The Role of Serology in Differential Diagnosis of IBD

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March 24-25, 2006
Diagnosis of IBD

- Compatible clinical manifestation
- Radiologic findings
- Endoscopic findings
- Histologic features
- Exclusion of intestinal infections is essential
- Long term follow-up: prolonged intestinal inflammation in the absence of GI infection
- Response to treatment and/or relapse after withdrawal of treatment
Diagnosis of UC
(Asia Pacific Working Party Concordence)

**Definite**

1. History of diarrhea or rectal bleeding or both for several weeks or more; with
2. either a record of at least one sigmoidoscopy or colonoscopy revealing one or more of the following:
   - friability of the mucosa with contact bleeding, petechial hemorrhages, ulceration with diffuse inflammation of the mucosa;
   - a barium enema with radiological evidence of ulceration or narrowing or shortening of the colon; or
   - characteristic macroscopic and microscopic changes in a specimen obtained by surgical resection, biopsy, or at autopsy.

APDW 2004
Diagnosis of UC
(Asia Pacific Working Party Concensus)

Probable
(1) An acceptable sigmoidoscopy, colonoscopy, or barium enema report but inadequate history;
(2) an acceptable history but sigmoidoscopic or colonoscopic appearances dubious (eg, mucosal erythema only or any of the changes described above but qualified as doubtful) and no barium enema;
(3) an adequate history but barium enema dubious (eg, loss of haustrations only) and no sigmoidoscopy or colonoscopy report
(4) characteristic macroscopic appearance of operative or postmortem specimen but histology indefinite.

Possible A medical record with a discharge diagnosis of ulcerative colitis, no findings inconsistent with the diagnosis, and an acceptable history.

APDW 2004
Diagnosis of CD
(Asia-Pacific Working Party Concensus)

**Definite** Characteristic positive pathological and histological reports from an operative or autopsy specimen indicating segmental, transmural lesions of the gut, fissuring ulcers, non-caseating granuloma and lymphoid aggregates in the lamina propria and submucosa of the bowel, no caseation detected either in the resected specimen or within the mesenteric lymph nodes.

**Probable** A laparotomy report of characteristic naked-eye appearance of the bowel but no specimen of gut resected for histology; (2) an equivocal histological report from an operative specimen with characteristic macroscopic features; (3) a colonoscopic report compatible with Crohn’s disease and biopsy with features strongly suggestive of Crohn’s disease, or (4) a radiologic examination strongly suggestive of intestinal or chronic inflammatory disease with obstructive or fistulous features.

**Possible** A medical record with a discharge diagnosis of Crohn’s disease, regional enteritis, or granulomatous colitis; no findings, clinical or radiological, inconsistent with the diagnosis; and an acceptable history.

APDW 2004
Difficult Situations in Making Diagnosis of IBD

- Infectious colitis (high prevalence area)
- Intermediate colitis
- Pediatric IBD
- Older IBD patients
- Autoimmune colitis e.g. Behcet’s disease, SLE, Wegener’s granulomatosis, Henoch Schoenlein purpura, intestinal vasculitis
Diagnosing Intermediate Colitis is clinically important because patients with IC are more likely to have pouch failure than in definite UC.

Sathaporn; 11.03.2006
Potential Role of Serologic Testings in IBD

- **Diagnosis**
  - For helping/confirming diagnosis of IBD
  - Screening for IBD patients
  - Identification of genetic susceptibility

- **Management**
  - Assessment of disease activities/severity
  - As a marker of disease course/prognosis
  - To predict outcome of therapy
Acute Phase Reactants of IBD

- ESR
- C-reactive protein
- Leukocyte and platelets counts (Thrombopoietin)
- α1 glycoprotein
- Fecal calprotectin
- Fecal lactoferrin
Serologic Markers of IBD

- pANCA (perinuclear antinutrophil cytoplasmic Ab)
- ASCA (anti-*Saccharomyces cerevisiae* Ab)
- Anti-OmpC (outer membrane porin C of *E. coli*)
- Anti-CBir1 (*Salmonella muenchen* flagellin)
- Anti-I$_2$ (*Pseudomonas fluorescens* DNA fragment)
- HupB (mycobacterial histone H1 protein)
- Anaerobic coccoid agglutinin
- Pancreatic antibody
- Anti-goblet cell
- Anti-colon
ANCA (Antinutrophile cytoplasmic antibody)

