Inflammatory bowel disease (IBD) is an enduring disease involving mostly young people, with symptoms of bloody diarrhea and abdominal cramps. Several antibodies have been associated with IBD, the 2 most comprehensively studied being autoantibodies to neutrophils (atypical perinuclear anti-neutrophil cytoplasmic antibodies) and anti-\textit{Saccharomyces cerevisiae} antibodies. This review focuses on the value of these antibodies for diagnosing IBD, differentiating Crohn disease from ulcerative colitis, indeterminate colitis, monitoring disease, defining clinical phenotypes, predicting response to therapy, and as subclinical markers. Pancreatic antibodies and newly identified anti-microbial antibodies (anti-outer membrane porin C, anti-I2, and anti-flagellin) are also reviewed.

Clinical Presentation and Diagnosis of IBD

CD may present with almost any gastroenterologic symptom depending on the site of the disease. Colonic disease (either alone or together with small intestine) is more common than terminal ileal or ileocecal disease, and usually presents with chronic abdominal pain and diarrhea. In more severe forms, a mass may be present in the right iliac fossa. Patients with CD can have typical perianal lesions, such as ulcers or multiple fistulas. In CD, serum concentrations of C-reactive protein (CRP) correlate well with disease activity and with other markers of inflammation as the CD activity index (2). Increased CRP (>45 mg/L) in patients with IBD predicts with a high certainty the need for colectomy (2). Patients with UC suffer from bloody diarrhea or rectal bleeding and tenesmus because of the rectal involvement.

There are several structural differences between CD and UC (3). In UC, the disease is restricted to the rectum and colon. The lesions are continuous and restricted to the mucosa. There is muscular thickening, mucin depletion, and glandular damage. In CD, the disease may affect any part of the gastrointestinal tract. The lesions are discontinuous and transmural (fissure, abscess, fistula). There may be fibrosis (stenosis) and lymphoid ulcers. Histiocytic granulomas are the hallmark of CD, but are found in only ~60% of cases.

Diagnosis of IBD and differentiation between CD and UC can be made accurately in most patients based on the patient’s history and physical examination, ileocolonoscopic examination, biopsy, double-contrast barium enema examination, and microbiology. The differential diagnosis includes irritable bowel disease; infective
diarrheas attributable to *Salmonella*, *Campylobacter*, or *Shigella*; lymphoma; carcinoma of the colorectum; and inflammation caused by ischemia or irradiation (3, 4).

Not all endoscopic biopsy specimens can be firmly assigned to either CD or UC. This is particularly the case for colonic biopsies from cases of proctitis. In ~10% of patients with IBD, the disease cannot be classified as CD or UC and the final diagnosis is “indeterminate colitis”, even if the whole colon is removed (5). The diagnosis of indeterminate colitis is usually a temporary diagnosis, and many patients with indeterminate colitis will be diagnosed with either UC or CD over time.

Disease behavior in CD can be classified into primary fibrostenotic (rather indolent behavior), primary perforating (abscesses and fistulas; aggressive behavior), and primary inflammatory (5). UC can be classified into pancolitis (associated with greater severity and greater risk of malignant change), left-sided colitis (no local therapy possible), distal colitis, and proctitis (may be resistant to local therapy) (5).

**Autoantibodies to Neutrophils: ANCAs and Atypical P-ANCAs**

ANCAs were originally associated with primary small vessel diseases, such as Wegener granulomatous, microscopic polyangiitis and its renal limited variant (pauci-immune crescentic glomerulonephritis), and Churg–Strauss syndrome (6). ANCAs are classically screened by indirect immunofluorescence using ethanol-fixed neutrophils, as agreed on in an international consensus statement (7). Indirect immunofluorescence shows 2 major staining patterns: a cytoplasmic granular (C-ANCAs) and a perinuclear pattern (P-ANCAs). The C-ANCA pattern shows granular cytoplasmic fluorescence, frequently accentuated between the nuclear lobes. C-ANCAs are primarily present in sera from patients with Wegener granulomatous and mainly recognize proteinase-3. The P-ANCA pattern shows a fine homogeneous rim-like staining of the perinuclear cytoplasm (just around the nucleus). Nuclear extension can be present. P-ANCAs are present in patients with microscopic polyangiitis and recognize myeloperoxidase. However, P-ANCA staining is also seen with antibodies to other neutrophil enzymes and with anti-nuclear antibodies (a neutrophil-specific anti-nuclear antibody or wrong reading because of the presence of anti-nuclear antibodies).

ANCAs have also been reported in patients with chronic inflammatory disorders, such as UC (60%–80%) (8, 9), primary sclerosing cholangitis (88%) (10), autoimmune hepatitis (81%) (11), and to a lesser extent, CD (5%–25%). In these disorders a (atypical) P-ANCA staining pattern is usually found. The antigen is not myeloperoxidase. The atypical p-ANCA is characterized by a broad inhomogeneous rim-like staining of the nuclear periphery (12).

