

## Perinuclear Antineutrophil Cytoplasmic Autoantibodies and Anti-*Saccharomyces cerevisiae* Antibodies: Serologic Markers in Inflammatory Bowel Disease

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Crohn's disease and ulcerative colitis are chronic, relapsing inflammatory disorders of the gastrointestinal tract. The two ailments are the major disease entities referred to as inflammatory bowel disease. The etiologies of CD and UC are still elusive. The current concept of the pathogenesis of IBD involves the interacting elements of multigenic host susceptibility, environmental factors and enteric microflora. Tissue damage is mediated by the immune system, although the contributory mechanisms are still unclear and may be multiple or varied in different individuals. A variety of immune abnormalities has been described at both the systemic and the intestinal level in IBD, of either the cellular or humoral type. Several autoantibodies have been identified so far in patients with IBD, including antipancreatic antibodies [1], anti-erythrocyte antibodies [2], anti-endothelial cell antibodies [3], antigoblet cell antibodies [4] and antibacterial/permeability-increasing protein antibodies [5].

Recently, two candidate antibodies, perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) have been suggested as possible diagnostic markers in IBD. The present review presents the current knowledge regarding the putative value of pANCA and ASCA in IBD.

### pANCA

ANCA constitute a heterogeneous group of antibodies that target antigens present mostly in azurophilic granules of polymorphonuclear leukocytes. ANCA were originally detected in serum from patients with Wegener's granulomatosis. Serum samples from

patients with Wegener's granulomatosis revealed a typical cytoplasmic staining pattern (cANCA) when tested by indirect immunofluorescence on ethanol-fixed neutrophils. The antigen recognized by cANCA proved to be a proteinase-3 constituent of azurophilic granules [6]. A second type of ANCA, characterized by a perinuclear staining pattern, has been detected in patients with other forms of vasculitis and glomerulonephritis. The first antigen recognized by pANCA was identified as myeloperoxidase, another constituent of azurophilic granules [7].

Lately, ANCA have been demonstrated in sera from patients with a variety of chronic idiopathic inflammatory disorders [Table 1]. In these disorders a pANCA staining pattern is usually found, but the antigen is not myeloperoxidase. Moreover, an increasing number of cytoplasmic proteins has been identified so far as ANCA target antigens, including bactericidal/permeability-increasing protein, lactoferrin, cathepsin G, human elastase, and lysozyme [8]. Some ANCA-targeted antigens are located directly in the cytoplasm, not within granules; for example, alpha-enolase and catalase. Other ANCA-targeted antigens are located in the nucleus, including the non-histone chromosomal proteins HMG1 and 2 (high mobility proteins). Another type of ANCA, producing a diffuse cytoplasmic staining pattern on ethanol-fixed neutrophils (atypical ANCA), has also been described [9].

In 1990, two different groups reported the presence of ANCA in serum from 84% and 59% of patients with UC, respectively [10,11]. Since then, numerous reports have confirmed this finding in different populations, including Israeli patients with IBD [12]. In addition, ANCA were also detected in CD, although with a much lower prevalence (10-20%), and in primary sclerosing cholangitis

- *Immunologic phenomena, as ANCA and ASCA antibodies, may help to advance our understanding of disease pathogenesis and provide the link between basic and clinical observations*
- *Further studies are warranted to identify and assess the clinical importance and applicability of the new markers in IBD*

