Fecal Calprotectin
Reliable, Novel, Noninvasive Biomarker

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Calprotectin

- First Described in 1980, initially called L1 protein.
- A 36 KD Calcium and Zinc binding protein found in neutrophils, monocytes, macrophages and squamous epithelial cells except those in normal skin.
Calprotectin

- It constitutes more than 60% of the total cytosolic protein content of neutrophils.
- After binding calcium, it can resist degradation by leukocytic and bacterial enzymes.
- By competing with different enzymes for limited local amounts of zinc, calprotectin may inhibit many zinc-dependent enzymes and thereby kill microorganisms.
Calprotectin

- Calprotectin is antimicrobial and has induced apoptosis in all cell types (human, animal, normal, malignant) tested.
Lack of calprotectin is not compatible with life!
Clinical Use

- Calpro resists metabolic degradation.
- It can be measured in urine, stool, saliva, plasma, CSF, synovial fluid, amniotic fluid and sputum.
- Stool samples can be sent by post, then frozen and batch analysed.
- Upper limit of normal in stool is 10mg/l.
- As little as 5gm stool sample is sufficient.
Clinical Use

- extensively validated, showing consistent abnormalities in patients with IBD, colorectal carcinoma, and nonsteroidal enteropathy.

- Proposed as a useful outpatient screening test for organic small bowel or colorectal pathology. *May be particularly useful in children.*

- Proposed as an IBD monitoring test, can predict steroid refractory disease, or which “well patients” are likely to relapse. Potential for monitoring the efficacy of new therapeutic regimes.
“Stool markers of gastrointestinal inflammation such as lactoferrin and, more recently, calprotectin, are of considerable research interest but, as yet, these have not been introduced into clinical practice.” Gut 2003
Calprotectin: Crohn’s versus Controls

![Graph showing Calprotectin levels in Crohn's disease versus controls.](image-url)
Calprotectin compared with CRP

Faecal calprotectin concn (mg/l)

C reactive protein (mg/l)

Crohn’s disease  IBS  Miscellaneous

Crohn’s disease  IBS  Miscellaneous
Calprotectin Levels in the Different Diagnostic Groups
General Background

- Levels relatively unaffected by GI bleeding
- Need > 100mls of blood per day to increase calprotectin level by 6mg/l
- In active Crohn’s disease, levels of calprotectin up to 40,000 mg/l reported
Why does calprotectin occur in the stool?

- In inflammation, the leukocytes migrate through the intestinal wall, leading to increased calprotectin level in the stool.
- The concentration of calprotectin correlates with the number of granulocytes in the intestinal lumen, and thus with the level of intestinal inflammation.
- Calprotectin measurement in a single sample correlates well with established markers of intestinal inflammation and permeability (4-day faecal collection of 111In labelled white cells, 51Cr-EDTA excretion, and lactulose/L-rhamnose ratio).
Diagnosis Of IBD

- Stool culture
- Blood tests
  - Inflammatory markers
  - Nutritional markers
- Endoscopy and histology
- Radiology
- Nuclear scans
  - White cell scans
  - DEXA
- ASCA/ANCA
- Fecal calprotectin
What are the serologic markers?

- The two jacks in IBD serological markers are pANCA and ASCA.
- ASCA is associated with CD, whereas increased levels of pANCA are more common among patients with UC.
- In a meta-analysis, combinations of tests for ASCA and pANCA distinguished patients with CD from those with UC with 40%–50% sensitivity and specificity of 90%.
Can Serological Markers Differentiate IBD from Non-IBD?

- pANCA and ASCA are specific for and have high positive predictive value for UC and CD respectively
  - Rule in disease
- The low sensitivity and negative predictive value preclude them as screening tests
  - Cannot rule out disease
Routine Blood tests in IBD

• CBC’s:
  - Anemia is common due to blood loss or malabsorption (iron, folate, B12) or may reflect the chronic disease state
  - Leukocytosis & thrombocytosis also common; modestly elevated WBC counts in active disease
• Erythrocyte sedimentation rate (ESR): Typically elevated; monitors disease activity
• Abnormal LFT’s: May represent pericholangitis or sclerosing cholangitis
• Low serum albumin (protein-losing enteropathy): Suggests extensive colitis
So... Challenges in the Diagnosis of IBD

1. Diagnosis is not straightforward
2. Symptoms are insidious
3. Multiple investigations may be required
4. Many other causes need to be excluded
Calprotectin detection is crucial

- The rapid identification of IBD is crucial as up to 15% of patients with CD have penetrating lesions (fistulae, phlegmons, or abscesses) at the time of diagnosis. The time to diagnosis in general seems to be acceptable, but long delays (> 12 months) exist for a considerable part of patients, especially in CD.

