Why FOCUS on Faeces!

Wednesday 20\textsuperscript{th} May 2009

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Conflicts of Interest
I have received grants, honorariums and consultancy fees from many companies:

- Novartis
- Solvay
- Genzyme
- GE Healthcare
- Oxford Outcomes
- Shire
- Astra Zeneca
- TechLab

I deserved them all!
‘The art of stool gazing is dead!’
Why FOCUS on faeces?

- Logical choice for non-invasive tests of gastrointestinal diseases

Examples include:

- Faecal pancreatic elastase
- Faecal lactoferrin
- Faecal occult blood
- Faecal Tumor M2-PK
- Faecal C. difficile
- Faecal H. pylori
Problems in pancreatology

- Pancreatic imaging is not adequate?!
  poor sensitivity, ‘damage’ tests not function

- EUS:
  invasive and time consuming but therapeutic potential

- Function testing:
  convenience, patient preference, sensitivity and specificity
Faecal pancreatic elastase-1

Proteolytic enzyme (28kD)

Initially identified as protease E and confirmed as elastase-1 in stool
~ 6% of pancreatic enzyme

Binds to bile salts & not degraded
Low in dilute specimens (lyophilize/centrifuge/dry)
Not affected by enzyme therapy
Identified using ELISA test

Intra-assay variation 5.8 – 6.4%
Inter-assay variation 7.7 – 8.8%
Sandwich ELISA for Pancreatic Elastase 1
(Enzyme Linked ImmunoSorbent Assay)

Monoclonal antibody to human pancreatic elastase 1 (E1)

Human pancreatic elastase 1 (antigen) from faecal sample or standards

2nd monoclonal antibody to E1, biotinylated (anti E1-bio) conjugated to Peroxidase-streptavidin

Peroxidase oxidises the ABTS substrate, oxidation product is dark green

Concentration of oxidised ABTS is then determined photometrically (405nm)
Sensitivity of faecal pancreatic elastase-1 versus other tests for recognising exocrine pancreatic insufficiency (EPI)

- In severe insufficiency = 100%
- In moderate insufficiency = 89 – 100%
- In mild insufficiency = 33 – 65%

Coeliac disease

- This association was first reported in 1957\(^1\)
- Validated using a variety of direct and indirect tests of pancreatic function
- Assumption that diarrhoea = gluten exposure

Dreiling DA. *J Mt Sinai Hosp NY* 1957;24:243-50

Mechanisms:
- Autoimmune phenomenon
- Impaired CCK release
- Impaired Plasma Peptide YY release
- Malnutrition
- Subclinical pancreatitis

Dimagno EP et al *Gastroenterology* 1972
Deprez P et al *Reg Peptides* 2002
Work Done in Sheffield!

Is exocrine pancreatic insufficiency in adult coeliac disease a cause of persisting symptoms?

Design: cross-sectional

Subgrouped:

- 1) New CD (<6 months) – Group A
- 2) Asymptomatic (On GFD)- Group B
- 3) Ongoing GI symptoms (On GFD) – Group C
- 4) Controls – Group D
p <= 0.0001
Response to therapy

Number of bowel motions per day before and after treatment

* p<0.0001
Summary of data on exocrine pancreatic insufficiency and coeliac disease

- N=259
- 20/66 had low Fel-1 (30%)
- Stool frequency reduced but no changes in weight
- Creon initially at 10,000 units tds then titrated
- Treating those with low Fel-1 leads to significant improvement in ~ 90% cases (p<0.001)

PATHOPHYSIOLOGY

GENETIC FACTORS

VISCERAL HYPERSENSITIVITY

CEREBRAL ABNORMALITIES

AUTONOMIC REACTIVITY

GASTROINTESTINAL INFECTION

IRRITABLE BOWEL SYNDROME

EFFECT OF MOOD ON GI FUNCTION

ABNORMAL ILLNESS BEHAVIOUR

CHILDHOOD EXPERIENCES

PSYCHOLOGICAL MORBIDITY
Patients and methods:

403 consecutive patients were referred to our unit over an 18 month period who met the Rome II criteria for D-IBS. Participants had baseline stool frequency and stool consistency recorded along with demographics and weight. Participants were then investigated as per BSG IBS guidelines (2000).