CD = Crohn's disease

UC = ulcerative colitis

IBD = inflammatory bowel disease

pANCA = perinuclear antineutrophil cytoplasmic autoantibodies

ASCA = anti-*Saccharomyces cerevisiae* antibodies

cANCA = cytoplasmic staining antineutrophil cytoplasmic autoantibodies

PR3 = proteinase-3

BIP = bactericidal/permeability-increasing protein

**Table 1.** ANCA staining and antigen targets in chronic inflammatory disorders

Disease	ANCA staining	ANCA antigens and their cellular localization				
		Azurophilic granules	Other granules	Cytosol	Nucleus	
Wegener's granulomatosis	cANCA	PR3				<b>Stone JH, et al.</b> Test characteristics of immunofluorescence and ELISA tests in 856 consecutive patients with possible ANCA-associated conditions. <i>Arthritis Care Res</i> 2000;13:424–34.
Amebiasis, endocarditis	cANCA	PR3				<b>Pudifin DJ, et al.</b> Invasive amoebiasis is associated with the development of ASCA. <i>Clin Exp Immunol</i> 1994;97:48–51. <b>Choi HK, et al.</b> Subacute bacterial endocarditis with positive cANCA and anti-proteinase 3 antibodies. <i>Arthritis Rheum</i> 2000;43:226–31.
Microscopic polyangiitis	pANCA	MPO				<b>Stone JH, et al.</b> Test characteristics of immunofluorescence and ELISA tests in 856 consecutive patients with possible ANCA-associated conditions. <i>Arthritis Care Res</i> 2000;13:424–34.
Idiopathic rapidly progressive glomerulonephritis	pANCA	MPO				
Churg-Strauss, JIA, RA, SLE, SS, Wegener's granulomatosis	cANCA pANCA	MPO	Lactoferrin			<b>Chikazawa H, et al.</b> Immunoglobulin isotypes of anti-myeloperoxidase and anti-lactoferrin antibodies in patients with collagen disease. <i>J Clin Immunol</i> 2000;20:279–86.
Other disorders (sarcoidosis, HIV)	cANCA pANCA	PR3 MPO, BPI	Lactoferrin			<b>Tervaert JW, et al.</b> Silicon exposure and systemic vasculitis. <i>Curr Opin Rheumatol</i> 1998;10:12–17. <b>Klaassen RJ, et al.</b> ANCA in patients with symptomatic HIV infection. <i>Clin Exp Immunol</i> 1992;87:24–30.
Cystic fibrosis	cANCA pANCA	PR3 BPI				<b>Mahadeva R, et al.</b> ANCA against bactericidal/permeability-increasing protein (BPI) and cystic fibrosis lung disease. <i>Clin Exp Immunol</i> 1999;117:561–7.
Autoimmune hepatitis	cANCA			Actin		<b>Schwarze C, et al.</b> IgA class ANCA in primary sclerosing cholangitis and autoimmune hepatitis. <i>Clin Exp Immunol</i> 2003;133:283–9.
Inflammatory bowel disease and primary sclerosing cholangitis	pANCA	BPI $\beta$ -glucuronidase	Lysozyme lactoferrin	Catalase $\alpha$ -enolase	Histone H1 HMG1/2 lamin	<b>Seibold F, et al.</b> Neutrophil antibodies (pANCA) in chronic liver disease and inflammatory bowel disease: do they react with different antigens? <i>Eur J Gastroenterol Hepatol</i> 1996;8:1095–100.

MPO = myeloperoxidase, JIA = juvenile idiopathic arthritis, RA = reactive arthritis, SLE = systemic lupus erythematosus, SS = systemic sclerosis.

(50–85%), a chronic cholestatic disease that is strongly associated with UC [13]. Identification of specific antigens recognized by ANCA in IBD revealed rather heterogeneous results [5]. Antibodies to PR3 and myeloperoxidase are rare in serum of IBD patients. The cytosolic enzymes catalase and  $\alpha$ -enolase as well as lactoferrin, elastase, cathepsin G and BIP were recently identified as ANCA antigens in both UC and CD [5,14]. Furthermore, antibodies to several nuclear antigens (histone I and nuclear non-histone chromosomal proteins high mobility group 1/2) have also been identified in IBD patients' sera [15].

Serum ANCA is thought to reflect mucosal ANCA production, and studies have shown that B cell priming and ANCA production take place in the colonic mucosa [16]. These findings imply that recognition of mucosal antigen(s) leads to local production of ANCA. The pathogenic role of ANCA in IBD is equivocal, so far. Whereas ANCA, which target PR3 and MPO, are probably involved in the pathophysiology of vasculitis disorders associated with these

autoantibodies, the pathophysiologic significance of ANCA in IBD is less clear. Most target antigens of ANCA have a physiologic role in the antimicrobial system of phagocytes. An indirect evidence of their pathogenicity, at least in vasculitis disorders, lies in the correlation of ANCA with the disease activity and the potential of ANCA to bind to target antigens and modify their physiologic function or induce endothelial cell injury. Because target antigens of ANCA are components of primary granules of neutrophils, the main focus has been on the influence of ANCA on neutrophil functions. Recently, it was documented that autoantibodies are able to penetrate into living cells; alternatively, ANCA can bind to target antigens that translocate to the cell membrane under different stimuli. The signal input produced by ANCA is most likely a combination of signals produced by both the binding of ANCA to target antigens and Fc receptor activation, resulting in neutrophil degranulation, oxygen radical production, and stimulation of inflammation [8].

