-ANCA patterns. Consequently, different laboratories may each observe the same pattern (i.e. the test performs consistently), but interpret the pattern differently.

An alternative methodology for the identification of the atypical P-ANCA reactivity developed by Targan and associates, uses a 3-step process that includes ELISA analysis, IIF assay on methanol-fixed neutrophils, followed by another IIF testing on deoxyribonuclease (DNase)-treated neutrophils. DNase-sensitive P-ANCA (i.e. not detectable on DNase-treated neutrophils) is present in the sera of 60% to 80% of the patients with UC and approximately 10% to 30% of patients with CD. In approximately 70% of UC sera, there is ablation of the P-ANCA pattern and the antigen recognition after DNase digestion of substrate cells[12]. This suggests that the epitope recognized by the UC-associated atypical P-ANCA is a protein-DNA complex or that the presence of intact DNA is necessary for maintaining the integrity of the epitope[4]. In up to 30% of the sera, there is conversion to homogeneous cytoplasmic staining, while in 3% of the sera the P-ANCA pattern was retained after DNase treatment of the substrate.

The overall specificity of the atypical P-ANCA is 84%-95%, sensitivity is 48%-63%, positive predictive value (PPV) is 69%, and negative predictive value (NPV) is 89%[5].

Anti-Saccharomyces cerevisiae antibodies

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are antibodies directed primarily against a 200 kDa-phosphopeptidomannan cell wall component of the common baker's or brewers yeast' *Saccharomyces (S.) cerevisiae*[13]. ASCA reactivity could be a result of cross-reacting antibodies to antigens found in a non-yeast organism and has not yet been identified[14,15]. Mannose is not only found in yeast but also in mycobacteria and other microorganisms[16]. Both IgA and IgG antibodies are formed. Separate and polyvalent ELISA configurations are available for ASCA IgG and IgA detection. ASCA are more frequently found in CD patients (50%-80%) compared to patients with UC (2%-14%) and to normal

healthy subjects (1%-7%)[17,18]. Approximately two-thirds of the CD patients with ASCA IgG are also positive for ASCA IgA, but 0% to 19% of the patients have only ASCA IgA antibodies. This suggests that both ASCA IgG and IgA antibodies should be measured. In CD, up to 90% specificity has been reported in specimens positive for both ASCA IgG and IgA antibodies, especially when the magnitude of both the IgG and IgA ASCA antibodies is high[19]. Sensitivity of ASCA testing ranges from 41%-76%, PPV 88% and NPV 68%[20].

ASCA IgG and IgA levels in CD patients are highly variable[19]. The prevalence of ASCA is much higher in cases of sporadic CD and in families with only CD (63%) compared to families with both CD and UC (33%). The familial trait to ASCA is obvious, but it is questionable whether this is due to the genetic background or environmental agents effect in childhood predisposing to the disease susceptibility.

A comparative study revealed a wide range in sensitivities and specificities among four assays, mainly as a consequence of the cutoff values chosen. Sensitivity was inversely related to specificity and PPV. Results correlated well overall and the different ROC curves showed good agreement[21].

Newly discovered serologic markers

Anti-OmpC antibody is directed against the outer membrane porin C transport protein of the *E. coli*. The detection of the IgA antibody is done with ELISA. Anti-OmpC has been reported in 55% of CD patients[22], but in children and young adults it was only reported 24%[23]. The prevalence of anti-OmpC was insignificant in UC patients and in healthy subjects (5%-11% and 5%, respectively). Anti-OmpC may be of value to aid diagnosis of ASCA negative CD patients. The prevalence of anti-OmpC among ASCA negative patients is 5%-15%.

A fragment of bacterial DNA (I2), a homolog of the tetR bacterial transcriptional factor family, has been identified from lamina propria mononuclear cells in active CD and shown to be associated with *Pseudomonas fluorescens*[24,25]. Anti-I2 antibody IgA has been detected by ELISA in IBD patients with a seroprevalance of 54% in CD and 10% in UC. Anti-I2 antibody was also found in patients with other inflammatory enteritis (19%) and also in healthy subjects (4%)[5].

