Use of Biomarkers in the Diagnosis and Prognosis of IBD

Schoepfer and Lewis. Gastroenterol 2015;148:889–892
**Biomarker Definition:** A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.


**Long-term Evolution of Disease Behavior in Crohn’s Disease**

Cumulative Probability (%)

**Penetrating**

**Inflammatory**

**Stricturing**

Patients at risk:  
N = 2002 552 229 95 37

Cosnes J et al. Inflamm Bowel Dis. 2002;8:244.
**PROBABILITY OF SURGERY FOR CROHN’S DISEASE**

<table>
<thead>
<tr>
<th>Years After Diagnosis</th>
<th>1 Surgery</th>
<th>2 Surgeries</th>
<th>≥3 Surgeries</th>
<th>No Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>37</td>
<td>7</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>11</td>
<td>12</td>
<td>39</td>
</tr>
<tr>
<td>15</td>
<td>34</td>
<td>14</td>
<td>22</td>
<td>30</td>
</tr>
</tbody>
</table>

5-year cumulative probability of first major surgery decreased from 44.7% in cohort (1979-1986) to 19.6% in cohort (2003-2011) \( p < 0.001 \)\(^*\)


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**Figure 1.** The potential role of biomarker assays in the care of patients with suspected or established IBD. Biomarkers might be used in all phases of the care.

Lewis. *Gastroenterol* 2011;140:1817–1826
Characteristics of a desirable IBD Biomarkers

- Rapid
- Non-invasive
- Widely available
- Inexpensive
- Reproducible
- Applicable:
  - Reflect the state of bowel inflammation
  - Identify IBD from non-IBD
  - Differentiate UC from CD
  - Have prognostic value

• Sensitivity: If a person has a disease, how often will the test be positive (true positive rate)
• Specificity: If a person does not have the disease how often will the test be negative (true negative rate)?
• Positive predictive value: Likelihood that the patient has the disease if the test is positive
• Negative predictive value: Likelihood that the patient does not have the disease if the test is negative

Test Accuracy (Believability)
Studies: Depends on Disease Prevalence
Individual Patients: Depends on Pre-test probability
IBD Biomarkers

- Blood cell counts or ratios
- Serology
- Blood proteins (ex CRP)
- Stool markers (ex lactoferrin, calprotectin)
- DNA
- Gene expression (RNA)
- Micro RNAs
- Metabolomics (serum, urine, stool)
- Gut bacterial profile

Prevalence of different serological markers in IBD

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD</th>
<th>UC</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical P-ANCA</td>
<td>2-28%</td>
<td>45-82%</td>
<td>1-7%</td>
</tr>
<tr>
<td>ASCA</td>
<td>39-76%</td>
<td>5-15%</td>
<td>5%</td>
</tr>
<tr>
<td>Anti-OMPC</td>
<td>24-55%</td>
<td>5-11%</td>
<td>5%</td>
</tr>
<tr>
<td>Anti-I2</td>
<td>54%</td>
<td>10%</td>
<td>4%</td>
</tr>
<tr>
<td>Anti-CBir 1</td>
<td>50%</td>
<td>6%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Papp et al World J Gastro 2007;13:2028-2036
Meta-analysis of IBD serology

Meta-analysis of 63 studies reporting on ASCA and pANCA in IBD (3,841 UC and 4,019 CD patients)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD vs non-IBD</td>
<td>63%</td>
<td>93%</td>
</tr>
<tr>
<td>ASCA+/ANCA- CD</td>
<td>55%</td>
<td>93%</td>
</tr>
<tr>
<td>ASCA-/ANCA+ UC</td>
<td>55.3%</td>
<td>88.5%</td>
</tr>
</tbody>
</table>

The low sensitivity of serological assays in IBD means that a negative test does not rule out the disease. The high specificity means that a positive test is useful in confirming a diagnosis of IBD.