**Autoantigens Recognized by Autoantibodies to Neutrophils (Atypical P-ANCAs) in IBD**

Overviews of the various studies (13–28) that have attempted to identify the antigenic species recognized by atypical P-ANCAs in UC and CD patients and in controls are given in Tables 1 through 3. The antigens that have been studied are located in the granules, the cytosol, and/or the nuclei or nuclear periphery. The serine pro-
teases cathepsin G and elastase, the hydrolase β-glucuronidase, the iron-binding protein lactoferrin (80 kDa), and the natural antibiotic bactericidal permeability increasing (BPI) are located in the granules of the neutrophils (and monocytes).

- Enolase (47 kDa), which is involved in glycolysis, and catalase (60 kDa), which catalyzes the dissociation of hydrogen peroxide to water and oxygen, are cytoplasmic proteins. High-mobility group (HMG)-1 nonhistone chromosomal proteins and HMG-2 nonhistone chromosomal proteins (28–29 kDa) are distributed in the nuclei and cytoplasm of eukaryotic cells and act as transcription factors. HMG-1 stimulates transcription by modulating the structure of chromatin in vitro and in cultured cells. HMG-2 (29 kDa) is closely related to cell proliferation. Antibodies to histone H1 have been suggested, but their prevalences have not been reported (29). Myeloperoxidase and proteinase-3, constituents of the azurophilic granules, are not autoantigens in IBD.

The various studies have revealed heterogeneous and conflicting results. Most studies have used ELISA to identify the antibodies, except for 2 studies in which Western blotting was used (see Tables 1–3). Many of the studies considered a sample positive if the absorbance value obtained by ELISA exceeded the mean of healthy control samples by 3 SD. Some studies used 2 SD instead of 3 SD (see Tables 1–3). This means that in the majority of the studies, the cutoff was chosen in a way such that positive results were found in only a small portion of the healthy control population. In one study, in which Western blot was used to detect antibodies, anti-BPI, anti-elastase, anti-catalase, and anti-α-enolase antibodies were found in 0%, 0%, 10%, and 5% of healthy controls, respectively (27).

A shortcoming of many studies is that the antibodies have been studied in well-defined CD or UC patients but not in diseased controls, i.e., non-IBD patients (e.g., infective diarrhea) who present with symptoms similar to the symptoms in IBD. This implies that data on the specificity of the antibodies studied are incomplete. One study reported anti-BPI and anti-cathepsin G antibodies in a substantial number (≥20%) of infectious enteritis cases (25).

### Table 2. Prevalence of autoantibodies to specific antigens in patients with CD.

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Controls</th>
<th>Patients, n</th>
<th>ANCAstr</th>
<th>BPI</th>
<th>β-Glu</th>
<th>Cathepsin G</th>
<th>Elastase</th>
<th>Lysozyme</th>
<th>Lactoferrin</th>
<th>Catalase</th>
<th>α-Enolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayet et al. (28)</td>
<td>EL</td>
<td>60</td>
<td>38</td>
<td>38</td>
<td>3</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schmitt et al. (17)</td>
<td>EL</td>
<td>ND</td>
<td>23</td>
<td>120</td>
<td>2</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peen et al. (18)</td>
<td>EL</td>
<td>52</td>
<td>27</td>
<td>218</td>
<td>2</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mulder et al. (19)</td>
<td>WB</td>
<td>35</td>
<td>40</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broekloelofs et al. (20)</td>
<td>EL</td>
<td>35</td>
<td>40</td>
<td>252</td>
<td>3</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kossa et al. (21)</td>
<td>EL</td>
<td>33</td>
<td>39</td>
<td>35</td>
<td>3</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yang et al. (22)</td>
<td>EL</td>
<td>37</td>
<td>5</td>
<td>190</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stoffel et al. (23)</td>
<td>EL</td>
<td>44</td>
<td>45</td>
<td>140</td>
<td>3</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walmsley et al. (25)</td>
<td>EL</td>
<td>30</td>
<td>27</td>
<td>46</td>
<td>3</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roozenaal et al. (27)</td>
<td>WB</td>
<td>38</td>
<td>11</td>
<td>112</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a Number of standard deviations above the mean of healthy controls that served as a cutoff point for a positive value.

