

(Auto)Antibodies in Inflammatory Bowel Diseases

Severine Vermeire, MD, PhD^{a,*},
Nathalie Vermeulen, MSc^a, Gert Van Assche, MD, PhD^a,
Xavier Bossuyt, MD, PhD^b, Paul Rutgeerts, MD, PhD, FRCP^a

^aDepartment of Gastroenterology, University Hospitals Leuven Belgium,
UZ Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

^bDepartment of Medical Diagnostic Sciences, University Hospitals Leuven Belgium, UZ
Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

Patients who have inflammatory bowel diseases (IBD) express strong antibody responses to a variety of epitopes. Their precise significance is unclear, but most are immunoglobulin (Ig)A and IgG antibodies directed toward commensals of the bowel or pathogens. Such multiple reactivity may represent cross-reactivity or a second phenomenon of mucosal injury and increased (microbial) exposure, leading to a diversely targeted immune response. The first demonstration of serum antibodies in ulcerative colitis (UC) and Crohn's disease (CD) patients dates from the late 1950s and early 1960s [1]. Since then, a number of (auto)antibodies have been described in patients who have CD or UC, and the markers that have undoubtedly been studied most include anti-*Saccharomyces cerevisiae* antibodies (ASCA) and antineutrophil cytoplasmic antibodies with perinuclear staining (pANCA). ASCA are directed against mannose residues on the cell wall of the yeast *Saccharomyces cerevisiae* and are observed in 40% to 60% of CD patients [2]. In contrast, pANCA (on indirect immunofluorescence [IIF]) have been associated with UC and with colonic CD [3]. Table 1 gives an overview of the most reported and studied antibodies in IBD.

ANTIBODIES STUDIED IN ULCERATIVE COLITIS

The first studies on antibodies in patients who had IBD focused on autoantibodies to colonic mucosa. These antibodies against a fraction of the crude colon mucosa were found to be specific for patients who had UC [1]. Cross-reactivity with the *Escherichia coli* bacterial antigen 0:14 had already been reported, suggesting a link with bacterial epitopes. In the following years, anti-globet cell antibodies were described and were very specific for UC, although

*Corresponding author. E-mail address: severine.vermeire@uz.kuleuven.ac.be (S. Vermeire).

Table 1
(Auto)antibodies studied in inflammatory bowel disease

Antibody	Directed against	CD	UC
Colon extract	Unknown	—	+
Antiglobet cell antibodies	Unknown	—	+
ASCA	Mannan epitope of <i>Saccharomyces cerevisiae</i>	+ (40%–60%)	—
gASCA	Mannan epitope of <i>Saccharomyces cerevisiae</i>	+ (56%–65%)	—
pANCA	Neutrophils	+ (UC-like CD)	+ (40%–60%)
PAB	Pancreas	+ (15%–30%)	+
Anti-Omp(C)	Outer membrane porin	+ (20%–40%)	+
Anti-I2	<i>Pseudomonas fluorescens</i>	+ (20%–40%)	—
Anti-cBir1	Flagellin	+ (20%–50%)	—
ACCA	Glycan (chitobioside)	+ (20%–40%)	—
ALCA	Glycan (laminaribioside)	+ (20%–40%)	—
AMCA	Glycan (mannobioside)	+ (28%)	—

Abbreviations: ACCA, antichitobioside antibodies; ALCA, antilaminaribioside antibodies; AMCA, antimannobioside antibodies; Anti-cBir1, antibody directed against bacterial flagellin epitopes; gASCA, anti-glycan ASCA; Anti-I2, antibody against an epitope of *Pseudomonas fluorescens*; Anti-Omp(C), antibody against outer membrane protein C of *Escherichia coli*; PAB, pancreatic antibodies.

their sensitivity was low [4]. It is still unclear whether both antibodies are directed to the same epitopes.