Serologic expression cloning was used by Lodes *et al* to identify commensal bacterial proteins in colitic mice. The dominant antigens were found to be flagellins. Strong B-cell and CD4+ T-cell responses were observed against one of these flagellins (anti-CBir1). Colitis was induced when the T-cell line specific for CBir1 was transferred into naive severe combined immunodeficient mice. Approximately 50% of patients with CD had IgG serum reactivity to CBir1 *versus* 6% of UC patients and 8% of healthy subjects. CBir1 is the first bacterial antigen to induce colitis in animal models of IBD and also leads to a pathological immune response in IBD patients[26]. Among the population of CD patients positive for atypical P-ANCA, but who do not react to other known antigens, 40%-44% are positive for anti-CBir1 whereas the antibody has only been found in 4% of atypical P-ANCA positive UC patients. Serum responses to CBir1 may be of help in differentiation between atypical P-ANCA positive CD and UC patients independently of ASCA[27].

Anti-pancreatic antibodies (PAB) are directed against the exocrine pancreatic tissue. The exact target antigen(s) however, has not yet been identified. The detection of PAB is done by IIF on human pancreas substrate. The reported prevalence of PAB is approximately 30% in CD patients compared to 2%-6% of UC patients and 0%-2% of healthy subjects[28]. The relevance of PAB in the pathogenesis of CD is unclear and whether the presence of PAB identifies a CD subgroup also remains to be determined[29].

Patients with CD express antibodies to cell wall carbohydrate epitopes found in different pathogenic bacteria and fungi. Using a glycan array (GlycoChip) and ELISA, anti-glycan antibodies have been found in CD patients including anti-laminaribioside carbohydrate antibody (ALCA) (18%-38%), anti-chitobioside carbohydrate antibody (ACCA) (21%-36%), and anti-mannobioside carbohydrate antibody (AMCA) (28%)[30,31]. Importantly, 24%-44% of the CD patients found ASCA negative in one study were positive for one or more of the anti-glycan antibodies. Patients with CD who were positive for at least one of ALCA, ACCA, or gASCA (very similar to conventional ASCA) could be differentiated from UC patients, with 77% sensitivity and more than 90% specificity. PPV and NPV was 91% and 77%, respectively. When the same criteria were applied to differentiation of CD from control patients, the specificity fell to 70.3%[30]. As one might expect, combination of 2 or more antiglycan antibodies resulted in a higher specificity and PPV in differentiation of CD from UC, but with loss of sensitivity, NPV, and efficiency. These data do not show a great increase in the sensitivity and specifity by the glycan assays. The low correlation between the presence of antibodies against mannan, laminaribioside, and chitobioside suggests that the antigens responsible for these antiglycan antibodies are directed against different microorganisms.

Prevalence of different serologic markers in IBD and their association with the disease phenotype is summarized in Table 1.

DIAGNOSTIC VALUE OF THE SEROLOGIC MARKERS IN IBD

The role of atypical P-ANCA and ASCA as diagnostic markers for IBD appears to be limited because of their moderate sensitivity and presence in other conditions. Atypical P-ANCA can also be observed in other colitis, e.g. collagenous or eosinophilc colitis and in various autoimmune liver diseases such as AIH and PSC[32,33]. ASCA has been found in AIH (20%) and gastrointestinal disorders such as celiac disease[34].

The combination of atypical P-ANCA and ASCA however, may be of help in patients in whom distinction between CD and UC is not obvious with the classic diagnostic tools (patient history, radiologic examination, endoscopy and biopsy). The $ASCA^+$/atypical $P\text{-}ANCA^-$ serologic pattern is mainly characteristic of CD, while the

Table 1 Prevalence of different serologic markers in IBD and their association with disease phenotype

	Crohn's disease (%)	Ulcerative colitis (%)	Healthy subjects (%)	Clinical significance
Atypical P-ANCA	2-28	45-82	1-7	Assists in differentiation between CD and UC: Atypical P-ANCA$^+$/ASCA$^-$: UC Atypical P-ANCA$^-$/ASCA$^+$: CD CD: ASCA$^+$: ileal involvement, complicated disease course, early need for surgery
ASCA	41-76	5-15	5	Atypical P-ANCA$^+$: left sided colitis, good therapeutical response, uncomplicated disease course UC: Atypical P-ANCA$^+$: severe left sided colitis, refractory to medical therapy, early need for surgery
Anti-OmpC (IgA)	24-55	5-11	5	Identify ASCA$^-$ CD patients Penetrating disease Faster disease progression Early need for surgery
Anti-I2 (IgA)	54	10	4	Inflammatory enteritis (19%) Stricturing form Early need for surgery
Anti-CBir1 (IgG)	50	6	8	Flagellin (CBir1) induce colitis in animal models of IBD Leads to a pathological immune response in IBD patients Differentiation between atypical P-ANCA$^+$ CD and UC Small bowel involvement Penetrating and stenosing form
Antiglycan antibodies (ALCA ACCA)	36	< 10	0	44% in ASCA- patients ALCA-penetrating; ACCA-stenosing form (small differences)
PAB (IIF)	27-39	2-6	0-2	High specificity, low sensitivity Significance? Distinct CD subgroup?