- In children, prompt diagnosis is of special importance, as IBD may affect **growth** and **sexual maturation**.

- The ultimate risk when not diagnosed properly and early ⇒ Colorectal Cancer
Supporting factors for calprotectin

- Can be conveniently assessed in small samples sent by mail, no dietary restrictions, not significantly increased by intestinal blood and unaffected by medication.
- Calprotectin is resistant to proteolytic degradation and is stable at room temperature for up to seven days in stool samples.
- The main advantage of fecal biomarkers is that the fecal stream is in direct contact with the mucosa and therefore, when measured in feces, *calprotectin detects inflammatory conditions far more precisely than biomarkers measured in serum.*
Limiting factors for calprotectin

- Fecal calprotectin not a disease-specific marker for IBD, can’t differentiate between CD and UC.
- Important biological variability has been reported for fecal calprotectin measurements on different days, also fluctuate depending on disease location. In Crohn’s disease, the release of calprotectin from site of ileal inflammation has been greater than from the inflamed colon.
- Increased levels are found in:
  - Neoplasia, polyps
  - NSAID abuse
  - Infections
How well does calprotectin level correlate with disease activity?

- Fecal calprotectin is elevated in patients with active IBD, but are much less elevated in quiescent IBD.
- From a diagnostic standpoint, there is a nice correlation between presence of endoscopic lesions & elevations in calprotectin.
- There is a very good correlation between absence of calprotectin and absence of intestinal and colonic inflammation, which can allow IBD to be ruled out.
How does Calprotectin facilitate diagnosis, predicting treatment response?

- Studies shown that this biomarker can predict disease relapse or announce a flare, so monitoring of this marker could allow clinicians to start earlier treatment or further testing.
- Improvements in fecal calprotectin nicely coincide with improvements in endoscopy among patients responding to therapy.
In patients with clinically quiescent IBD, FC 50 mg/l can predict the likelihood of clinical relapse of disease within a few months, with over 80% sensitivity.

Most patients with quiescent IBD have low-grade inflammation, and it is suggested that symptomatic relapse occurs only when the inflammatory process reaches a critical intensity.
Fecal Calprotectin vs. histology and severity score

$r = 0.85$
$p < 0.001$

J Pediatr Gastroenterol Nutr;33: 2001
Calprotectin levels in IBD patients with active disease and when mucosal healing had been achieved.
Calprotectin levels during treatment of a patient with ulcerative colitis

Prednisolone 80 mg
5 ASA 3 g/daily

Faecal calprotectin mg/l

Weeks

J. Gastroenterol NEMA ;20: 2011
Recent researches

- **Usefulness of a Novel and Rapid Assay System for Fecal Calprotectin in Pediatric Patients with Inflammatory Bowel Diseases.**
  Inoue K, Aomatsu T, Yoden A, Okuhira T, Kaji E, Tamai H.

- **Markers of gut mucosal inflammation and cow's milk specific immunoglobulins in non-IgE cow's milk allergy.**
  Merras-Salmio L, Kolho KL, Pelkonen AS, Kuitunen M, Mäkelä MJ, Savilahti E.

- **Pre-clinical Crohn's disease: Diagnosis, treatment and six year follow-up.**
  Sorrentino D, Avellini C, Geraci M, Vadalà S.
  J Crohns Colitis. 2014 Jan 8. pii: S1873-9946(13)00441-8

- **The effect of probiotics on fecal calprotectin in patients with cystic fibrosis.**
Diagnosis of IBD

Recommended position of faecal calprotectin in diagnostic pathway

Source: BMJ 2010; 341
Significance of Calprotectin

- Screening of IBD
- Differential Diagnosis of IBD & IBS
- Reduces Endoscopies
- Therapy Monitoring
- Relapse Prediction

Reduces Endoscopies
Screening of patients with suspected IBD by fecal calprotectin