A subset of pANCA on IIF has been associated with colonic IBD: for UC, a sensitivity and a specificity of 60% to 80% and 90%, respectively, were reported, as was 10% sensitivity for UC-like CD [3,5,6]. A high prevalence of pANCA has also been associated with primary sclerosing cholangitis and autoimmune hepatitis [7–9]. The role of pANCA in the pathogenesis of UC is not clear; one of the reasons is the target antigen against which pANCA is directed is not known. A number of nuclear and cytoplasmic antigens have been proposed as the target epitopes for the atypical pANCA pattern in UC (eg, myeloperoxidase, proteinase-3, lactoferrin, cathepsin G and C, elastase, lysozyme, bactericidal permeability increasing protein, glucosidase, and galactosidase); however, none has proved to be the causing antigen [9]. In addition, alpha-enolase has been proposed as a target antigen. In a recent study, the authors evaluated the prevalence and diagnostic value of anti-alpha-enolase antibodies in IBD and related disorders [10]. By means of Western blot analysis, a cohort of 525 subjects was screened for the presence of anti-alpha-enolase antibodies. Anti-alpha-enolase antibodies were detected in 49.0% of patients who had UC, in 50.0% of patients who had CD, in 30.5% of patients who had primary sclerosing cholangitis, in 37.8% of patients who had autoimmune hepatitis, in 34.0% of patients who had ANCA-positive vasculitis, in 31.0% of non-IBD gastrointestinal control subjects, and in 8.5% of healthy control subjects. Gene array experiments on RNA extracted from colonic mucosal biopsies from 35 IBD and 6 control subjects showed a significant up-regulation of

alpha-enolase mRNA in colonic mucosal biopsy samples from patients who had IBD, but not from control subjects. There was no association, however, between the presence of pANCA and anti-alpha-enolase antibodies. In addition, preabsorption with alpha-enolase did not eliminate the pANCA pattern on IIF. From these results, it seems that alpha-enolase is not the target epitope for the atypical pANCA in UC. Although anti-alpha-enolase antibodies are present in a substantial proportion of patients who have IBD, they are not specific for this disease and are also found in patients who have various inflammatory/autoimmune disorders and in non-IBD gastrointestinal control subjects. Therefore, anti-alpha-enolase antibodies are of limited diagnostic value for the diagnosis of IBD.

Because the exact epitope for pANCA has not been found, the current assays to detect these antibodies consist of an IIF technique, with an atypical perinuclear fluorescence on ethanol-fixed neutrophil slides. Although pANCA refers to a perinuclear fluorescence, it is the inner border of the nucleus that is stained. Because a 50-kd nuclear envelope protein was identified as the target for the pANCA in UC [11], it was proposed that the terminology of pANCA should be changed to “perinuclear antineutrophil antinuclear antibodies (pANNA).” Further identification of this 50-kd protein is anticipated and may lead to a better understanding of the nature of these antibodies.

A comparative study using five different pANCA IIF assays showed very heterogeneous results among laboratories, with sensitivities among UC patients ranging from 0% to 63% (Table 2). Methodological differences (preparation of neutrophils) were the most important determinants for the heterogeneity in results [12]. When the epitope of pANCA is identified, future studies using standardized ELISAs may overcome the methodological problems as they are now seen.

ANTIBODIES STUDIED IN CROHN’S DISEASE

The search for antibodies in CD has been limited by the lack of specificity. Although lymphocytotoxic antibodies have been reported in approximately 40% of CD patients and in 30% of unaffected relatives, unaffected spouses of IBD patients also express seroreactivity [13]. Up to 70% of CD patients carry antibodies to epithelial cell components, but these antibodies lack specificity and are detected in other gastrointestinal diseases (30%) and systemic autoimmune

Table 2
Results of a comparative study of perinuclear antineutrophil cytoplasmic antibodies with indirect immunofluorescence assays

Type of IBD	Laboratory				
	Prometheus	Oxford	Wurzburg	Mayo	SKB
UC	63%	39%	32%	48%	0%
CD	25%	22%	14%	19%	1%

Values represent sensitivities of assays.
Abbreviation: SKB, Smith Kline Beecham.

diseases (10%) [14]. Anti-erythrocyte antibodies are directed against the heavy chain of the Vh3-15 IgG gene product [15]. They occur at a high frequency (90%) in CD patients, in 30% of UC patients and healthy control subjects, and in a high percentage of patients who have infectious gastroenteritis.

