Serum Markers: What, Who, When and Why?

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### Crohn’s Disease: Microbial Antibodies

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCA</td>
<td>Anti- Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Anti-I2</td>
<td>Protein from pseudomonas fluorescens</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>Escherichia coli outer membrane porin</td>
</tr>
<tr>
<td>Bir1 Flagellin</td>
<td>Antibodies to CBir Flagellin</td>
</tr>
</tbody>
</table>

Targan S. Gastroenterology 2005;128(7): 2020-8
Combining Markers Improves Sensitivity but Not Overall Accuracy

Antibodies to CBir1 Flagellin

- Antigen of the gut flora
- Induces a strong B cell and CD4+ T cell response in colitic mice
- 50% of patients with Crohn’s disease have serum reactivity
- Little or no reactivity in UC, inflammatory controls, and normal controls
Immune Response
CD Patients

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCA</td>
<td>56</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>55</td>
</tr>
<tr>
<td>pANCA</td>
<td>23</td>
</tr>
<tr>
<td>Anti-CBir1</td>
<td>55</td>
</tr>
</tbody>
</table>

Anti-CBir1 Presence
CD Patients

Anti-CBir1 Helps Distinguish Between pANCA+ Patients

$P < 0.001$ (level)

44%

11/25

4%

1/25

0.255

0.623

In a clinical study, serum samples from 100 CD, 101 UC and 27 colitis/diarrheal patients and 163 controls were analyzed using pANCA IFA and ASCA ELISA

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**Test Result**

<table>
<thead>
<tr>
<th>IBD Predicted</th>
<th>IBD Not Predicted</th>
<th>PROMETHEUS IBD Serology 7 Overall Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>IBD</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NPV</td>
</tr>
</tbody>
</table>

**PROMETHEUS Predictive Algorithm Description:**
- Utilizes Smart Diagnostic Algorithm (SDA) technology to characterize complex relationships between markers to produce a diagnostic prediction with greater accuracy than simple comparison of assay results to a reference range.
- Developed (n=1813; 36% CD, 24% UC, 20% IBS, 20% normal) and validated (n=500; 38% CD, 21% UC, 41% normal) using serology results for samples with a known diagnosis.

**Assay Information**

<table>
<thead>
<tr>
<th>Assay</th>
<th>ASCA IgA ELISA</th>
<th>ASCA IgG ELISA</th>
<th>Anti-OmpC IgA Elisa</th>
<th>Anti-CBir1 ELISA</th>
<th>Neutrophil-Specific Nuclear Auto Antibodies (NSNA) (IBO Specific pANCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Auto Antibody ELISA</td>
</tr>
<tr>
<td>Assay Value</td>
<td>109.4 EU/ml</td>
<td>113.8 EU/ml</td>
<td>26.0 EU/ml</td>
<td>50.2 EU/ml</td>
<td>&lt;12.1 EU/ml</td>
</tr>
</tbody>
</table>

Note: Test result determined by the PROMETHEUS Predictive Algorithm without direct consideration of assay values relative to reference values. However, interpretation of prognostic information should be made based on relative differences between assay values and reference values.

**Reference Values**

|                | <20.0 EU/ml | <40.0 EU/ml | <16.5 EU/ml | <21.0 EU/ml | <12.1 EU/ml | Not Detected | Not Detected |
**Progression of CD and Response to Microbial Antigens Scottish Population**

<table>
<thead>
<tr>
<th>Antibodies tested: ASCA, anti-OmpC, anti-I2</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Number of Positive Antibodies* (%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>$P$ Value</th>
<th>OR (3:0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Progression</td>
<td>24</td>
<td>52</td>
<td>73</td>
<td>87</td>
<td>$&lt;0.001$</td>
<td>20</td>
</tr>
<tr>
<td>Surgery</td>
<td>32</td>
<td>57</td>
<td>52</td>
<td>89</td>
<td>$&lt;0.001$</td>
<td>17</td>
</tr>
</tbody>
</table>

Association of ASCA and Early Surgery Newly Diagnosed CD Patients

<table>
<thead>
<tr>
<th></th>
<th>Early Surgery (n=35)</th>
<th>No Early Surgery (n=35)</th>
<th>P Value</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCA IgA</td>
<td>63%</td>
<td>20%</td>
<td>.001</td>
<td>8.5</td>
</tr>
<tr>
<td>ASCA IgG</td>
<td>40%</td>
<td>14%</td>
<td>.027</td>
<td>5.5</td>
</tr>
<tr>
<td>ASCA IgA &amp; IgG</td>
<td>37%</td>
<td>14%</td>
<td>.043</td>
<td>5.0</td>
</tr>
</tbody>
</table>

High ASCA IgA & IgG in CD Associated With More Aggressive Small Bowel Disease

*Other Investigators: Sands, Dassopoulos, Riis → Similar results
Frequency of Complications Increases With Number of Serologic Markers Children With CD

![Graph showing the frequency of disease behavior (%) with increasing number of immune responses.]

- **Nonperforating Nonstenotic**
- **Perianal Perforating Only**
- **Internal Perforating/Stenotic Only**

Serologic markers:
- ASCA
- Anti-OmpC
- Anti-CBir1
- Anti-I2

*Odds ratios

*Odds ratios (P trend=0.002)

Anti-microbial responsiveness is increased in NOD2 mutant carriers

- Mutations in Nod2 may decrease innate immune clearance of bacteria leading to secondary increase in adaptive immune responses
- Analyzed 732 Crohn’s disease patients, 220 healthy relatives, 200 controls
- Nod2 mutant carriers have higher titers of anti-microbial antibodies: CBir1, I2, ASCA, OmpC

One way ANOVA
Potential Roles for IBD Serology

• Help identify IBD in patients with unclear diagnosis (but chronic inflammation already established)
• Help assess the need for endoscopy in patients who are suspected of having IBD (pediatric population)
• Help differentiate between CD and UC
• Help improve the accuracy of diagnosis prior to surgery (eg, colectomy, IPAA)
• Help identify patients at risk for aggressive disease behavior
Indeterminate Colitis: Value of Serology

Joossens S. Gastroenterology 2003. 124: A323
IBD Serologies: When to order?

- IC / Colectomy is planned (UC vs CD, pouchitis risk)
- Pediatric: low suspicion
- Mainly extra-intestinal manifestations (is it IBD?)
  - Ankylosing spondylitis
  - Arthritis
  - Pyoderma gangenosum
  - Uveitis
- Second opinion / questionable diagnosis
- Prognostication: ready for prime time?
  - Expensive: will insurance cover?
IBD Serologies: Summary

- Identify IBD in cases of diagnostic uncertainty
- Assess the need for invasive endoscopy in patients who are suspected of having IBD
- Differentiate between CD and UC
- Identify patients at risk for aggressive disease behavior
- Insurance / cost still an issue