### Table 3. Prevalence of autoantibodies to specific neutrophilic antigens in controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Controls</th>
<th>Infectious enteritis</th>
<th>Collagenous colitis</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walmsley et al. (25)</td>
<td>EL</td>
<td>46</td>
<td>190</td>
<td>WB</td>
</tr>
<tr>
<td>Yang et al. (22)</td>
<td>EL</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Koza et al. (27)</td>
<td>WB</td>
<td>30</td>
<td>38</td>
<td>78</td>
</tr>
</tbody>
</table>

a EL, ELISA; WB, Western blot.

b Number of standard deviations above the mean of healthy controls that served as a cutoff point for a positive value.
IBD and that also may explain the wide range of results observed among the studies reported. The differences between the studies could be attributable not only to variations in methodology and the choice of the controls for determination of the ELISA cutoff values, but also to differences in the patient population (diagnostic criteria, severity of disease, treatment). Most studies found that the (azurophilic) granule proteins of the neutrophil were not recognized by most of P-ANCAs in IBD patients. Multiple antibodies against different antigens were found in 1 patient, and antibodies to several antigens were also found in immunofluorescent-negative samples (30). This is particularly the case for the cytoplasmic autoantigens, which are not packed and concentrated in granules. For example, only 21% and 38% of IBD samples positive for antibodies to catalase and α-enolase, respectively, were positive for ANCA antibodies by indirect immunofluorescence (27).

Various target antigens of atypical P-ANCAs, such as catalase, enolase, histone H1, and high-mobility group nonhistone chromosomal proteins, are not uniquely present in neutrophils or other myeloid cells, but are found in most higher eukaryotic somatic cells. β-Glucuronidase is also found in bacteria. It is not clear why these antigens cause atypical P-ANCAs. Specific epitopes of these proteins recognized by atypical P-ANCAs may be unique or only immunoinaccessible in neutrophils.

Taken together, most studies support the conclusion that the neutrophil (azurophilic) granule components characterized to date are not UC-associated P-ANCA-specific antigens and that the main target antigen has not yet been identified. The idea that a nuclear antigen is the target of these antibodies has been suggested by several authors.

Vidrich et al. (31) reported loss of antigenic recognition after DNA digestion of neutrophils for UC P-ANCAs but not for P-ANCAs of primary sclerosing cholangitis and autoimmune hepatitis. The P-ANCA patterns in primary sclerosing cholangitis and autoimmune hepatitis convert to a C-ANCA staining pattern after DNase treatment. This suggests that the epitope recognized by the UC P-ANCA is a protein–DNA complex or that the presence of intact DNA is necessary for maintaining the integrity of the epitope. The atypical P-ANCA of UC and primary sclerosing cholangitis is specific for neutrophils, whereas that of autoimmune hepatitis reacts with both neutrophils and monocytes. Confocal microscopy revealed a nuclear reaction for 88% (22 of 25) of the sera, with 72% (18 of 25) showing the reaction localized to the inner side of the nuclear (membrane) periphery (32). Immunoelectron microscopy showed that the UC-associated P-ANCA reaction localized primarily over chromatin concentrated toward the nuclear periphery. The sera did not recognize double-stranded DNA. Sobajima et al. (24) identified high-mobility group nonhistone chromosomal proteins as a possible target. Terjung et al. (33) described a 50-kDa protein (pI 6) that colocalized with proteins of the nuclear envelope of neutrophils. This protein was confined to myeloid cells and was recognized by >90% of atypical P-ANCAs in individuals with IBD, primary sclerosing cholangitis, and autoimmune hepatitis. Terjung et al. (12) reported that the atypical P-ANCAs also show multiple intranuclear fluorescent foci, which likely correspond to infoldings of the nuclear envelope.

As long as the target antigen recognized by atypical P-ANCAs remains unidentified, sensitive and specific solid-phase assays cannot be developed, leaving immunofluorescence microscopy as the only widely available technique for the detection of these antibodies.

Joossens et al. (34) evaluated the interassay and interobserver variability in the detection of UC-associated ANCAs. In the interobserver study, the same assay was used by different readers in 4 geographically distinct laboratories [INOVA Diagnostics (San Diego, CA), University of Iowa Hospital, Ospedale Mauriziano Umberto I (Torino, Italy), and University Hospital Leuven, (Leuven, Belgium)]. In the interobserver study, the authors found moderate to substantial agreement (κ-values between 0.4 and 0.65–0.8). The prevalence of ANCAs varied between 56% and 70%. In the intermethod study, ANCAs were assayed by 1 experienced reader using substrates from 4 different commercial sources (The Binding Site, Bio-Rad, INOVA, and Immunocorecepts). The prevalence of ANCAs varied between 18% and 68%, with κ-values <0.2, indicating poor agreement. Similar results were obtained by Sandborn et al. (35). The sensitivity for ANCA detection in 150 UC patients varied between 0% and 63% in 5 different laboratories (Prometheus, Oxford, Wuerzburg, Mayo, and Smith Kline Beecham).