Pancreatic antibodies (PAB) are very specific (95%) for CD and are found at a frequency of 30% in these patients. They are absent in healthy relatives of CD patients [16,17]. PAB are very stable over time and do not correlate with disease activity, pattern of disease, or extraintestinal manifestations. Patients who have CD and concomitant pancreatic insufficiency, however, are significantly more frequently PAB positive than CD patients who do not have pancreatic insufficiency [16]. On the contrary, in patients who have CD and acute pancreatitis, PAB do not occur more often than in CD patients who do not have acute pancreatitis. These antibodies are directed against a not-yet-further-identified 1300-kd antigen localized within the pancreatic secretions. Several studies focused on defined pancreatic proteins but could not identify them as the causative antigens. Further studies are needed to identify the antigen, which in turn could lead to increased utility of this marker.

ASCA, an antibody against the bakers' and brewers' yeast *Saccharomyces cerevisiae*, has been associated with CD [2,18,19]. The reported prevalence is 60% to 70% in patients who have CD, 10% to 15% in patients who have UC, and 0% to 5% in control subjects. The epitope against which ASCA is directed consists of oligomannosidic sequences in the yeast's cell wall [20]. It is not clear, however, whether ASCA are truly directed against this particular yeast or whether they represent cross-reactivity with other, yet-unidentified microorganisms that also carry mannan sequences. A number of other microorganisms such as *Candida* and *Mycobacteria* have been studied in this context but results were negative [20]. How these antibodies are formed is unclear, but immunization by yeasts is one hypothesis. Dietary yeasts and yeast species that are natural inhabitants of the gastrointestinal tract must be considered immunogen candidates. Most CD patients have increased intestinal permeability, therefore exposing the epithelium to food antigens such as yeasts, consequently leading to increased exposure of yeast to immune reactive cells. Some investigators have postulated that abnormal permeability is a primary defect. The fact that patients who have celiac disease also show ASCA may point toward an increased small bowel permeability as a common etiology. In patients who have CD, ASCA are found especially in small bowel disease. Finally, it could also be that the oligomannosides share structural homologies with oligomannosides expressed on human glycoconjugates as autoantigens [2].

Accuracy data on ASCA reported a sensitivity for CD of 45% to 60% and a specificity of 85% to 95% for differentiating CD from control subjects in adults [20,21]. A similar study performed in a pediatric IBD population showed similar results [22].

In previous years, more antibodies directed against bacterial epitopes have been discovered in patients who have CD. One is the antibody against outer membrane protein C of *E coli* (anti-OmpC) and an epitope of *Pseudomonas*

fluorescens (anti-I2) [23,24]. The most recent antibody is cBir1, directed against bacterial flagellin epitopes [25].

Two years ago, a new panel of antiglycan antibodies was described. These markers include antilaminaribioside antibodies (ALCA), antichitobioside antibodies (ACCA), and antimannobioside antibodies (AMCA) [26].

CLINICAL IMPACT OF ANTIBODIES

The first studies on autoantibodies in IBD focused on their significance in the pathogenesis of IBD, but the results were disappointing. Later, the question was posed whether these antibodies could have a diagnostic relevance, similar to tissue transglutaminase antibodies in gluten-sensitive enteropathy or antinuclear antibodies in systemic lupus erythematosus. It was also wondered whether they could be of value in screening for IBD, in differentiating CD from UC in cases of indeterminate colitis, and for the prediction of disease behavior.