- **cANCA** = targeting at proteinases for diagnosis of Wegener’s granulomatosis
- **pANCA** = targeting at myeloperoxidase (on the outer nuclear membrane) for diagnosis of small vessel vasculitis
- **pANCA** = targeting at histones H1 (on the inner nuclear membrane) for diagnosis of ulcerative colitis (present in 50-80% of UC, 10-30% of CD)

Test by ELISA method
Confirm by immunofluorescence method
Disappear with DNAase enzyme that digest the nuclear membrane
S16  histones = a small protein intimately involved coiling or packing DNA within the nucleus of cells, 50kDa protein

cathepsin G, lysozymes, elastase, lactoferrin
Sathaporn 15.03.2006
Atypical P-ANCA: on formalin

Ethanol Fixation: p-ANCA → Formaldehyde Fixation: c-ANCA

(a) Ethanol Fixation: atypical p-ANCA → Formaldehyde Fixation: atypical p-ANCA

(c) Ethanol Fixation: p-ANCA

(d) Formaldehyde Fixation: c-ANCA

- Cytoplasm
- Cytoplasmic Proteins
- Endoplasmic Reticulum
- Perinuclear Cytoplasm
- Inner nuclear membrane
- Nucleus
- Nuclear Envelope Proteins
Ethanol

Formalin

P-ANCA

C-ANCA

Granulocytes EOH / HCHO: anti-myeloperoxidase (pANCA)

Granulocytes EOH / HCHO: anti-proteinase 3 (cANCA)
Differential diagnosis of IBD from Non IBD using various markers

<table>
<thead>
<tr>
<th></th>
<th>UC</th>
<th>CD</th>
<th>Non IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pANCA</td>
<td>50-70%</td>
<td>10-25%</td>
<td>2-3%</td>
</tr>
<tr>
<td>Anti-Goblet</td>
<td>39%</td>
<td>30%</td>
<td>2%</td>
</tr>
<tr>
<td>Anti-colon</td>
<td>36%</td>
<td>13%</td>
<td>13%</td>
</tr>
<tr>
<td>ASCA</td>
<td>10-15%</td>
<td>55-70%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>2%</td>
<td>38-50%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Anti-I2</td>
<td>2%</td>
<td>54%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Pancreatic Ab</td>
<td>4%</td>
<td>30-40%</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>

Modified from Harrison’s 15th ed. and Simon W 2004
### Sensitivity, specificity and PPV of each seromarkers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Disease</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pANCA</td>
<td>UC</td>
<td>57-83%</td>
<td>65-97%</td>
<td>69-88%</td>
</tr>
<tr>
<td>ASCA</td>
<td>CD</td>
<td>46-72%</td>
<td>87-95%</td>
<td>69-89%</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>CD</td>
<td>25%</td>
<td>92%</td>
<td>67%</td>
</tr>
<tr>
<td>Anti-I2</td>
<td>CD</td>
<td>54%</td>
<td>84%</td>
<td>-</td>
</tr>
<tr>
<td>Pancreatic Ab</td>
<td>CD</td>
<td>31-39%</td>
<td>89-100%</td>
<td>-</td>
</tr>
<tr>
<td>Anti-Goblet</td>
<td>UC</td>
<td>28-39%</td>
<td>57%</td>
<td>-</td>
</tr>
<tr>
<td>Anti-colon</td>
<td>UC</td>
<td>36%</td>
<td>73%</td>
<td>-</td>
</tr>
<tr>
<td>Anaerobic coccoid agglutinin</td>
<td>CD</td>
<td>52-58%</td>
<td>90%</td>
<td>-</td>
</tr>
</tbody>
</table>
Prevalence of p-ANCA in Ulcerative Colitis in Asia

- Western: 65.0
- Japanese: 63.0
- Chinese: 35
- Indian: 31.0
- Thai: 30.4

Suki K, 1999
Kaneko K, 1995
Sung JY, 1994
Habeeb MA, 1997
Osangthamnont C, 2001
Results: pANCA in UC

Chinese  Caucasian

Percent

Sens
Spec
PPV

P = 0.046

Lawrance, Leong et al. Am J Gastroenterol 2004
Serology: ASCA in CD

Lawrance, Leong et al. *Am J Gastroenterol* 2004
### Impact of ANCA/ASCA on diagnosis stratified by indication for testing