ASCA$^-$/atypical P-ANCA$^+$ is characteristic of UC. Several independent studies found that these combinations had sensitivities of from 30% to 64%, specificity more than 90%, and PPV from 77% to 96%[12,17,18,35,36].

It must be emphasized that neither ASCA, nor atypical P-ANCA negativity rules out IBD. Similarly, the presence of these antibodies does not confirm the diagnosis of IBD.

Serologic evaluation may be of help in patients with indeterminate colitis (IC) to increase the diagnostic accuracy. Ninety-seven patients with IC were enrolled, analyzed for atypical P-ANCA and ASCA, and followed up prospectively in a multicentre study of Joosens *et al*. After the 1-year follow-up, a definitive diagnosis was reached in 31 of 97 patients (37%). In IC patients, ASCA$^+$/atypical P-ANCA$^-$ results correlated with CD in 80%, whereas ASCA$^-$/atypical P-ANCA$^+$ correlated with UC in 63%. The remaining ASCA$^-$/atypical ANCA$^+$ patients were eventually determined to be CD, but clinically showed a UC-like CD phenotype. Remarkably, during the 9.9 year follow-up, 48.5% of the patients did not show antibodies against ASCA or atypical P-ANCA. In 85% of these seronegative patients the diagnosis remained indeterminate. In contrast, 48% of the seropositive patients became CD or UC on follow-up[37]. Adding anti-OmpC and anti-I2 to the serologic panel in patients with IC did not add diagnostic clarification[38].

Several groups have studied whether atypical P-ANCA and ASCA are preclinical markers of IBD in families. Some studies[39,40] showed that presence of atypical P-ANCA occurred frequently in healthy first-degree relatives of UC patients, whereas other studies were not able to confirm this observation[41,42]. ASCA positivity was obviously found at a higher rate in unaffected first-degree relatives of CD patients than in the general population (20%-25% *vs* 5%)[43,44]. Further studies are necessary to clarify whether the unaffected, but ASCA positive, family members face an increased risk of disease development.

Israeli *et al*[45] demonstrated that the presence of ASCA and atypical P-ANCA in healthy subjects can predict for IBD before the emergence of overt clinical manifestations. Serum samples were obtained systematically and stored from 5% of all military recruits. ASCA were detected in 31% of CD patients before clinical diagnosis. The mean interval between ASCA detection and diagnosis was 38 mo. There was no ASCA positivity in control population. Atypical P-ANCA was present in 25% of patients with available sera before the diagnosis of UC. None of their 24 matched controls were positive.

ASSOCIATION WITH DISEASE PHENOTYPES AND PROGRESSION

The occurrence of atypical P-ANCA in UC is associated with a characteristic clinical appearance and represents a distinct subgroup which is often characterized by specific HLA markers. These patients have a higher probability to develop a severe left-sided ulcerative colitis, which is more resistant to treatment than the usual case. The disease has a more aggressive course requiring surgery earlier[47]. Some authors suggest that pouchitis develops more frequently after ileal pouch anastomosis[48], whereas others were not able to confirm this observation. The presence of

atypical P-ANCA identifies a subgroup of CD patients characterized by UC-like colitis; the inflammation usually involves the left side of the colon and the response to therapy is generally good. The atypical P-ANCA in CD patients associated with later age of onset and a relative decreased incidence of complications such as stricture and/or perforation[49-51].