Diagnosis of IBD

Although the sensitivity of individual markers is too low to defend their general application for diagnosing IBD, using a panel of markers may reach sensitivity values of approximately 80%. Using the new panel of antiglycan antibodies, 76% of CD patients had at least one of the tested antibodies [27]. For differentiation between CD and UC, however, the combination of antiglycan (g)ASCA and pANCA was still the most accurate. For differentiation between IBD, healthy control subjects, and non-IBD gastrointestinal inflammation, the combination of gASCA, pANCA, and ALCA had the best accuracy.

Even a combination panel, however, will not replace endoscopy and pathology in the diagnosis of the disease. Nevertheless, in children in whom invasive examinations are avoided as often as possible, ASCA and pANCA may prioritize diagnostic workup as shown by Dubinsky and colleagues [28]. Of the 128 children studied for ASCA and pANCA with a sequential testing (a sensitive assay followed by more a specific assay in the case of positive serology), only 3.1% (4/128) of children would have received unnecessary investigations. Serology is often also negative, and in the same study, in 17 (13%) children in whom this was the case, the diagnosis would have been delayed despite IBD on colonoscopy and pathology.

Where these antibody markers could be of help is in the setting of colitis-type unclassified (previously also called indeterminate colitis). This entity is one in which a formal diagnosis of CD or UC cannot be made using the conventional diagnostic modalities and is seen in approximately 10% of IBD patients. More than 50% of these patients also test negative for these antibodies and seem to behave differently because they do not evolve toward a clear diagnosis of CD or UC [29,30].

Stability of Antibodies

There is no solid correlation between disease activity and the various antibodies or the magnitude of seroreactivity. Most antibodies are believed to be

stable over time, although few studies have specifically investigated this. The stability of the seroreactive response has been studied in detail for ASCA and pANCA. pANCA are very stable and persist up to 3 years after colectomy, which would argue against a secondary phenomenon (as a consequence of inflammation) of these antibodies [31]. In addition, for ASCA, the authors could not demonstrate significant variability in seroreactivity over time (Fig. 1) [19]. More recently, however, the stability of the newer antiglycan antibodies has been questioned. In a cohort of 373 CD patients followed at the authors' center, patients who were positive for gASCA, ACCA, AMCA, and OmpC had significantly longer disease duration compared with patients who were negative for these serologic markers. To study whether there was a correlation between the disease duration and the level of antibody response, the authors divided 373 CD patients into four relatively equal groups based on their disease duration. From these 373 CD patients, 98, 103, 82, and 90 patients had a disease duration of 3 years or less, 4 to 9 years, 10 to 15 years, and 16 years or longer, respectively. Significantly higher antibody responses were found against gASCA, AMCA, and OmpC with increasing disease duration. A trend for ACCA was also found. The antibody response against ALCA was not influenced by disease duration [27].

Antibodies as Preclinical Markers?

There are conflicting studies on the presence of IBD antibodies in unaffected relatives of patients. A French group and the authors' group showed an increased prevalence of ASCA in unaffected relatives in contrast to spouses [19,32]. Sutton and colleagues [33] did not find an increased frequency of anti-mannan immunoglobulin in unaffected relatives of CD patients (9.3%) compared with healthy control subjects (4.0%); however, a familial concordance and intrafamilial aggregation of ASCA and absence of this marker in unaffected spouses was seen, pointing toward a genetic origin or a common childhood exposure. The same has been observed for pANCA in relatives of UC

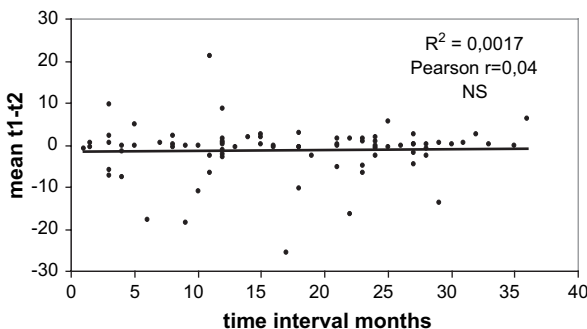


Fig. 1. Stability of ASCA in CD. Figure shows the mean difference between ASCA levels ($t_1 - t_2$) related to time interval.

patients: some groups reported pANCA in unaffected UC relatives [7,34,35], whereas other investigators were not able to confirm this finding [36–38].