A stool sample was provided and faecal elastase-1 (Fel-1) was determined.

Those patients with a Fel-1 level of less than 100 µg/g of stool were offered pancreatic enzyme supplementation in the form of Creon 40,000 units tds.

Age and sex matched D-IBS (therapeutic controls) with a Fel-1 greater than 100 were also offered the same pancreatic enzyme supplementation.

Pancreatic imaging was performed using ultrasound or CT. Patients were reassessed at six weeks. We also assessed Fel-1 in 50 individuals without IBS (prevalence controls).
**IBS Data**

<table>
<thead>
<tr>
<th>Group</th>
<th>D-IBS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fel-1 &lt;100</td>
<td>19 (6.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fel-1 &gt;100</td>
<td>295 (93.9%)</td>
<td>50 (100%)</td>
</tr>
</tbody>
</table>
IBS Data

![Graph showing Fel-1 levels in D-IBS and Controls](image-url)
Pancreatic enzyme supplementation reduced median stool frequency from 6/day to 1.5/day in 18/19 (94.7%) D-IBS with a Fel-1 <100 (p<0.001)

Pancreatic enzyme supplementation reduced median stool frequency in 1/15 (6.7%) D-IBS with Fel-1 >100 (p=0.66)

Leeds JS et al Gut 2007;56:Suppl II A65
What is Faecal Lactoferrin (FEL)?

- Lactoferrin is an iron binding glycoprotein secreted by most mucosal membranes.
- Expressed by activated neutrophils.
- Inflammation in the bowel results in acute phase reaction & migration of leukocytes to the gut.
- Production of large number of proteins detectable in serum and stool.
- A significant rise may occur prior to a flare-up?
- Differentiates IBS from (active) IBD?
- Differentiates active from inactive IBD?

Kane et al Am J Gastro 2003
# Faecal lactoferrin publications

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Number of Patients</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker 2007</td>
<td>148 (IBS n=7)</td>
<td>84%</td>
<td>97%</td>
</tr>
<tr>
<td>Langhorst 2008</td>
<td>140 (IBS n=54)</td>
<td>85%</td>
<td>77%</td>
</tr>
<tr>
<td>Schoepfer 2008</td>
<td>136 (IBS n=30)</td>
<td>87%</td>
<td>96%</td>
</tr>
<tr>
<td>Schroder 2007</td>
<td>88 (IBS n=31)</td>
<td>82%</td>
<td>100%</td>
</tr>
<tr>
<td>Kane 2003</td>
<td>271 (IBS n=31)</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>Dai 2007</td>
<td>177 (IBS n=25)</td>
<td>92%</td>
<td>88%</td>
</tr>
<tr>
<td>Silberer 2005</td>
<td>120 (IBS n=40)</td>
<td>AUC 0.69</td>
<td></td>
</tr>
</tbody>
</table>
Sheffield Data

- Patient recruitment over 23 months: Nov 06- Oct 08: 465 patients
- Irritable bowel syndrome n=137
- Ulcerative colitis n=126
- Crohn’s disease n=104
- Healthy volunteers n=98

Sidhu R et al *Gut* 2009;58: Suppl I A108
## Modified Harvey Bradshaw Index (HBI)

Can be influenced by subjectivity (2,3) and by non-GI conditions (4)