Phenotype and the disease course of CD are heavily dependent on the presence and extent of serologic responses against various microbial antigens. In patients with an ASCA$^+$ (IgG and/or IgA)/atypical P-ANCA$^-$ phenotype, small bowel involvement (with or without colonic disease) is more typical than the pure colonic disease (68%-76% *vs* 34%-46%)[18,51,52]. ASCA positivity predicts a more aggressive disease course with a higher rate of complications. ASCAs have been associated with stricturing (70%) and penetrating (51%) type of disease as opposed to the inflammatory one (14%) and a higher risk of small bowel resection[46,49,53]. Several studies suggest that ASCA positivity is associated with an earlier onset of disease[16,48]. ASCA IgA positivity in children may represent a higher risk for relapses [OR 2.9 (95% CI 1.33-6.33)][54].

The presence of anti-OmpC in adult CD patients is associated with an increased prevalence of the penetrating form only[21,49,50], while in children both the penetrating and stenosing forms[55] are more frequent. Moreover, antibody positivity may lead to a more aggressive course of disease and a higher risk for surgical interventions.

Like ASCA and anti-OmpC, anti-I2 also appears to be associated with an increased risk for complications in adult CD patients. It is an independent risk factor for the development of the stenosing form and the need for surgical interventions[21,49,50].

Recent research has shown that the anti-CBir1 antibody is associated with ileal involvement in adult CD patients independent of other serologic markers, and it predisposes to the development of both stenosing and penetrating forms[26].

Among the anti-glycan antibodies in CD, ALCA is more often positive in the penetrating form (34% *vs* 25%) and ACCA in the stenosing one (29% *vs* 18%) when compared to the inflammatory type; although, the differences are small. No correlation was found between the anti-glycan positivity and the need for small-bowel resections[30].

The number of antibodies produced against microbial antigens in CD shows a positive correlation with the severity of the disease course. Mow *et al*[50] analyzed 303 patients retrospectively and found that simultaneous presence of 3 antibodies (ASCA, anti-OmpC and anti-I2) resulted in an increased risk of complications [stenosing form (72% *vs* 23%), penetrating form (58.7% *vs* 27.9%) and need for surgical intervention (72% *vs* 23%)], as compared to the seronegative group. When all three antibodies were present, the OR was 8.6 (95% CI 4.0-18.9). In addition to qualitative correlations, quantitative correlations with serologic responses are also present. Patients expressing serologic markers at high titers are more likely to have complicated small bowel CD. In a prospective pediatric cohort (n = 196), Dubinsky *et al*[52], as in a related study by Mow *et al*[50], found that the presence and magnitude of immune responses to microbial antigens (ASCA, anti-OmpC, anti-I2 and anti-CBir1) were significantly associated with more aggressive disease phenotype. The risk of developing penetrating and/or stricturing CD was increased 11-fold in those individuals with immune responses to all four microbial antigens compared to seronegative cases [95% CI (1.5-80.4)]. Moreover, they demonstrated that the time to develop a disease complication during the 18 mo of follow-up period was significantly faster in those children who had a serologic response against at least one antigen. There is a difference between the cohort studies performed in children and adults indicating differences as to which immune response has the greatest effect on the course of the disease in children and adults. The reason for this difference is not yet understood.

An elevated titer of several antiglycan antibodies also predispose to the development of complicated ileal CD[30]. In patients positive for more than 2 anti-glycan antibodies (ALCA, ACCA, gASCA), ileal involvement can be seen in 93% of the cases, as compared to seronegative patients (60%) [OR 9.0; 95% CI (3.3-24.5)]. High ALCA titers seem to be correlated with small bowel involvement and the development of stenosing and penetrating disease forms. ACCA titers, on the other hand, demonstrated no such associations.

One should be aware that really striking differences in serologic response are demonstrated only in a minority of patients. About 1/4 of the patients are positive for several antibodies and have markedly elevated antibody titers at the same time. The proportion of seronegative patients, or patients being positive for only 1 antigen with a low antibody titer, is about the same. The remaining 50% of all CD patients have an intermediate phenotype based on the serologic assessments. The aim is to find new serologic markers with which we shall be able to identify certain homogenous groups of patients in this grey zone regarding disease progression and response to therapy[21,30].

SEROLOGICAL MARKERS IN THE FOLLOW-UP AND TREATMENT OF IBD

In patients with UC, no correlation was found between the presence and titer of atypical P-ANCA and the activity of the disease. The titer of atypical P-ANCA remains unchanged even after a colectomy[45]. Similarly, the presence of ASCA in CD patients is relatively constant during the course of the disease and seems to be independent of the disease activity[45,48,51]. As a consequence, neither atypical P-ANCA, nor ASCA is suitable for monitoring the disease. The ALCA and ACCA titers also seem to be independent of disease activity[30]. The prevalence of ASCA, anti-I2, anti-OmpC, and the presence of multiple serologic responses are more frequent when the disease persists for a long time[49,56].