It is unclear when (before or during the disease course) these antibodies appear, but ASCA has been described before onset of CD [39]. Recent data suggest that their presence might represent a genetic susceptibility because patients who have positive antibodies more or less often carry mutations in the NOD2/CARD15 gene or in toll-like receptor genes [40,41].

Antibodies and Clinical Phenotypes

The presence of antibodies and the magnitude of the seroreactivity is associated with specific clinical phenotypes, most often complicated CD of the small bowel, with stricture or fistula formation and need for surgical intervention. ASCA, anti-OmpC, anti-I2, and anti-cBir1 are also found mainly in patients who have fibrostenosing or perforating disease [24]. Using a different panel of novel (mainly) antiglycan antibodies, the same conclusions were reached because increasing amounts and levels of antibody responses against gASCA, ALCA, ACCA, AMCA, and anti-Omp were associated with more complicated disease behavior (44.7% versus 53.6% versus 71.1% versus 82.0%, $P < .001$) and a higher frequency of CD-related abdominal surgery (38.5% versus 48.8% versus 60.7% versus 75.4%, $P < .001$) (Fig. 2) [27].

There is a need for large prospective studies to validate these findings. A small prospective study in children using a panel of anti-cBir1, ASCA, anti-I2, and anti-OmpC showed that the odds of developing internal penetrating or stenosing disease were highest in patients positive for all four markers (odds ratio: 11; 95% confidence interval: 1.5–80.4; $P = .03$), and the rate of disease progression was faster in seropositive patients compared with patients who did not have antibodies [42].

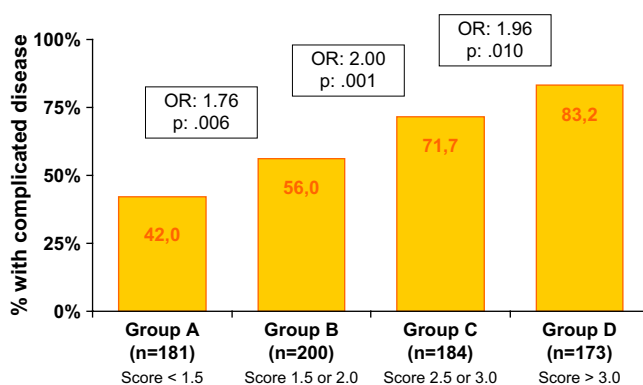


Fig. 2. Using a panel of markers (gASCA, AMCA, ALCA, ACCA, and anti-Omp), a correlation between the magnitude of serologic response and complicated CD is shown. OR, odds ratio; p, P value. (From Ferrante M, Henckaerts L, Joossens M, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007;56:1394–403; with permission.)

In UC patients, pANCA tend to occur mainly in UC patients who have a severe course of the disease and who would be associated with early surgery [43] and treatment-resistant left-sided disease [44]. High ANCA titers have also been shown to predict the occurrence and severity of pouchitis in patients undergoing colectomy and ileal pouch-anal anastomosis (IPAA). Data in the literature, however, are conflicting and not solid enough to justify the use of pANCA before or after IPAA to predict who will develop pouchitis.

Further Areas of Research

Prospective studies looking at the stability of markers and linking the presence and magnitude of antibody responses to disease course are greatly needed to confirm the previous findings.