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patients</th>
<th>Patient population</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limburg (2000) (35)</td>
<td>180</td>
<td>UC/CD</td>
<td>94</td>
<td>83</td>
</tr>
<tr>
<td>Tindle (2000) (36)</td>
<td>226</td>
<td>CD</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Brown (2001) (41)</td>
<td>68</td>
<td>UC/CD</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>Sammartino (2002) (29)</td>
<td>116</td>
<td>UC/CD</td>
<td>70</td>
<td>n/a</td>
</tr>
<tr>
<td>Costa (2003) (42)</td>
<td>236</td>
<td>UC/CD</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Carroccio (1995) (43)</td>
<td>70</td>
<td>CD</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>Langford (2005) (66)</td>
<td>31</td>
<td>UC</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td>Silverman (2005) (58)</td>
<td>159</td>
<td>UC/CD</td>
<td>68</td>
<td>n/a</td>
</tr>
<tr>
<td>Canani (2006) (10b)</td>
<td>27</td>
<td>UC/CD</td>
<td>95</td>
<td>89</td>
</tr>
<tr>
<td>Dinsa (2007) (64)</td>
<td>144</td>
<td>UC</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>Chauve-Faye (2007) (55)</td>
<td>148</td>
<td>UC/CD</td>
<td>78</td>
<td>90</td>
</tr>
<tr>
<td>Kaiser (2007) (67)</td>
<td>171</td>
<td>UC/CD</td>
<td>63</td>
<td>86</td>
</tr>
<tr>
<td>Silverman (2007) (68)</td>
<td>88</td>
<td>UC/CD</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Langford (2008) (29)</td>
<td>139</td>
<td>UC</td>
<td>100</td>
<td>37</td>
</tr>
<tr>
<td>Orten (2006) (71)</td>
<td>114</td>
<td>UC/CD</td>
<td>100</td>
<td>n/a</td>
</tr>
<tr>
<td>Scharf (2008) (72)</td>
<td>136</td>
<td>UC/CD</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Stiller (2008) (73)</td>
<td>63</td>
<td>UC</td>
<td>100</td>
<td>64</td>
</tr>
</tbody>
</table>

For each study the number of included patients (No. of patients) and the type of inflammatory bowel disease (IBD) of the patient population is given. UC/CD = ulcerative colitis/CD; UC = ulcerative colitis; CD = Crohn’s disease; UC/CD/CD = both UC/CD/CD; Sensitivity and specificity indicate the diagnostic ability to distinguish between IBD and non-IBD.

A rapid test for calprotectin measurement was used. Adapted in parts from Gobert et al. (58).

**Meta-analysis of 40 studies**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Reduction in endoscopy</th>
<th>False negative test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adults</strong></td>
<td>0.93 (0.85-0.97)</td>
<td>0.96 (0.79-0.99)</td>
<td>67%</td>
<td>6%</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td>0.92 (0.84-0.96)</td>
<td>0.76 (0.62-0.86)</td>
<td>35%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Reduction in false positive test is 40 studies.

*BI = inflammatory bowel disease; CRC = colorectal cancer; IBS = irritable bowel syndrome; HC = healthy controls.

Adapted in parts from Gobert et al. (58).
**Future: Screening Test for Intestinal Allograft Monitoring**

- Intestinal allograft rejection is difficult to distinguish from other causes of diarrhea and can rapidly lead to severe exfoliation or death.
- Protocol biopsies are standard for allograft monitoring but may cause serious complications.

Fecal Calprotectin is a non-invasive test showing clinical utility for monitoring of the intestinal Allograft

*Annals of Surgery; Volume 246, Number 2, August 2007*
A person with positive Rome criteria and a normal Calprotectin (< 50 µg/g) has virtually No Chance of having IBD
Easy & reliable lab diagnostics with monoclonal Calprotectin-ELISAs

- Various ELISA-systems are available for laboratory determination of fecal calprotectin.
- Assays with monoclonal antibodies have been proven to be more sensitive and more specific with better performance than polyclonal antibodies and therefore deliver more reliable results.
- Assays with monoclonal antibodies avoid the danger of many false positives up to 50% of samples may need to be re-run, costing time and money.
## How do I interpret test results?