Reese et al. AJG 2006;101:2410–2422

ASCA in Celiac Sprue

Eur J Gastroenterol & Hep 2006;18:75-78
ASCA in Intestinal TB

Table 2  Results of ASCA IgA, ASCA IgG, and ANCA in healthy controls and patients with ulcerative colitis, Crohn’s disease, and intestinal tuberculosis

<table>
<thead>
<tr>
<th>Serological test</th>
<th>Healthy controls (n=21)</th>
<th>Ulcerative colitis (n=25)</th>
<th>Crohn’s disease (n=59)</th>
<th>Intestinal tuberculosis (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCA IgA</td>
<td>1 (4.7%)</td>
<td>7 (28%)</td>
<td>20 (33.9%)</td>
<td>13 (43.3%)</td>
</tr>
<tr>
<td>ASCA IgG</td>
<td>1 (4.7%)</td>
<td>6 (24%)</td>
<td>30 (50.8%)</td>
<td>14 (46.6%)</td>
</tr>
<tr>
<td>Either ASCA IgA or ASCA IgG</td>
<td>2 (9.5%)</td>
<td>10 (40%)</td>
<td>36 (61%)</td>
<td>20 (66.6%)</td>
</tr>
<tr>
<td>Both ASCA IgA and ASCA IgG</td>
<td>0</td>
<td>3 (12%)</td>
<td>14 (23.7%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>ANCA</td>
<td>0</td>
<td>8 (32%)</td>
<td>6 (10.1%)</td>
<td>2 (6.6%)</td>
</tr>
</tbody>
</table>


Antibodies to CBir1 Are Associated With Glycogen Storage Disease Type Ib

N=19 (7 with IBD-like disease, 12 without known IBD-like disease).

17 of 19 (89%) patients had elevated anti-CBir1 levels (6/7 in the IBD group and 11/12 in the no clinical evidence of IBD group).

13 of 19 (68%) had elevated anti-OmpC levels (5/7 in the IBD group and 8/12 in the no clinical evidence of IBD group).

11 of 19 (58%) patients had elevated ASCA IgA levels (4/7 in the IBD group and 7/12 in the no clinical evidence of IBD group).

Davis et al. JPGN 2010;51:14–18
“Although the test characteristics in regards to sensitivity and specificity are reasonably good for the most comprehensive serologic panel for IBD, the serologic tests should not be considered a diagnostic tool, and treatment for IBD should not be initiated solely on the results of serologic testing.”


Increased Immune Reactivity Predicts Complicating Crohn’s Disease in Children

Multicenter, prospectively recruited pediatric cohort
Sera were collected from 796 pediatric CD cases
The median age at diagnosis was 12 yrs (0.6–18 yrs)

32% of patients developed at least 1 disease complication within a median of 32 months, and 18% underwent surgery.

The number of (+) antibodies and the magnitude of immune response were predictive of aggressive disease phenotypes.

Further studies needed to demonstrate meaningful added value over the prediction by ileal location


Table 3
When should IBD serologies be ordered?

<table>
<thead>
<tr>
<th>Indication</th>
<th>Utility</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea/abdominal pain. Unusual gastrointestinal symptoms</td>
<td>0</td>
<td>High probability of false-positives. Surrogate markers of inflammation more helpful</td>
</tr>
<tr>
<td>Diagnosis of IBD</td>
<td>+</td>
<td>Serologies should not (cannot) replace conventional diagnostic modalities</td>
</tr>
<tr>
<td>Diagnosis of pediatric IBD</td>
<td>+++</td>
<td>Threshold for endoscopy may be higher in pediatric population, but fecal markers may be preferred</td>
</tr>
<tr>
<td>Diagnosis of Crohn vs UC</td>
<td>++</td>
<td>May be helpful in differentiating UC from Crohn.</td>
</tr>
<tr>
<td>IBDU</td>
<td>++</td>
<td>In a minority of cases of IBDU, serologies may be diagnostic</td>
</tr>
<tr>
<td>First-degree relatives of IBD patients</td>
<td>0</td>
<td>High percentage of first-degree relatives may have positive results, but never have disease, contributing to needless testing and anxiety</td>
</tr>
<tr>
<td>Risk of pouchitis</td>
<td>++</td>
<td>Serologies may identify those with high risk of pouchitis after IPAA, but unclear whether that would preclude surgery</td>
</tr>
<tr>
<td>Prognosis/risk stratification in Crohn disease</td>
<td>+++</td>
<td>Panel of IBD antibodies may identify those at greater risk for progression to fistulizing/penetrating disease or surgery. Not clear if it significantly exceeds clinical predictive factors. Statistically significant, but clinically significant?</td>
</tr>
</tbody>
</table>

Utility graded on a 0 (not indicated) to ++++ (clearly necessary) scale.