SUMMARY

Many patients who have CD have (auto)antibodies circulating in the serum. These markers reflect a loss of tolerance toward bacterial and fungal flora and have been studied for their clinical value in IBD patients. These antibodies have no place in the diagnostic workup, except perhaps in cases of colitis-type unclassified, in which ASCA and pANCA still have the best accuracy, although they often test negative in this setting. Their real promise may lie in their use as surrogate markers of complicated aggressive disease as shown in various retrospective studies, but prospective data are lacking.

References

- [1] Broberger O, Perlmann P. Autoantibodies in human ulcerative colitis. *J Exp Med* 1959;110:657–74.
- [2] Sendid B, Colombel JF, Jacquinet PM, et al. Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* 1996;3:219–26.
- [3] Rump JA, Scholmerich J, Gross V, et al. A new type of perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) in active ulcerative colitis but not in Crohn's disease. *Immunobiology* 1990;181:406–13.
- [4] Harrison WJ. Autoantibodies against intestinal and gastric mucous cells in ulcerative colitis. *Lancet* 1965;1:1346–50.
- [5] Vasilias EA, Plevy SE, Landers CJ, et al. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996;110:1810–9.
- [6] Satsangi J, Landers CJ, Welsh KI, et al. The presence of anti-neutrophil antibodies reflects clinical and genetic heterogeneity within inflammatory bowel disease. *Inflamm Bowel Dis* 1998;4:18–26.
- [7] Seibold F, Slametschka D, Gregor M, et al. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 1994;107:532–6.
- [8] Targan SR, Landers C, Vidrich A, et al. High titer antineutrophil cytoplasmic antibodies in type-1 autoimmune hepatitis. *Gastroenterology* 1995;108:1159–66.
- [9] Seibold F, Weber P, Schöning A, et al. Neutrophil antibodies in chronic liver disease and inflammatory bowel disease: do they react with different antigens? *Eur J Gastroenterol Hepatol* 1996;8:1095–100.
- [10] Vermeulen N, Arijis I, Joossens S, et al. Anti- α -enolase antibodies in patients with inflammatory bowel disease. *Clin Chem* 2008;54:534–41.

- [11] Terjung B, Spengler U, Sauerbruch T, et al. "Atypical p-ANCA" in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. *Gastroenterology* 2000;119:310–22.
- [12] Sandborn WJ, Loftus EV, Colombel JF, et al. Utility of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) as serologic markers in a population based cohort of patients with Crohn's disease and ulcerative colitis. *Gastroenterology* 2000;118:A696.
- [13] Korman SJ, Williams RC, Wilson D, et al. Lymphocytotoxic antibody in inflammatory bowel disease. *N Engl J Med* 1975;22:1117–20.
- [14] Fiocchi C, Roche JK, Michener WM. High prevalence of antibodies to intestinal epithelial antigens in patients with inflammatory bowel disease and their relatives. *Ann Intern Med* 1989;110:786–94.
- [15] Berberian LS, Valles-Ayoub Y, Gordon LK, et al. Expression of a novel autoantibody defined by the Vh3-15 gene in inflammatory bowel disease and *Campylobacter jejuni* enterocolitis. *J Immunol* 1994;153:3756–63.
- [16] Seibold F, Weber P, Jens H, et al. Antibodies to a trypsin-sensitive pancreatic antigen in chronic inflammatory bowel disease: specific markers for a subgroup of patients with Crohn's disease. *Gut* 1991;32:1192–7.
- [17] Joossens S, Vermeire S, Van Steen K, et al. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis* 2004;10(6):771–7.
- [18] Main J, Mc Kenzie H, Yeaman GR, et al. Antibody to *Saccharomyces cerevisiae* (baker's yeast) in Crohn's disease. *BMJ* 1988;297:1105–6.
- [19] Vermeire S, Peeters M, Vlietinck R, et al. Anti-*Saccharomyces cerevisiae* antibodies (ASCA), phenotypes of IBD, and intestinal permeability: a study in IBD families. *Inflamm Bowel Dis* 2001;7(1):8–15.
- [20] Quinton JF, Sendid B, Reumaux D, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with anti-neutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998;42:788–91.
- [21] Peeters M, Geyens B, Claus D, et al. Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterology* 1997;113:802–7.
- [22] Ruemmele FM, Targan SR, Levy G, et al. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. *Gastroenterology* 1998;115:822–9.
- [23] Landers CJ, Cohavy O, Misra R, et al. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002;123:689–99.
- [24] Mow WS, Vasilias EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004;126:414–24.
- [25] Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005;128:2020–8.
- [26] Dotan I, Fishman S, Dgani Y, et al. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006;131:366–78.
- [27] Ferrante M, Henckaerts L, Joossens M, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007;56:1394–403.
- [28] Dubinsky MC, Ofman JJ, Urman M, et al. Clinical utility of serodiagnostic testing in suspected pediatric inflammatory bowel disease. *Am J Gastroenterol* 2001;96:758–65.
- [29] Joossens S, Reinisch W, Vermeire S, et al. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology* 2002;122:1242–7.
- [30] Joossens S, Colombel JF, Landers C, et al. Anti-outer membrane of porin C and anti-I2 antibodies in indeterminate colitis. *Gut* 2006;55:1667–9.
- [31] Seibold F, Weber P, Klein R, et al. Clinical significance of antibodies against neutrophils in patients with inflammatory bowel disease and primary sclerosing cholangitis. *Gut* 1992;33:657–62.