<table>
<thead>
<tr>
<th>Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 µg/g</td>
<td>No significant inflammation</td>
</tr>
<tr>
<td>50-100 µg/g</td>
<td>Indicates some GI inflammation: IBD, infection</td>
</tr>
<tr>
<td></td>
<td>Polyps, neoplasia, NSAIDS</td>
</tr>
<tr>
<td>100 µg/g</td>
<td>Significant inflammation:</td>
</tr>
<tr>
<td></td>
<td>Referral may be indicated</td>
</tr>
<tr>
<td>&gt; 250 µg/g</td>
<td>Active disease present or predicts relapse in treated patients</td>
</tr>
</tbody>
</table>
How do I interpret test results?

- In patients with clinically quiescent IBD, studies have shown at the cutoff level of 150 µg/g, the sensitivity of calprotectin for predicting relapse within 3 months was 90% with a specificity of 83%.
- This suggests that symptomatic relapse occurs when inflammation in the gut reaches a critical intensity.
Fecal Lactoferrin

- Well known and established with many physicians. Valuable but less specific for differentiation than Calprotectin.
  - Often used in combination with Calprotectin to ”be on the safe side...“ or simply with conservative physicians.
  - Often used with infants and children (not when breast fed).
  - Detection of relapse in CD later than Calprotectin.
Novel fecal and luminal markers of inflammatory bowel disease

Lundberg JO *et al.* (2005) Technology Insight: calprotectin, lactoferrin and nitric oxide as novel markers of inflammatory bowel disease


<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Calprotectin</th>
<th>Lactoferrin</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>36 kDa protein</td>
<td>75 kDa protein</td>
<td>30 Da gas</td>
</tr>
<tr>
<td>Cellular source</td>
<td>Neutrophils</td>
<td>Neutrophils</td>
<td>Intestinal epithelium</td>
</tr>
<tr>
<td>Physiological role</td>
<td>Host defense</td>
<td>Host defense</td>
<td>Host defense</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Fecal</th>
<th>Fecal</th>
<th>Luminal gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of sample</td>
<td>ELISA</td>
<td>ELISA</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>Detection limit</td>
<td>&lt;5 mg/l</td>
<td>?</td>
<td>1 ppb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference valuesa</th>
<th>Healthy controls</th>
<th>Active IBDb</th>
<th>Nonactive IBD</th>
<th>IBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin</td>
<td>0.5–50 mg/l</td>
<td>500–50,000 mg/l</td>
<td>50–500 mg/l</td>
<td>1–150 mg/l</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>1–5 µg/g</td>
<td>100–1,000 µg/g</td>
<td>150–200 µg/g</td>
<td>1–5 µg/g</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>50–250 ppb</td>
<td>1,000–50,000 ppb</td>
<td>50–500 ppb</td>
<td>50–200 ppb</td>
</tr>
</tbody>
</table>

*a*Reference values are approximated from the literature; it is important that future studies are carried out to define these levels more clearly. *b*Active inflammatory bowel disease includes ulcerative colitis and Crohn’s disease. ELISA, enzyme-linked immunosorbent assay; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ppb, parts per billion.
Why Calprotectin is the ideal marker?

<table>
<thead>
<tr>
<th>Performance</th>
<th>Qualities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>Be disease specific: identify individuals at risk for IBD and differentiate IBD from non-IBD</td>
</tr>
<tr>
<td>Easy to perform</td>
<td>Able to objectively measure disease activity</td>
</tr>
<tr>
<td>Not or minimally invasive</td>
<td>Able to predict the disease course (relapse or recurrence)</td>
</tr>
<tr>
<td>Cheap</td>
<td>Able to monitor the effect of treatment</td>
</tr>
<tr>
<td>Rapid</td>
<td>Have a prognostic value in assessing morbidity/mortality</td>
</tr>
<tr>
<td>Reproducible between labs and individuals</td>
<td></td>
</tr>
</tbody>
</table>

IBD, inflammatory bowel disease.
Final Message

- Simple, reliable, and noninvasive
- Distinguishing between patients with Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD) with nearly 100% Positive Predictive Value in patients with Rome Criteria and negative calprotectin (<50 ug/g)
- Determining disease activity and risk of relapse in IBD while assessing the level of mucosal healing.
- Assisting in the selection of patients with abdominal symptoms who may require further diagnostic procedures.
Final Message

- Selecting patients for endoscopy as well as monitoring the response to treatment. This is especially useful for children, who may require general anesthesia to undergo more invasive analyses also.
- Predicting relapse in patients with the IBD, allowing an objective marker to decide when to treat also. The use of fecal calprotectin levels promises to offset morbidity by enabling early intervention treatment while disease relapse is still subclinical.