Serum Biomarkers of Inflammation

• **CRP**
  • Acute Phase protein
  • Produced in Liver under influence of (gut?) IL-6/TNFα
  • Short T½ of ~19 hours

  **Caveats and Considerations**
  • CD > UC
  • Colon > isolated Ileal CD?
  • Elevated in other conditions
    – Virus/Bacterial/Obesity/CAD
  • 10-40% have no or minimal response

• **ESR**
  • Rate RBCs settle in 1 hour
    – Fibrinogen

  **Caveats/Considerations**
  • Influenced by
    – Anemia, gender, pregnancy
  • Verses CRP
    – Peaks less rapidly
    – Resolves more slowly
CRP and Predicting Crohn’s Relapse

Infliximab Failure After Azathioprine Withdrawal in Crohn's Disease Treated With Combination Therapy

Cumulative probability of a steroid-free course after steroid induced remission in patients with high and normal CRP value at steroid weaning

Fecal Biomarkers of Inflammation

**Calprotectin**
- Granulocyte cytosolic protein
- Stable in feces for days

**Lactoferrin**
- Neutrophil granule protein
- Stable in feces as well
- May have slightly lower sensitivity and specificity than calprotectin

Both are more sensitive and specific than serum tests for gut inflammation

**Caveats and Considerations**
- Also elevated in Cancer, NSAIDS damage, celiac and microscopic colitis
- Colon > SI disease
- Specificity and Sensitivity vary greatly based on cutoff value (50 vs 100)
Correlation between CRP and endoscopic activity is < fecal markers and endoscopic activity

### Table 3. Sensitivity and Specificity of Biomarkers to Identify Active Disease, Based on Endoscopy

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient population</th>
<th>Assessment of endoscopic disease activity</th>
<th>Lactoferrin (Sensitivity/specificity)</th>
<th>Calprotectin (Sensitivity/specificity)</th>
<th>CRP (Sensitivity/specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosent et al(^{(1)}) (2004)</td>
<td>CD and UC</td>
<td>Farup method(^{(12)})</td>
<td>100%/100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solen et al(^{(4)}) (2005)</td>
<td>CD</td>
<td>Study specific index(^{(a)})</td>
<td></td>
<td></td>
<td>54%/75%</td>
</tr>
<tr>
<td>Sipinen et al(^{(5)}) (2008) (^{(b)})</td>
<td>CD</td>
<td>CDEIS</td>
<td>77%/100%</td>
<td>87%/100%</td>
<td></td>
</tr>
<tr>
<td>Sipinen et al(^{(5)}) (2008) (^{(c)})</td>
<td>CD</td>
<td>CDEIS</td>
<td>66%/71%/83%/92%</td>
<td>70%/91%/44%/92%</td>
<td>48%/91%</td>
</tr>
<tr>
<td>Sipinen et al(^{(5)}) (2010) (^{(d)})</td>
<td>CD</td>
<td>SES-CD</td>
<td>80%/67%</td>
<td>80%/80%</td>
<td></td>
</tr>
<tr>
<td>D’Inca et al(^{(2)}) (2007) (^{(e)})</td>
<td>CD</td>
<td>SES-CD</td>
<td>77%/80%</td>
<td>81%/80%</td>
<td></td>
</tr>
<tr>
<td>D’Inca et al(^{(2)}) (2007) (^{(f)})</td>
<td>UC</td>
<td>Mayo score</td>
<td>75%/60%</td>
<td>78%/70%</td>
<td></td>
</tr>
<tr>
<td>Schoepfer et al(^{(2)}) (2009) (^{(g)})</td>
<td>UC</td>
<td>Rachmilewitz index</td>
<td>86%/93%/71%/88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schoepfer et al(^{(2)}) (2010) (^{(h)})</td>
<td>CD</td>
<td>CDEIS</td>
<td>89%/58%</td>
<td></td>
<td>68%/58%</td>
</tr>
</tbody>
</table>

CDEIS, Crohn’s Disease Endoscopic Index of Severity; SES-CD, Simple Endoscopic Score for Crohn’s Disease.

Fecal Calprotectin Increases Prior to Symptomatic Relapse

### Table 5. Studies Associating Increased Concentrations of Fecal Calprotectin With Relapse in Patients With Exacerbations in Ulcerative Colitis