<table>
<thead>
<tr>
<th>HBI for CD</th>
<th>HBI for UC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) No of liquid stools/day</strong></td>
<td><strong>1) No of liquid stools/day</strong></td>
</tr>
<tr>
<td><strong>2) Abdominal pain: sum of 7 days ratings</strong>&lt;br&gt;(none,1=mild,2=moderate,3=severe)</td>
<td><strong>2) Abdominal pain: sum of 7 days ratings</strong>&lt;br&gt;(none,1=mild,2=moderate,3=severe)</td>
</tr>
<tr>
<td><strong>3) General well being</strong>&lt;br&gt;(0=very well,1=slightly below par, 2=poor,3=very poor,4=terrible)</td>
<td><strong>3) General well being</strong>&lt;br&gt;(0=very well,1=slightly below par, 2=poor,3=very poor,4=terrible)</td>
</tr>
<tr>
<td><strong>4) Complications</strong></td>
<td><strong>4) Complications</strong></td>
</tr>
<tr>
<td>● Arthritis/arthralgia</td>
<td>● Arthritis/arthralgia</td>
</tr>
<tr>
<td>● Skin/mouth lesions</td>
<td>● Skin/mouth lesions</td>
</tr>
<tr>
<td>● Iritis/uveitis</td>
<td>● Iritis/uveitis</td>
</tr>
<tr>
<td>● Anal fissure,fistula/ perianal abscess</td>
<td>● Anal fissure,fistula/ perianal abscess</td>
</tr>
<tr>
<td><strong>5) Abdominal mass</strong></td>
<td><strong>5) Bleeding per rectum</strong></td>
</tr>
<tr>
<td></td>
<td>(0=none,1=slight,2=moderate,3=severe)</td>
</tr>
</tbody>
</table>
Faecal lactoferrin concentrations in all patients

HV-healthy volunteers
### Table: Faecal Lactoferrin (FEL) Concentration

<table>
<thead>
<tr>
<th></th>
<th>UC</th>
<th>Crohn’s</th>
<th>IBS</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>126</td>
<td>104</td>
<td>137</td>
<td>98</td>
</tr>
<tr>
<td>Mean FEL (±SD)</td>
<td>69.5 (168)</td>
<td>41.4 (139)</td>
<td>1.39 (3.4)</td>
<td>2.4 (7.2)</td>
</tr>
<tr>
<td>Median FEL (± IQ)</td>
<td>6.6 (42)</td>
<td>4 (13)</td>
<td>0 (1.4)</td>
<td>0.5 (2)</td>
</tr>
</tbody>
</table>

*FEL concentrations > in IBD patients compared to IBS (p=0.001) and healthy controls (p=0.001)*

*Comparison between UC & CD groups: p=0.051*
## Comparison of activity in IBD groups

<table>
<thead>
<tr>
<th></th>
<th>UC (n=126) : No of pts median FEL (IQ)</th>
<th>CD (n=104): no of pts mean FEL (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active disease</strong></td>
<td>N=51</td>
<td>N=51</td>
</tr>
<tr>
<td></td>
<td>Median: 26 (102) ug/g</td>
<td>Median: 8.4 (32) ug/g</td>
</tr>
<tr>
<td><strong>Inactive disease</strong></td>
<td>N=75</td>
<td>N=53</td>
</tr>
<tr>
<td></td>
<td>Median: 3 (8.5) ug/g</td>
<td>Median: 1 (6) ug/g</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>p&lt;0.001</td>
<td>p=0.002</td>
</tr>
</tbody>
</table>
## Quantitative Stool Test – *IBD-SCAN*

<table>
<thead>
<tr>
<th></th>
<th>All IBD (active &amp; inactive) vs IBS &amp; controls</th>
<th>Active IBD vs IBS &amp; controls</th>
<th>Active IBD vs inactive IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>44%</td>
<td>67% (CD 60%, UC 79%)</td>
<td>67%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>95.7%</td>
<td>95.7%</td>
<td>74%</td>
</tr>
<tr>
<td><strong>Positive Predictive Value</strong></td>
<td>91%</td>
<td>87%</td>
<td>67%</td>
</tr>
<tr>
<td><strong>Negative Predictive Value</strong></td>
<td>64%</td>
<td>86.8%</td>
<td>74%</td>
</tr>
</tbody>
</table>
ROC: Active IBD vs. IBS and healthy controls

Auc: 0.929
**Table 1.** Descriptive Statistics for PMN-e, Cal, and LF for Endoscopy-Based Classification of Inflammation for UC, CD, and IBS