Landers *et al* found that following anti-TNF-α treatment the prevalence and titer of various antibodies (ASCA, atypical P-ANCA, anti-I2 and anti-OmpC) remained unchanged in the majority of patients[30]. Mesalamine treatment also leaves the ASCA level unchanged in active CD patients[57]. ASCA positivity

remains even after steroid treatment, but the antibody titer decreases[58].

The role of the serologic response in the prediction of therapeutical effectiveness is yet to be determined. A Belgian study involving 279 CD patients failed to find any correlation between the atypical P-ANCA or ASCA positivity and the rate of response of patients given anti-TNF-α treatment. The investigators observed a generally poorer responsiveness in the case of atypical P-ANCA$^+$/ASCA$^-$ status, but the difference was not significant[59].

Patients exhibiting serologic responses against various microbial antigens (OmpC and I2) should expect a higher remission rate if the budesonide treatment was supplemented with ciprofloxacin and metronidazole, while in anti-OmpC/I2 seronegative group budesonide treatment alone proved to be more effective[60]. The study brings up the possibility that certain antibiotics are more effectively used in those CD patients who present a marked immunologic response against microbial antigens. This group of patients may be the one that can be most effectively treated by manipulating the bacterial flora.

MUTATIONS OF THE SEROLOGIC MARKERS AND RECEPTORS TAKING PART IN INNATE IMMUNITY

Genetic heterogeneity may be responsible for the differences found in the serologic response. Literature is split regarding the possible correlation between antibody production in patients with CD and the NOD2/CARD 15 status. Several studies found the serologic response (atypical P-ANCA, anti-I2, anti-OmpC, ASCA IgG/IgA) to be independent of the NOD2/CARD 15 status[50,53]. A Belgian workgroup, on the other hand, confirmed the proposed association in a large (n = 913) cohort of patients. When more than 1 NOD2/CARD 15 mutation was present, the investigators found the rate of gASCA positivity and its titer to be significantly higher. Moreover, they found a positive correlation between the number of mutations and the prevalence of gASCA and ALCA, which may indicate a gene dose effect[61].

Investigating the association of the serologic response with the toll-like receptor (TLR)-4 gene D299G (Asp299Gly) polymorphism led to results in contrast to those found with the NOD2/CARD 15 assessments. If the patient had a variant TLR-4 genotype, the rate of ACCA positivity was less frequent as compared to the wild type, which again suggests a gene dose effect. Polymorphism may also play a role in the development of serologic responses against the Gram-negative *E. coli* membrane protein. The Belgian workgroup also found a correlation between anti-Omp positivity and TLR4 gene polymorphism in patients with UC (n = 272)[59]. Anti-Omp positivity was significantly less frequent among patients with a TLR4 variant genotype and prevalence of the antibody showed an inverse correlation with the number of the variants[58].

It must be mentioned that the immune response against microbial antigens seems to be more closely correlated with disease phenotype and the frequency of complications in CD than the known predisposing genetic factors.

SEROLOGIC MARKERS IN THE PATHOPHYSIOLOGY OF IBD

The real importance of the antibodies produced against various microbial and autoantigens is still unclear. A fundamental question that remains to be answered is whether these antibodies play a role in the immunopathogenesis of IBD, or their appearance is merely a consequence of the inflamed, leaky bowel mucosa. Several studies suggest that the main reason for the antibody production is loss of the immune tolerance, rather than increased bowel permeability[48,62]. Clinical studies could not find any correlation between the serologic response and bowel permeability in CD patients, suggesting that the presence of different serologic markers is not an epiphenomenon related to disease activity[21,45,48,51].

Lindbergh *et al*[63] demonstrated that CD was associated with a high serologic response against yeast and mannose, but no antibodies were produced against food antigens. Konrad *et al*[64] found strong and specific lymphocyte reaction against mannose in ASCA positive CD patients, but not against the nutritional antigen, ovalbumin. Both studies concluded that the serologic response in CD is a highly selective process against certain antigens and not a generalized immune hyperreactivity against the intestinal content.