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient population</th>
<th>Duration of remission at entry</th>
<th>Calprotectin concentration to define elevated level</th>
<th>Relapse rate with low calprotectin, %</th>
<th>Relapse rate with high calprotectin, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gisbert et al(^{(1)})</td>
<td>UC</td>
<td>&gt;6 mo</td>
<td>&gt;150 µg/g</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>Tibble et al(^{(1)})</td>
<td>UC</td>
<td>1-4 mo</td>
<td>&gt;50 µg/g</td>
<td>10(^{p})</td>
<td>85(^{p})</td>
</tr>
<tr>
<td>Tibble et al(^{(1)})</td>
<td>CD</td>
<td>1-4 mo</td>
<td>&gt;50 µg/g</td>
<td>15(^{p})</td>
<td>85(^{p})</td>
</tr>
<tr>
<td>Costa et al(^{(2)})</td>
<td>UC</td>
<td>1-12 mo</td>
<td>&gt;150 µg/g</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td>Costa et al(^{(2)})</td>
<td>CD</td>
<td>1-12 mo</td>
<td>&gt;150 µg/g</td>
<td>57</td>
<td>87</td>
</tr>
<tr>
<td>Costa et al(^{(2)})</td>
<td>UC</td>
<td>3-36 mo</td>
<td>&gt;130 µg/g</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Sipinen et al(^{(5)})</td>
<td>UC + CD</td>
<td>&gt;3 mo (51% &gt;12 mo)</td>
<td>&gt;150 µg/g</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>Wackiwetz et al(^{(6)})</td>
<td>CD</td>
<td>Not stated</td>
<td>&gt;400 µg/g</td>
<td>11</td>
<td>56</td>
</tr>
</tbody>
</table>

\(^{p}\)Estimated from Kaplan-Meier curves

Lewis. Gastroenterol 2011;140:1817–1826
140 Crohn’s; 43 healthy controls undergoing ileocolonoscopy were prospectively enrolled and scored according to the SES-CD and the CDAI.

The overall accuracy for the detection of endoscopically active disease was 87% for calprotectin (cutoff 70 μg/g), 66% for elevated CRP, 54% for blood leukocytosis, and 40% for the CDAI ≥150.

Fecal calprotectin was the only marker that reliably discriminated inactive from mild, moderate, and highly active disease.

Figure 2. Fecal calprotectin concentrations in different groups of endoscopic Crohn’s disease activity illustrated by boxplots. The figure shows that calprotectin concentrations are directly correlated with Simple Endoscopic Score for Crohn’s disease (SES-CD). The vertical line in the middle of the box is the median, and the box represents the lower and upper quartiles, whereas the whiskers indicate the 95% confidence interval of the values. The values outside the whiskers represent individual outliers.
EMerging BiomARKers in Inflammatory Bowel Disease (EMBARK) Study

- UC \( (n=107) \) and CD \( (n=157) \) patients were characterized and underwent ileocolonoscopy (ICO).
- A subset of CD patients \( (n=66) \) also underwent computed tomography enterography (CTE).
- An extensive list of serum and fecal biomarkers were evaluated.
- Individual biomarkers with a moderate degree of correlation \( (P \leq 0.3) \) were evaluated using multivariate analysis.
- 36 patients had no detectable inflammation by ICO (SESCD=0). Of these patients, 20 patients had evidence of disease by ICO-CTE.


**Figure 3.** Relationship between ileocolonoscopy (ICO)-computed tomography enterography (CTE), individual biomarkers, and a biomarker combination. Fecal calprotectin \( (a) \) correlated well with ICO-CTE (Pearson \( r=0.613 \) ), whereas both serum interleukin-22 (IL-22), \( (b) \) and serum matrix metalloproteinase 9 (MMP9), \( (c) \) were less well correlated. Fecal calprotectin, serum IL-22, and serum MMP9 were combined in a linear regression model to predict ICO-CTE, increasing the Pearson correlation coefficient to \( r=0.699 \).

Table. General summary of data supporting the roles of biomarkers in IBD

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>IBD vs non IBD</th>
<th>CD vs UC</th>
<th>Disease activity</th>
<th>Mucosal healing</th>
<th>Prediction of clinical relapse</th>
<th>Prognosis</th>
<th>CD Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP/ESR</td>
<td>Useful</td>
<td>Not used</td>
<td>Useful</td>
<td>Possibly</td>
<td>Possibly</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Fecal biomarkers</td>
<td>Useful</td>
<td>Not used</td>
<td>Useful</td>
<td>Possibly</td>
<td>Possibly</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Serologic markers</td>
<td>Useful</td>
<td>Useful</td>
<td>Not used</td>
<td>Not used</td>
<td>Not used</td>
<td>Not used</td>
<td>Useful</td>
</tr>
</tbody>
</table>

Abbreviations: IBD, inflammatory bowel diseases; CD, Crohn’s disease; UC, ulcerative colitis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Know your pretest probability and how the test will influence management

Costs: including chasing a false (+)  
Useful information