The perinuclear nature of some ANCA reactions (e.g., antibodies to myeloperoxidase or elastase) is an artifact of the alcohol fixation of neutrophils, which causes positively charged cytoplasmic granular proteins to redistribute around the negatively charged nucleus. When neutrophils are fixed with non-alcohol-based reagents (e.g., paraformaldehyde or formalin), the perinuclear reaction obtained with either myeloperoxidase–P-ANCA or elastase–P-ANCA is abolished and converted to a cytoplasmic reaction pattern.

Published data about the reactivity of atypical P-ANCAs on formaldehyde-fixed neutrophils in IBD and autoimmune liver disorders are rare and controversial. Some investigators did not detect any fluorescence on formaldehyde-fixed neutrophils in most patients with IBD (34), whereas others reported a cytoplasmic (19, 30) or an atypical P-ANCA (33) formalin-positive reactivity in a high portion of the sera. Variations in the formaldehyde fixation techniques and the resolution of the immunofluorescence microscope used might be responsible for the controversial reports about the microscopic features of atypical P-ANCAs on formaldehyde-fixed neutrophils. Even the use of the same technique in the same laboratory may lead to inconsistent results.

The serum dilution for the determination of P-ANCA
was 1:40 or 1:20 for most of the studies. Some studies were done with the Prometheus UC Diagnostic system. This is a 3-step process that includes ELISA analysis, an indirect immunofluorescence assay, and DNase treatment. Samples were considered positive if a certain cutoff value on ELISA analysis was exceeded.

**Anti-Saccharomyces cerevisiae Antibodies**

Increased concentrations of antibodies to the baker’s and brewer’s yeast *Saccharomyces cerevisiae* (ASCAs) are found in patients with CD (36). Both IgG and IgA antibodies are formed. They have been demonstrated in 60%–70% of patients with CD, 10%–15% of patients with UC, and 0%–5% of control individuals (Table 4). The antibodies recognize carbohydrate epitopes in phosphopeptidomanan, a 200-kDa glycoprotein of the cell wall. The major epitope was identified as mannotetraose (38). The highest sensitivity and specificity of the serologic response was found with strain Sul1 (38).

A comparative study revealed a wide range in sensitivities and specificities among 4 assays (3 commercially available assays and the assay developed in Lille, Centre Hospitalier Régional Universitaire, France), mainly as a consequence of the cutoff values chosen (39). The agreement among the assays was good (39).

In contrast to P-ANCAs, ASCAs do not seem to be autoantibodies but rather antibodies against bacterial or fungal species. The presence of ASCAs in patients with IBD may be the result of a response to either the antigens on *S. cerevisiae* itself or to an as yet unidentified antigen that cross-reacts with *S. cerevisiae* antigens.

**Diagnostic Value of Autoantibodies to Neutrophils**

**Table 4. Prevalence of ASCA and P-ANCA in CD, UC, diseased controls, and healthy controls.**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Study</th>
<th>Positive for antibodies, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD</td>
<td>UC</td>
</tr>
<tr>
<td>ASCAs</td>
<td>147/407/74/157</td>
<td>60</td>
</tr>
<tr>
<td>Koutrobakis et al. (42)</td>
<td>97/56/0/150</td>
<td>39</td>
</tr>
<tr>
<td>Quinton et al. (41)</td>
<td>101/100/27/163</td>
<td>61</td>
</tr>
<tr>
<td>Ruemmele et al. (43)</td>
<td>36/131/78/0</td>
<td>55</td>
</tr>
<tr>
<td>Hoffenberg et al. (44)</td>
<td>25/20/74/0</td>
<td>60</td>
</tr>
<tr>
<td>Peeters et al. (40)</td>
<td>147/407/74/157</td>
<td>6</td>
</tr>
<tr>
<td>Koutrobakis et al. (42)</td>
<td>97/56/0/150</td>
<td>16</td>
</tr>
<tr>
<td>Quinton et al. (41)</td>
<td>101/100/27/163</td>
<td>15</td>
</tr>
<tr>
<td>Ruemmele et al. (43)</td>
<td>36/131/78/0</td>
<td>13</td>
</tr>
<tr>
<td>Hoffenberg et al. (44)</td>
<td>25/20/74/0</td>
<td>60</td>
</tr>
</tbody>
</table>

* UC/CD/diseased controls/healthy controls.

**P-ANCAs**

The prevalences of P-ANCAs and ASCAs in UC, CD, diseased controls, and healthy controls (35, 40–44) are summarized in Table 4. Atypical P-ANCAs are (50%–67%) found mainly in UC, but also in CD (6%–15%) and to a lesser extent in diseased controls (<11%). Atypical P-ANCAs are also found in autoimmune hepatitis and primary sclerosing cholangitis. The prevalence of ASCAs is higher in CD (40%–60%), but they are also found in UC and in diseased controls (4%–14%). In healthy controls, the antibodies are present in <5%. The clinical value of ANCA or ASCA testing in diarrheal diseases is limited because of insufficient sensitivity (45) and imperfect specificity.