Dubinsky *et al*[52] reported that the immune response against bacteria is not a consequence of the transmural penetration, since it develops earlier. It remains, however, an open question as to what was the extent of the immune response before the onset of the complications in the symptom-free patients, where no serologic assessments were performed due to the lack of clinical signs. Results of this study coincide with earlier observations that the presence of serologic response in a given patient does not change during the course of disease.

The currently accepted theory for the etiopathogenesis of IBD suggests that susceptibility to the disease, the clinical manifestations, and the progression depend equally on genetic factors, impairment of immune regulation, and environmental effects. The chronic inflammation of the intestinal mucosa and the related systemic reactions in IBD are consequences of an exaggerated immune response against the endogenous gut flora.

When the immune and/or barrier function of the bowel mucosa are genetically impaired, certain environmental factors may easily initiate a pathologic process. Animal models[65-67] highlight the importance of the interaction between host and its intraluminal bacteria. In the presence of a particular genetic defect, not every member of the commensal bacterial flora is able to induce pathologic immune response and the extent of the serologic reaction-and thus the clinical appearance of the disease-may differ. A higher rate of antibody production may reflect the extent of loss of tolerance against the various microbial antigens. A clinical interpretation of this observation may be that the effect of bacteria on the

prevalence and extent of the inflammatory reaction may affect the course of disease. Loss of immune tolerance in CD develops against targeted microbial and autoantigens and is not a global process[52].

CONCLUSION

ASCA and atypical P-ANCA remain the best-characterized markers in IBD. Unlike ASCA assays, which are generally ELISA tests and both simple to run and well-standardized, atypical P-ANCA testing is dependent on experienced personnel for both running and interpreting the test results. Results of the various assays used for the detection of atypical P-ANCA may differ significantly from each other and must therefore be compared very carefully. Individually ASCA and atypical P-ANCA tests have moderate sensitivity and specificity. Atypical P-ANCA and ASCA cannot be used for monitoring, because the antibody titers are relatively stable and do not correlate with disease activity.

Assessing both ASCA and atypical P-ANCA reactivity allows better differentiation of CD from UC and from non-CD than using the individual tests alone. The $ASCA^+$/atypical $P\text{-}ANCA^-$ phenotype is characteristic of CD, while the $ASCA^-$/atypical $P\text{-}ANCA^+$ phenotype is seen primarily in UC. Viewed together, the two assays offer a differential diagnostic tool, which may be particularly helpful in those cases when the diagnosis of CD or UC cannot be safely established using conventional investigation methods (medical history, radiological assessments, endoscopy and biopsy). These markers can help in the assessment of about half of the patients in the indeterminate colitis (CD or UC) category. In Crohn's disease ASCA positivity carries a higher chance of a complicated disease behavior and the need for early surgical intervention. In UC the presence of atypical P-ANCA one can expect a dominantly left-sided disease, which is often severe and resistant to therapy, with a high risk of early surgical intervention.

Newer markers derived from various microbial inhabitants of the gut, such as Omp, I2, and CBir1, as well as various glycan markers offer new ways to stratify patients into serologic subgroups. The cumulative presence and extent of the serologic response against these various markers may act as prognostic indicators of the severity and behavior of the ileal disease. Although the prevalence of antibodies is higher in healthy relatives of IBD patients than in the control population, their role as subclinical markers is yet to be established.

There is considerable overlap of the reactivity of many of the new serological markers and additional studies to more fully understand the basis for their development as well as their clinical significance are required. Addition of these, as well as yet to be discovered new markers, to the serologic IBD diagnostic algorithm will likely result in incremental increases in sensitivity. Increases in sensitivity however, can often be accompanied by reductions in specificity and this outcome must be carefully assessed and recognized. Evolution of effective serological test panels will involve shifting through the various markers to arrive at optimal diagnostic utility balanced with practical economic realities.

We have made significant progress in understanding the clinical features associated with various serologic markers in IBD. The ongoing challenge is how to best utilize these new assays to provide clinically relevant information in a cost-effective manner. Assembly of logical panels of serologic markers to identify patients at increased risk of more severe disease and who may benefit from early intensive monitoring and therapy to improve long-term outcome is a primary practical goal. Further prospective clinical trials will be needed to determine the evolving role and practical clinical importance of serologic assessments in IBD.

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