The gold standard for ANCA determination is currently represented by an indirect immunofluorescence on ethanol-fixed neutrophils, combined with testing of the target antigen specificity by enzyme-linked immunosorbent assay (standard or capture) or Western blot. Other methods for ANCA detection are represented by a fixed neutrophil ELISA and an immuno-alkaline phosphatase staining method [17]. The sensitivity of these three methods varies in different disorders and laboratories, and large comparative studies on these detection methods have not been performed.

### ASCA

Anti-*Saccharomyces cerevisiae* antibodies were discovered a decade ago in the course of studies designed to search for a putative dietary antigen involved in the pathogenesis of CD. Increased antibody levels to dietary or bacterial antigens in both CD and UC have been reported often and were attributed to increased absorption of antigens across the inflamed gut mucosa. Alternatively, these antibodies may represent the response to a still not identified antigen involved in the initiation or pathogenesis of the local immune response characteristic of IBD. In their original report, Main et al. [18] showed that immunoglobulins G and A antibodies to *S. cerevisiae* are present in high titers in patients with CD but not in those with UC or in normal control subjects. Subsequent reports confirmed the findings of Main et al., and using the initially described ELISA assay, with crude mannan as an antigen, ASCA were identified in 60–70% of patients with CD, 10–15% of patients with UC and 0–5% of control subjects [19–23] [Table 2].

Heelan and collaborators [24] provided evidence that the relevant antibodies recognize carbohydrate epitopes present on a high molecular weight constituent of the crude *S. cerevisiae*, the composition of which is identical to that of the cell wall mannoprotein. The yeast cell wall mannan (phosphopeptidomannan) is a 200 kDa water-soluble glycoprotein component that may be extracted by autoclaving. Using several yeast strains, Sendid and team [25] documented the highest specificity and sensitivity using Su1 and Su2 strains of *S. cerevisiae* brewer's yeast. Graded chemical degradation performed on the most reactive phosphopeptidomannans demonstrated that the most important polysaccharide epitope was shared by both acid-stable and alkali-labile domains and was identified as a mannotetraose [25].

In contrast to pANCA, ASCA do not seem to be autoantibodies, but rather antibodies against bacterial or fungal species. *S. cerevisiae*, a yeast commonly used in the food industry for baking and brewing, has not been directly implicated in the pathogenesis of CD. The yeast is a common microorganism that rarely causes significant disease in humans. The presence of ASCA in patients with IBD may be the result of a response to either the antigens on

**Table 2.** Prevalence of ANCA and ASCA in UC and CD

Study	No. of participants	ANCA prevalence (%)			ASCA prevalence (%)		
		UC	CD	Controls	CD	UC	Controls
Quinton JF, et al. [19]	201 patients 163 controls	66	15	0.6	61	12	0
Koutroubakis IE, et al. [20]	157 patients 150 controls	67	16	0.7	39	11	1.3
Peeters M, et al. [21]	582 patients 157 controls	49.7	5.7	2.5	59.7	13.6	3.2
Hisabe T, et al. [22]	98 patients 31 controls				45.6	13.3	6.4
Oudkerk Pool M, et al. [23]	225 patients 141 controls	79	13	9			

*S. cerevisiae* itself, or to an as yet unidentified antigen(s) that cross-reacts with *S. cerevisiae* antigens.

Four ASCA assays are currently available: the assay developed by Dr. D Poulain (Laboratoire de Parasitologie-Mycologie, Center Hospitalier Regional Universitaire, Lille, France), and three commercially available assays from Prometheus Laboratoires Inc. (San Diego, CA, USA), Medipan Diagnostica (Selchow, Germany) and Quanta Lite ASCA (Inova Diagnostics, San Diego). Each assay uses a different *S. cerevisiae* strain and purification process, as well as different assay conditions and specific cutoff values. A recent report showed that the four different ASCA assays agree well enough to be used interchangeably [26].