The combination of atypical P-ANCAs and ASCAs may be useful in the differential diagnosis of UC and CD in patients with IBD. A survey of studies (35, 40–42, 46) that combined the 2 markers to distinguish UC from CD is given in Table 5. The CD-associated pattern was ASCA+/P-ANCA−, whereas the UC-associated pattern was ASCA−/P-ANCA+. The combined evaluation of ANCAs and ASCAs had a higher specificity (>90% in most studies and >80% in all studies) to differentiate CD from UC than the separate use of either ANCAs or ASCAs. The increased specificity, however, was associated with decreased sensitivity.

In addition to sensitivity and specificity, positive predictive values are provided in some studies. For example, in patients with IBD, the positive predictive value of the combination of a positive ASCA test with a negative P-ANCA test for UC has been reported to be 92.5% by Quinton et al. (41), 88% by Peeters et al. (40), and 93% by Koutrobakis et al. (42). The positive predictive values of the combination of a negative ASCA test with a positive P-ANCA test for UC has been reported to be 96% by Quinton et al. (41), 95% by Peeters et al. (40), and 77% by Koutrobakis et al. (42). The positive predictive value places test specificity in the context of disease prevalence and indicates what percentage of patients with positive test results actually have the disease. In these studies, however, the authors artificially controlled the prevalence of the disease by focused unnatural recruiting of patients and healthy and diseased controls. The prevalence of disease in a clinical situation is different from the prevalence in most research studies.

The likelihood ratio is less likely to change with the prevalence of the disorder and incorporates both the sensitivity and specificity of the test. It provides a direct estimate of how much a test result will change the odds of...
Table 5. Ability of ANCAs and ASCAs to differentiate UC from CD in patients with IBD.

<table>
<thead>
<tr>
<th>Study</th>
<th>UC, n</th>
<th>CD, n</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinton et al. (41)</td>
<td>101</td>
<td>100</td>
<td>88 (CD)</td>
<td>97 (UC)</td>
<td>5</td>
<td>0.44</td>
</tr>
<tr>
<td>Linskens et al. (46)</td>
<td>51</td>
<td>50</td>
<td>82 (CD)</td>
<td>94 (UC)</td>
<td>4</td>
<td>0.41</td>
</tr>
<tr>
<td>Koutoubakis et al. (42)</td>
<td>97</td>
<td>56</td>
<td>89 (CD)</td>
<td>58 (UC)</td>
<td>3.5</td>
<td>0.39</td>
</tr>
<tr>
<td>Sandborn et al. (35)</td>
<td>83</td>
<td>79</td>
<td>87 (CD)</td>
<td>84 (UC)</td>
<td>3.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Peeters et al. (40)</td>
<td>147</td>
<td>407</td>
<td>86 (CD)</td>
<td>94 (UC)</td>
<td>4.2</td>
<td>0.46</td>
</tr>
<tr>
<td>ASCA⁺ / P-ANCA⁻ (CD)</td>
<td>61</td>
<td>65</td>
<td>69 (CD)</td>
<td>67 (UC)</td>
<td>4.3</td>
<td>0.43</td>
</tr>
<tr>
<td>ASCA⁻ / ASCA⁻ (CD)</td>
<td>97</td>
<td>97</td>
<td>97 (CD)</td>
<td>97 (UC)</td>
<td>16</td>
<td>0.41</td>
</tr>
<tr>
<td>ASCA⁺ / P-ANCA⁺ (CD)</td>
<td>49</td>
<td>49</td>
<td>58 (CD)</td>
<td>58 (UC)</td>
<td>19</td>
<td>0.43</td>
</tr>
<tr>
<td>ASCA⁺ / P-ANCA⁻ (UC)</td>
<td>57</td>
<td>57</td>
<td>79 (CD)</td>
<td>55 (UC)</td>
<td>19</td>
<td>0.43</td>
</tr>
<tr>
<td>ASCA⁻ / ASCA⁻ (UC)</td>
<td>97</td>
<td>97</td>
<td>97 (CD)</td>
<td>97 (UC)</td>
<td>19</td>
<td>0.43</td>
</tr>
</tbody>
</table>

having a disease (47, 48). The likelihood ratio for a positive result tells how much the odds of the disease increase when a test is positive; conversely, the likelihood ratio for a negative result tells how much the odds of the disease decrease when a test is negative. The likelihood ratio can be combined with information about the prevalence of the disease and the characteristics of the patient group and the patient to determine the posttest odds of disease in a particular patient. Thus, the likelihood ratios allow the clinician to estimate whether there will be a significant change in the pretest to posttest probability of a disease as a result of obtaining the test (47, 48). A likelihood ratio of 1 implies that the test is of no clinical value. Likelihood ratios >10 or <0.1 indicate very large, clinically important differences in pretest–posttest probability. Likelihood ratios between 5 and 10 or between 0.1 to 0.2 often lead to more modest, but still substantial differences, in pretest–posttest probability. Ratios from 2 to 5 or from 0.5 to 0.2 generate small differences that may be relevant in certain clinical settings. Likelihood ratios between 1 and 2 or 0.5 and 1 generate very small differences that are seldom clinically important (47, 48).