<table>
<thead>
<tr>
<th></th>
<th>UC No Inflammation (N = 15)</th>
<th>UC Inflammation (N = 27)</th>
<th>CD No Inflammation (N = 10)</th>
<th>CD Inflammation (N = 33)</th>
<th>IBS No Inflammation (N = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>4.34&lt;sup&gt;b&lt;/sup&gt; (0–104)</td>
<td>51.1&lt;sup&gt;b&lt;/sup&gt; (1–1,669)</td>
<td>6.4±&lt;sup&gt;±&lt;/sup&gt; (0.01–103)</td>
<td>55.1&lt;sup&gt;b&lt;/sup&gt; (1.3–1,795)</td>
<td>1.82±&lt;sup&gt;±&lt;/sup&gt; (0–90)</td>
</tr>
<tr>
<td>Cal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>34.5&lt;sup&gt;b,±&lt;/sup&gt; (5.0–134)</td>
<td>108.6&lt;sup&gt;b&lt;/sup&gt; (8.7–311)</td>
<td>11.2&lt;sup&gt;b&lt;/sup&gt; (0–168)</td>
<td>105.0&lt;sup&gt;b&lt;/sup&gt; (12.4–347)</td>
<td>7.1±&lt;sup&gt;±&lt;/sup&gt; (0–77)</td>
</tr>
<tr>
<td>PMN-e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>0.035±&lt;sup&gt;±&lt;/sup&gt; (0–1.3)</td>
<td>0.18&lt;sup&gt;±&lt;/sup&gt; (0.01–0.81)</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt; (0–0.48)</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt; (0.01–1.04)</td>
<td>0.02±&lt;sup&gt;±&lt;/sup&gt; (0–0.3)</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt; (0–2.9)</td>
<td>0.70&lt;sup&gt;a&lt;/sup&gt; (0–3.9)</td>
<td>0.45 (0–3.2)</td>
<td>0.9 (0–13.7)</td>
<td>0.20±&lt;sup&gt;±&lt;/sup&gt; (0–1.2)</td>
</tr>
<tr>
<td>CDAI/CDAI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Range</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt; (0–5)</td>
<td>7.0&lt;sup&gt;b&lt;/sup&gt; (3–13)</td>
<td>76.0 (7–132)</td>
<td>98.0 (5–237)</td>
<td></td>
</tr>
</tbody>
</table>

Inflammation was defined as endoscopic score of ≥1; no inflammation was defined as endoscopic score = 0.

Data are shown as µg/mL. PMN-e = polymorphonuclear-elastase; Cal = calprotectin; LF = lactoferrin. (Published cutoffs: ≥0.062 µg/mL for PMN-e, >6 µg/mL for Cal, >7.25 µg/mL for LF).

<sup>a,b</sup> Letters indicate significant differences between inflamed and noninflamed patients (Mann-Whitney tests: <sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01).

<sup>±</sup> For all indices, IBS patients had significantly lower scores than UC or CD patients with endoscopic inflammation (all P < 0.001, Mann-Whitney tests).

<sup>Î±,Î±</sup> IBS patients had significantly lower scores than UC without endoscopic inflammation (for Cal P < 0.01, for PMN-e P < 0.05) and CD without inflammation (for LF P < 0.05) (all Mann-Whitney tests).
Conclusions

Faecal elastase:
- Established first-line test for exocrine pancreatic insufficiency in chronic pancreatitis and cystic fibrosis patients
- Has an evolving role in coeliac patients with persisting symptoms
- In due course it may become an established test in the first line investigation of irritable bowel syndrome (IBS)
- Further research/work is required in the areas of inflammatory bowel disease (IBD), diabetes and alcohol related liver disease to establish its clinical utility in these patient groups

Faecal Lactoferrin:
- Is an inexpensive and non invasive test that can provide the clinician with a marker to differentiate between IBD and IBS
- FEL can also be used as an adjunct to blood parameters and clinical symptoms to determine IBD patients who have ongoing inflammation
- Faecal lactoferrin may help stratify patients with GI symptoms into those who do /don't require endoscopic investigations (work ongoing)
Thank you and Questions!