<table>
<thead>
<tr>
<th>Indication</th>
<th>Significant Role in Diagnosis</th>
<th>Supportive Role in Diagnosis</th>
<th>Not Helpful</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 76)</td>
<td>28% (n = 21)</td>
<td>26% (n = 20)</td>
<td>46% (n = 35)</td>
</tr>
<tr>
<td>Subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeterminate colitis CD versus UC (n = 10)</td>
<td>20% (2)</td>
<td>40% (4)</td>
<td>40% (4)</td>
</tr>
<tr>
<td>Atypical inflammation (n = 38)</td>
<td>32% (12)</td>
<td>29% (11)</td>
<td>39% (15)</td>
</tr>
<tr>
<td>Chronic diarrhea (n = 17)</td>
<td>24% (4)</td>
<td>12% (2)</td>
<td>64% (11)</td>
</tr>
<tr>
<td>Family history (n = 3)</td>
<td>0% (0)</td>
<td>33% (1)</td>
<td>67% (2)</td>
</tr>
<tr>
<td>Pouchitis versus CD (n = 3)</td>
<td>67% (2)</td>
<td>0% (0)</td>
<td>33% (1)</td>
</tr>
<tr>
<td>Other (n = 5)</td>
<td>20% (1)</td>
<td>40% (2)</td>
<td>40% (2)</td>
</tr>
</tbody>
</table>
Clinical Indications of the Use of Antineutrophil Cytoplasmic Antibodies and Anti-Saccharomyces cerevisiae Antibodies in the Evaluation of Inflammatory Bowel Disease at an Academic Medical Center


Sathaporn: 09.10.2005
## Diagnostic usefulness of the combination of pANCA+/ASCA- for UC

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prometheus 2001</td>
<td>55%</td>
<td>94%</td>
<td>86%</td>
</tr>
<tr>
<td>Oxford 2001</td>
<td>34%</td>
<td>92%</td>
<td>82%</td>
</tr>
<tr>
<td>Wuerzburg 2001</td>
<td>27%</td>
<td>92%</td>
<td>82%</td>
</tr>
<tr>
<td>Mayo 2001</td>
<td>42%</td>
<td>93%</td>
<td>84%</td>
</tr>
<tr>
<td>Stephen B 2002</td>
<td>55%</td>
<td>81%</td>
<td>75%</td>
</tr>
<tr>
<td>Linskin RK 2002</td>
<td>51%</td>
<td>94%</td>
<td>90%</td>
</tr>
</tbody>
</table>

Source: Sandborn WJ et al. 2001
### Diagnostic usefulness of the combination of ASCA+/pANCA- for CD

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prometheus</td>
<td>38%</td>
<td>94%</td>
<td>86%</td>
</tr>
<tr>
<td>Oxford</td>
<td>39%</td>
<td>92%</td>
<td>82%</td>
</tr>
<tr>
<td>Wuerzburg</td>
<td>41%</td>
<td>92%</td>
<td>82%</td>
</tr>
<tr>
<td>Mayo</td>
<td>41%</td>
<td>93%</td>
<td>84%</td>
</tr>
<tr>
<td>SKB</td>
<td>44%</td>
<td>87%</td>
<td>76%</td>
</tr>
<tr>
<td>Stephen B</td>
<td>39%</td>
<td>94%</td>
<td>86%</td>
</tr>
</tbody>
</table>

*Sandborn WJ et al. 2001*
Differentiating UC from CD in Intermediate Colitis

<table>
<thead>
<tr>
<th></th>
<th>PPV</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>pANCA+/ASCA-</td>
<td>88-92.5%</td>
</tr>
<tr>
<td>CD</td>
<td>ASCA+/pANCA-</td>
<td>95-96%</td>
</tr>
</tbody>
</table>

40% remain indeterminate

Peeters M et al. 2001
Quinton JF et al 1998
Differentiating UC from CD in Intermediate Colitis

<table>
<thead>
<tr>
<th></th>
<th>Standard markers</th>
<th>PPV</th>
<th>New markers</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>ASCA+/pANCA-</td>
<td>67%</td>
<td>ASCA+/OmpC+/pANCA-</td>
<td>75%</td>
</tr>
<tr>
<td>UC</td>
<td>pANCA+/ASCA-</td>
<td>70%</td>
<td>pANCA+/ASCA-/OmpC-/I2-</td>
<td>83%</td>
</tr>
</tbody>
</table>

72% remain indeterminate

Joossens S et al. 2003
Patients tested for panel IBD seromarkers (pANCA, ASCA IgA&IgG, Anti-OmpC IgA)