I have calculated the likelihood ratios of P-ANCA and ASCA results (a) to distinguish IBD from non-IBD and (b) to distinguish CD from UC in IBD patients. The likelihood ratios to discriminate IBD from non-IBD were calculated based on data obtained from the study of Peeters et al. (40). The likelihood ratio of a positive result for ASCAs, P-ANCAs, ASCA⁺ / P-ANCA⁻, and ASCA⁻ / P-ANCA⁺ to distinguish patients with IBD (n = 554) from controls (n = 231) was, respectively, 6.6, 10, 9.3, and 14.6, and the likelihood ratio of a negative result was 0.43, 0.52, 0.46, and 0.57, respectively. This indicates that in the context of distinguishing IBD patients from non-IBD individuals, a positive test result for ASCAs, P-ANCAs, ASCA⁺ / P-ANCA⁻, or ASCA⁻ / P-ANCA⁺ substantially affects pretest–posttest probability, whereas a negative test result insignificantly affects pretest–posttest probability. The likelihood ratios of a positive result for atypical P-ANCAs and for ASCAs to differentiate between UC and CD in patients with IBD are shown in Table 5 and was between 2 and 5 in all studies, indicating that these assays affect pretest–posttest probability to a small degree. The likelihood ratio of a negative test result for atypical P-ANCAs and ASCAs was between 0.3 and 0.7 in all conditions, signifying poor clinical importance of a negative test result for differentiating CD from UC. By contrast, the combined evaluation of atypical P-ANCAs and ASCAs had a positive likelihood ratio > 5 in almost all studies and >10 in one-half of the studies (see Table 5). This implies that the combined use of atypical P-ANCAs and ASCAs substantially affects pretest–posttest probability in distinguishing UC from CD in patients with IBD.

Thus, the combined use of ASCA and P-ANCA results could be an addition to conventional techniques (the patient’s history, radiologic examination, endoscopy, and biopsy) in the differential diagnosis between CD and UC. It should be mentioned, however, that a high percentage of CD patients with pure colonic disease and UC-like colitis have been reported positive for P-ANCAs (49), thereby constraining the sensitivity of the ASCA⁺ / P-ANCA⁻ combination as a specific marker for CD.
**Uses of Test Results for Autoantibodies to Neutrophils (Atypical P-ANCAs) and ASCAs**

**Indeterminate Colitis**
Serologic evaluation of ANCA and ASC could be of help in patients with indeterminate colitis. In these patients, early knowledge of the exact diagnosis could be of clinical importance with regard to therapeutic decisions and prognosis. In a multicenter prospective study, 97 patients with indeterminate colitis were analyzed for ANCAs and ASCAs (50). After a 1-year follow-up, a definite diagnosis was reached in 31 of the 97 patients. The combination ASCA⁺/ANCA⁻ predicted CD in 80% of IC patients (sensitivity, 67%; specificity, 78%; positive likelihood ratio, 3), whereas ASCA⁻/ANCA⁺ was predictive for UC in 64% of the patients (sensitivity, 78%; specificity, 67%; positive likelihood ratio, 2.3). ASCA⁻/ANCA⁺ patients who did not progress to UC all developed UC-like CD. A remarkable finding in this study was that 48.5% of the patients did not have antibodies to either ASCAs or ANCs and that these seronegative patients remained indeterminate after a mean duration of 9.9 years (50).

**As Tools for Disease Monitoring**
There is no relationship between the presence or titer of ANCAs and UC activity (49, 51). The ANCA titer remains positive after colectomy (51). In addition, the presence of ASCAs is stable over time and is independent of CD activity and duration (49, 52). ASCA titers most often remain stable after treatment (52). Hence, serial measurement of ANCA and ASCA titers in IBD is not useful for follow-up of disease activity and prediction of relapses.

The authors of one study have suggested that the presence of ANCAs may correlate with chronic pouchitis after ileal pouch–anal anastomosis (52), but this has not been confirmed in other studies (51, 54, 55).