- [32] Sendid B, Quinton JF, Charrier G, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies in familial Crohn's disease. *Am J Gastroenterol* 1998;93:1306–10.
- [33] Sutton CL, Yang H, Rotter JI, et al. Familial expression of anti-*Saccharomyces cerevisiae* mannan antibodies in affected and unaffected relatives of patients with Crohn's disease. *Gut* 2000;46:58–63.
- [34] Shanahan F, Duerr R, Rotter JI. Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. *Gastroenterology* 1992;103:456–61.
- [35] Folwaczny C, Noehl N, Endres SP, et al. Antinuclear autoantibodies in patients with inflammatory bowel disease—high prevalence in first-degree relatives. *Dig Dis Sci* 1997;42:1593–6.
- [36] Reumaux D, Colombel JF, Delecourt L, et al. Antineutrophil cytoplasmic autoantibodies in relatives of patients with ulcerative colitis [letter]. *Gastroenterology* 1992;103:1706.
- [37] Yang P, Jarnerot G, Danielsson D, et al. pANCA in monozygotic twins with inflammatory bowel disease. *Gut* 1995;36:887–90.
- [38] Lee CW, Lennard-Jones JE, Cambridge G. Antineutrophil antibodies in familial inflammatory bowel disease. *Gastroenterology* 1995;108:428–33.
- [39] Israeli E, Grotto I, Gilburd B, et al. Anti-*Saccharomyces cerevisiae* and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005;54:1232–6.
- [40] Devlin SM, Yang H, Ippoliti A, et al. NOD2 variants and antibody response to microbial antigens in Crohn's disease patients and their unaffected relatives. *Gastroenterology* 2007;132:576–86.
- [41] Henckaerts LC, Pierik M, Joossens M, et al. Mutations in pattern recognition receptor genes modulate seroreactivity to microbial antigens in patients with inflammatory bowel disease. *Gut* 2007;56:1536–42.
- [42] Dubinsky MC, Lin YC, Dutridge D, et al. Western regional pediatric IBD research alliance. Serum immune responses predict rapid disease progression among children with Crohn's disease: immune responses predict disease progression. *Am J Gastroenterol* 2006;101:360–7.
- [43] Vecchi M, Bianchi MB, Calabresi C, et al. Long-term observation of the perinuclear antineutrophil cytoplasmic antibody status in ulcerative colitis patients. *Scand J Gastroenterol* 1998;33:170–3.
- [44] Sandborn WJ, Landers CJ, Tremaine WJ, et al. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996;71:431–6.