<table>
<thead>
<tr>
<th></th>
<th>No of patients</th>
<th>Percent detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD patients</td>
<td>275</td>
<td>76.1</td>
</tr>
<tr>
<td>CD patients</td>
<td>175</td>
<td>76.1</td>
</tr>
<tr>
<td>UC patients</td>
<td>100</td>
<td>72.0</td>
</tr>
<tr>
<td>Normal or other disease controls</td>
<td>127</td>
<td>6.3</td>
</tr>
</tbody>
</table>

“Negative test results does not rule out the possibility of IBD”

Nakamura RM 2003
All tested by Prometheus
### Diagnostic Accuracy of Serologic Markers in Children

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CD</th>
<th>UC</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>44</td>
<td>41</td>
<td>7</td>
</tr>
<tr>
<td>ASCA+</td>
<td>58%</td>
<td>7%</td>
<td>14%</td>
</tr>
<tr>
<td>pANCA+</td>
<td>16%</td>
<td>73%</td>
<td>0%</td>
</tr>
<tr>
<td>Anti-OmpC+</td>
<td>11%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>ASCA-,pANCA-</td>
<td>27%</td>
<td>17%</td>
<td>85%</td>
</tr>
<tr>
<td>ASCA-,pANCA+</td>
<td>13%</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>ASCA+,pANCA-</td>
<td>54%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>ASCA+,pANCA+</td>
<td>5%</td>
<td>3%</td>
<td></td>
</tr>
</tbody>
</table>

ANCA and ASCA antibodies were both available in 29 of 41 UC patients and in 37 of 44 CD patients.

Elitsur Y et al, J Clin Gastroenterol 2005
Value of ANCA/ASCA Testing in a Subset of Middle-aged and Elderly Patients with Sigmoid Inflammation

<table>
<thead>
<tr>
<th>Age</th>
<th>Pretest Diagnosis</th>
<th>ANCA/ASCA</th>
<th>Effect on Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>IBD vs. ischemia</td>
<td>++</td>
<td>IBD</td>
</tr>
<tr>
<td>49</td>
<td>Segmental colitis and PSC</td>
<td>--</td>
<td>None</td>
</tr>
<tr>
<td>57</td>
<td>IBD vs. ischemia</td>
<td>++</td>
<td>CD</td>
</tr>
<tr>
<td>79</td>
<td>IBD vs. ischemia vs. diverticular</td>
<td>+/−</td>
<td>UC</td>
</tr>
<tr>
<td>54</td>
<td>IBD vs. diverticular</td>
<td>++</td>
<td>IBD</td>
</tr>
<tr>
<td>70</td>
<td>IBD vs. ischemia vs. diverticular</td>
<td>+/−</td>
<td>UC</td>
</tr>
<tr>
<td>69</td>
<td>IBD vs. diverticular vs. radiation</td>
<td>−/+</td>
<td>CD</td>
</tr>
<tr>
<td>67</td>
<td>IBD vs. diverticular</td>
<td>−/+</td>
<td>CD</td>
</tr>
</tbody>
</table>
Clinical features of pANCA+ UC (50-80% of UC)

- Left-sided colitis
- More resistant to treatment
- More aggressive disease
- Requiring surgery early in the course
- Developing pouchitis after ileal pouch-anal anastomosis
- Having specific HLA markers
Clinical features of pANCA+ CD
(10-30% of CD)

- “ulcerative colitis-like” features
- Later age of onset
- Decreased incidence of fibrostenosis and penetrating disease
- Less likely to respond to anti-TNFα (esp. in pANCA+/ASCA- CD)
Clinical features of ASCA+ CD (70% of CD)

- Ileal involvement
- Obstruction
- Perforation
- More aggressive disease

ASCA as a predictor of CD

ASCA were detected in 31% of patients before the clinical diagnosis of CD

Israeli E et al. IBD 2005;54:1232
UC

Negative

pANCA+
Anti-goblet+
Anti-colon+

CD

ASCA+
Anti-OmpC+
Anti-I2

Negative
Role of serologic testing in IBD

- Diff. Dx between IBD and non IBD: Helpful
- Between UC and CD (esp. IC): Fair/Good
- Pediatric colitis: Fair
- IBD in elderly: Good
- Screening for IBD patients: Fair
- Predict genetic susceptibility: Fair
- Predict disease behavior: Fair/good
- Predict response to treatment: Possible
Situations which benefits from seromarkers testing

- CD patients who are at risk of requiring multiple surgery
- CD patients whom anti-TNFα is being considered
- IBD in children whom invasive test are unavailable or undesirable
- UC patients undergoing ileal pouch anal anastomosis