**Association with Clinical Phenotypes**
Atypical P-ANCAs are not associated with clinical characteristics such as age of onset, need for surgery, location of the disease, or familial history (40). It has been suggested that in patients with CD, the presence of atypical P-ANCAs in serum characterizes a UC-like clinical phenotype (49). Patients with “UC-like CD” have 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. In the study by Vasiliauskas et al. (49), the number of patients meeting the criteria of UC-like phenotype was 18 of 18 (100%) in the P-ANCA-positive CD subgroup, 9 of 20 (45%) of the C-ANCA subgroup, and 12 of 31 (39%) in the ANCA-negative CD subgroup.

On the other hand, ASCAs have been associated with several CD clinical phenotypes. ASCAs have been associated ileal disease (small-bowel rather than colonic disease) (40, 41, 52, 57–59) and strictureing as well as penetrating (56–59) disease behavior. For example, Quinton et al. (41) reported that 70% of CD patients with small bowel involvement (with or without colonic disease) had ASCA antibodies vs 46% of the patients with pure colonic disease, and Walker et al. (57) reported that 68% of patients with CD involving the ileum had ASCAs vs 38% of patients with colonic disease. Mow et al. (59) reported that 71.7% of CD patients with fibrostenosing disease had ASCAs vs 36.1% of patients without fibrostenosing disease and that 50.9% of patients with internal perforating disease had ASCAs vs 27.1% of patients without internal perforating disease. ASCAs have been associated with young age at diagnosis in several studies (40, 41, 52, 58) but not in others (57).

**Response to Medical Therapy**
ANCA positivity has been reported to be associated with resistance to treatment of left-sided UC (60). Sanborn et al. (60) reported that 90% of patients with refractory left-sided colitis were ANCA-positive vs 62% of patients with treatment-responsive UC (P = 0.03). ASCAs and P-ANCAs were not associated with response to anti-tumor necrosis factor-α therapy (61). A trend toward poor response to anti-tumor necrosis factor-α therapy in CD patients carrying the ANCA⁺/ASC⁻ combination has been suggested (61). This was, however, not statistically significant and needs to be confirmed.

**As Subclinical Markers**
The strongest risk factor for IBD is having a relative with the disease. The occurrence of antibodies in unaffected family members could reflect a genetic and/or environmental factor predisposing to disease. In addition, the presence of antibodies could also be indicative of presymptomatic disease. Several groups have studied whether P-ANCAs and ASCAs are subclinical markers of IBD in families.

Some studies showed that 16%–30% of healthy first-degree relatives of UC patients were P-ANCA-positive (62, 63), whereas other studies were unable to find P-ANCAs in first-degree relatives (64–66).

Several studies have found an increased prevalence of ASCAs in unaffected first-degree relatives of patients with CD. Sendid et al. (67) detected ASCAs in 35 of 51 (69%) patients with CD and in 13 of 66 (20%) healthy relatives vs 1 of 163 healthy controls. In that study, the health status of the healthy relatives was assessed by an interviewer practitioner who visited the families. None of the healthy relatives had a previous history or symptoms of IBD. Seibold et al. (68) found ASCAs in 25% of 193 healthy first-degree relatives. Sutton et al. (69) reported familial aggregation of ASCA concentrations for affected relatives and even stronger for unaffected relatives. Vermiere et al. (52) found that ASCA prevalence was the same in both sporadic and familial CD. Within pure CD families, ASCAs were present in 54% of CD patients with 2 family members affected vs 74.7% in CD patients with 3 or more family members affected (52). There was no
concordance of ASCA reactivity in marital pairs. These data support the suggestion that ASCAs reflect the familial load of the disease. Whether the presence of ASCAs is a familial trait attributable to a genetic factor or to an increased childhood environmental exposure that predisposes to the disease is unknown. A recent study in twins suggested that ASCAs are a marker of a response to an environmental antigen and that genes other than CARD15 determine the extent of the response (58).

Israeli et al. (70) demonstrated that ASCAs and p-ANCAs may predict development of IBD years before the disease is clinically diagnosed. ASCAs were present in 10 of 32 (31.3%) CD patients before clinical diagnosis compared with 0 of 95 (0%) controls ($P < 0.001$). ASCA test results were positive in 54.5% of patients after diagnosis of CD. The mean interval between ASCA detection and diagnosis was 38 months. p-ANCAs were present in 2 of 8 (25%) patients with available sera before the diagnosis of UC. None of the 24 matched controls were positive ($P = 0.014$).

## Pancreatic Antibodies

Antibodies against exocrine pancreas have been described in patients with CD, and have been reported to be specific (not present in nondiseased individuals), albeit at a low prevalence (~30%) (71–73). The antigen has not been elucidated, and the antibodies are detected by indirect immunofluorescence. Joossens et al. (74) found pancreatic antibodies not only in CD patients but also, in low titers, in UC patients. The antibodies were also found in first-degree relatives of IBD patients, a finding that had not been observed in earlier studies (65, 75).