### ANCA and ASCA as genetic and immunologic markers of IBD

The value of autoantibodies as markers of disease susceptibility is well established in the case of islet cell autoantibodies and insulin-dependent diabetes mellitus. ANCA and ASCA may be markers of genetic susceptibility to UC and CD, as suggested by the finding of an increased prevalence of ANCA and ASCA in unaffected first-degree relatives of patients with these disorders [27–33] [Tables 3 and 4]. However, the reports of the prevalence of ANCA in relatives of patients with UC have been contradictory. Whereas North American and German studies reported 15–30% ANCA prevalence in first-degree relatives of UC patients, other European studies failed to detect ANCA in unaffected family members [27–29]. Several studies examined the association between ANCA status and the carriage of certain genetic markers (human leukocyte antigen class I and II antigens), cytokines and adhesion molecules. So far, the association between ANCA and genetic markers was not consistent and seems highly dependent on the ethnic background of the population studied [8].

Similarly, an increased prevalence of ASCA has been reported in healthy relatives of patients with CD. Furthermore, ASCA were more prevalent in familial than in sporadic cases of CD [30–33]. The increased occurrence of ASCA in first-degree relatives of patients with CD is compatible with a role of these antibodies as markers for genetic susceptibility. Increased permeability could be the basis for the presence of ASCA; one large study [34] found that approximately the same proportion (20–25%) of first-degree relatives of

ELISA = enzyme-linked immunosorbent assay

**Table 3.** ANCA prevalence in IBD patients and first degree healthy relatives

Study	No. of patients	ANCA prevalence (%)				
		Ulcerative colitis		Crohn's disease		Controls
		Patients	Relatives	Patients	Relatives	
Papo M, et al. [27]	80 patients 217 relatives 62 controls	64	3.9	12.5	6.7	1.6
Lee JC, et al. [28]	168 patients 197 relatives 64 controls	46% sUC 44% fUC	3	3	0	3
Seibold F, et al. [29]	65 patients 215 relatives 47 controls	70	30	27	6	0

sUC = sporadic UC, fUC = familial UC

**Table 4.** ASCA prevalence in IBD patients and first-degree healthy relatives

Study	No. of patients	ANCA prevalence (%)				
		Ulcerative colitis		Crohn's disease		Controls
		Patients	Relatives	Patients	Relatives	
Sendid B, et al. [30]	51 patients 66 relatives 163 controls	69	20			0.61
Sutton CL, et al. [31]	420 CD patients 249 UC patients 333 relatives 190 controls	54.1	9.3	9.2		3.8
Annese V, et al. [32]	335 patients 216 relatives 50 controls	35% sCD 55% fCD	28	12% sUC 25% fUC	26	4
Glas J, et al. [33]	361 patients 206 relatives 100 controls	54.4	16.2	9.7	5	4

sUC/sCD = sporadic UC/CD, fUC/fCD = familial UC/CD.

patients with CD also had abnormal intestinal permeability. However, in patients with CD, abnormal intestinal permeability was not associated with ASCA positivity in the respective patients. Accordingly, ASCA and intestinal permeability are most likely independent phenomena. Therefore, whether familial aggregation of ANCA and ASCA serologic markers may reflect environmental or genetic factors remains to be determined.

### The diagnostic value of ANCA and ASCA in IBD

A higher prevalence of pANCA in ulcerative colitis and ASCA in Crohn's disease was initially reported. Later on, it was shown that pANCA was also present in CD, and ASCA in UC, although with much lower prevalences (10–40%) [19–23]. This limited the usefulness of both antibodies for distinguishing between UC and CD. Nevertheless, these antibodies may be used as serologic markers to differentiate IBD from other diarrheal or colitic disorders.