## New Serologic Markers for IBD

Screening lysates of cultures of colonic bacteria with a monoclonal P-ANCA antibody revealed that *Escherichia coli* outer membrane porin (OmpC) is an antigen in IBD (76). Landers et al. (77) reported anti-OmpC antibodies in 55% of CD patients. Patients with anti-OmpC antibodies were more likely to have internal perforating CD (59). In children and young adults, anti-OmpC antibodies (IgG and IgA) were found in 24% of CD patients, 11% of UC patients, and 5% of controls (78).

Microbial sequence I2 was identified as a homolog of the tetR bacterial transcription factor family, which is frequently present in CD colonic lesions (43%) but not in other colonic specimens (79). This sequence has been shown to be associated with *Pseudomonas fluorescens* (80). IgA anti-I2 antibodies were found in 54% of CD patients, 10% of UC patients, 19% of patients with other inflammatory enteric diseases (infectious colitis, radiation-associated proctitis, *Shigella* colitis, eosinophilic colitis, and collagenous colitis), and 4% of healthy controls (79). Patients with anti-I2 antibodies were more likely to have fibrostenosing disease and require surgery (59).

The flagellin CBir1 has been identified as a dominant antigen capable of inducing colitis in C3H/HeJ Bir mice and eliciting IgG antibody responses in a subpopulation (~50%) of patients with CD (81, 82). Anti-CBir1 expression is independent of other antibody responses, is associated with small-bowel, internal-penetrating, and fibrostenosing disease features, and defines a subgroup of CD patients not previously recognized by other serologic responses (82). Among the population of CD patients positive for P-ANCAs but who do not react to other known antigens, 40%–44% are positive for anti-CBir1 antibodies, whereas anti-CBir1 antibodies have been found in only 4% of P-ANCA+ UC patients (82).

There is evidence that the number and magnitude of immune responses to different microbial antigens (ASCA, OmpC, and I2) in a given patient are associated with the severity of the disease course; i.e., the greater the number of responses and the greater their magnitude, the more severe the disease course (fibrostenosis, internal perforating disease, and the need for small-bowel surgery) (59, 82). Patients positive for I2, OmpC, and ASCA were more likely to undergo small-bowel surgery (72%) than were patients without reactivity (23%) (59). These findings must be confirmed in future studies evaluating the association between the presence of antibodies to microbial antigens and the development of strictures and perforations and subsequent need for surgery. If such studies can confirm that antibodies can identify patients likely to undergo a severe and problematic disease course, then the determination of antibodies could be helpful in clinical practice.

## Conclusions

Atypical P-ANCAs and ASCAs are markers for UC and CD, respectively. Their role as diagnostic serologic markers for IBD appears to be limited, however, mainly because of their lower sensitivity. A positive test result for either P-ANCAs or ASCAs modestly influences pretest–posttest probability in distinguishing IBD from non-IBD, and a negative test result has no clinical value. The combined use of atypical P-ANCA and ASCA test results substantially affects pretest–posttest probability in distinguishing UC from CD in patients with IBD. The P-ANCA+/ASCAN− combination is specific for UC, whereas the ASCAN+/P-ANCA− combination is specific for CD. This may be of help in patients in whom distinction between CD or UC is not obvious with the classic diagnostic tools (patient history, radiologic examination, endoscopy, and biopsy). The discriminative value of ASCAs and P-ANCAs to predict definitive diagnosis (CD or UC) in patients with indeterminate colitis is modest. Almost 50% of these patients do not develop ASCA or P-ANCA antibodies. Future studies should unravel whether this seronegative subgroup of patients represents a separate clinical entity.

Serial measurement of P-ANCAs and ASCAs is not useful. Titers of both antibodies are stable over time and do not correlate with disease activity. ASCA positivity is
correlated with ileal involvement of CD disease and stricturing as well as with penetrating disease behavior. ASCAs are detectable in 20%–25% of first-degree relatives of patients with CD, but whether these antibodies are markers of future disease has yet to be determined.

The assays that detect atypical P-ANCA and ASCA lack standardization, which leads to large interlaboratory variation. Efforts should be undertaken to harmonize these assays, and future research should aim to identify the main autoantigens targeted by atypical P-ANCA.

Pancreatic antibodies are specific markers for IBD. Their sensitivity, however, is limited (30%). New microbial target antigens (OmpC, I2, and the flagellin CBir1) have been described in patients with CD. There is evidence that the number and magnitude of immune responses to different microbial antigens are associated with the severity of the disease course. This should be confirmed by additional studies.

Upcoming studies should further explore the potential to cluster patients in more homogeneous subgroups based on antibody responses. Correlating serologic markers with genotypes and clinical phenotypes should enhance our understanding of the pathophysiology of IBD. Hopefully this will lead to the introduction of new and accurate tools for diagnosis, stratification, and follow-up of patients with IBD.

References


59. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treat-