The measurement of ANCA or ASCA alone has been found to have limited clinical diagnostic value in IBD because of a relatively

low sensitivity. Several studies reported an overall sensitivity of 50–60% and specificity of 79–88% for ASCA in CD, and almost the same percentages for ANCA and UC, when used independently [19–23]. The combined determination of ANCA and ASCA has been evaluated in several studies and found to be a valuable diagnostic tool in IBD [19–23]. Quinton et al. [19] and Peeters et al. [21] reported that the combination of ANCA positivity and ASCA negativity yielded 44–57% sensitivity but 97% specificity for UC, whereas ANCA negativity with ASCA positivity had 49–56% sensitivity with 94–97% specificity for CD. In these studies the combined use of both tests dropped sensitivity by approximately 10%, but increased specificity to more than 95%, yielding a very high positive predictive value for the diagnosis of either UC or CD.

The diagnosis of CD and UC is based on clinical, radiologic, endoscopic and pathologic criteria. Some patients with colonic disease (10–15%), however, cannot be classified as having either CD or UC and are categorized as having "indeterminate colitis." The diagnosis of indeterminate colitis is usually a temporary diagnosis, and most patients in this category will be diagnosed with either UC or CD over time. Early knowledge of the exact diagnosis could be of clinical importance with regard to therapeutic decisions and future prognosis. The prospective study of Joossens et al. [35] showed that the combined use of ASCA and ANCA was helpful and discriminative in

categorizing indeterminate colitis. In their study ASCA+/ANCA-predicted evolution to CD in 80% of patients and ASCA-/ANCA+ predicted evolution to UC in 63.6% of patients with indeterminate colitis.

### The clinical significance of ANCA and ASCA in IBD

The clinical significance of ANCA and ASCA in IBD is not defined. In a relatively small study, ANCA positivity in UC was associated with an aggressive disease course (treatment-resistant disease and early surgery in the disease course) [36]. However, ANCA remained positive after total colectomy in UC patients, questioning any relationship between antibodies and disease activity [37]. In some studies, positive or high ANCA titers predicted the development of pouchitis following total colectomy and ileal anal-pouch anastomosis [38]. Nevertheless, other studies failed to show differences in ANCA prevalence between patients with or without pouchitis [37]. The expression of ANCA in CD has been claimed as additional evidence that ANCA is a marker of a distinct mucosal inflammatory

process. ANCA-positive CD patients had clinical features of left-sided colitis and endoscopic or histologic features of typical UC [39]. This UC-like phenotype has been confirmed in several studies [19,39]. However, in other studies the presence of ANCA could not be associated with disease activity or particular clinical features in either disorder [10,19].

A number of clinical studies on ASCA-positive CD patients has suggested a close relationship between ASCA positivity and several clinical features of CD. Quinton and co-workers [19] reported that the age at diagnosis was significantly lower in patients with CD who were ASCA positive (21 vs. 24 years), and the same was reported in the studies of Peeters et al. [21] and Vasiliauskas et al. [39]. Additionally, ASCA seroprevalence was significantly higher in patients with small bowel involvement than in those with pure colonic disease (70 vs. 46%), and higher ASCA levels were present in patients with fibrostenosing and fistulizing disease [19]. Other studies, however, found no relationship between ASCA status and disease activity, location, complications or response to treatment [20,22].

Predicting response to a specific therapy could represent a significant application of serologic testing in IBD. The presence of definite microsatellite haplotypes of the tumor necrosis factor genes and ANCA positivity may allow prediction of resistance to anti-TNF therapy in CD. For UC, the TNF microsatellite A2B1C2D4E1 and ASCA positivity was suggested to predict medically resistant disease. However, in a recent study neither ANCA nor ASCA, alone or in combination, could predict response to anti-TNF treatment [40].

## Conclusion

Serologic markers have attracted considerable attention as possible elements involved in IBD pathogenesis and clinical manifestations. The relative specificity of these antibodies for either CD or UC indicates that their expression is not a simple epiphenomenon of intestinal injury or mucosal inflammation. Detection of disease-related antibodies may have particular value as markers of heterogeneity within IBD, as markers of underlying immune dysregulation or cross-reactivity with an environmental agent, or as possible immunologic traits related to disease susceptibility. At present, the clinical diagnostic utility of antibody detection is of limited value, although combined detection of multiple serologic markers seems promising.

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TNF = tumor necrosis factor

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