No defined biomarker for IBS

Diagnosis by well defined clinical features.....?
- Manning criteria 1978
- Rome I and Rome II 1999, at least 12 weeks or more during the last year have experienced abdominal pain/discomfort that have 2/3 features
  * Relief with defecation
  * Change in frequency of stools
  * Change in form (appearance) of stools

Gut 2000;47:506
IBS, a significant economic burden to the society.

Affect approx 12-15% of the population in EU and USA (India 25%)

About 40% of the IBS pts seek medical advice from family physician

- 12 % of all primary care consultations
- 40% referred for colonoscopy

Typical work-up in a single patient; $ 2387 = € 2000

Cost the American health system $ 25 000 000 000 !!!
What does IBS cost in the EU countries?

IBS and health cost in 8 European countries

UK, Fr, Ge, Sp, It, Ne, Be, Su = 330 mill people

- Total cost US-$ 40 000 000 000

- 1/3 is direct cost like colonoscopy etc

- 2/3 Indirect cost, absenteeism, disability

Quigley Dig Liver Dis 2006
Alarm symptoms like rectal bleeding in young persons; what does it really mean??

20% of the population will have rectal bleeding in a year

Only 50% of CRC do actually bleed, and if so usually intermittently..........

A study from the USA, showed that you will have to do 11500 scopes to diagnose one CRC in a patient below 40 years of age ............
The incidence of Colorectal cancer by different age groups in Norway

The average incidence between 2004-2008

Total  3535 (m/f: 1756 / 1779)

Total < 50 years of age  10%

Total < 45 years of age  3%

Total < 40 years of age  1%
Faecal Calprotectin in Irritable Bowel Syndrome; “the London experience”

- Average waiting list for colonoscopy in the UK is 28 weeks
- Kings college implemented F-Cpt in clinical practice 5 years ago
- They pre-screen all younger (< 45) referred for colonoscopy, a negative test leads to “no colonoscopy”
- “Waiting list” for colonoscopy in the UK, 28 weeks, Kings College, 8 days

Prof. Bjarnasson, pers com.
The use of laboratory analysis in Sweden
Quality and cost-effectiveness in test utilization
PhD thesis from University of Uppsala 2010
Mirja Mindemark

“F-Calprotectin screening could lead to cost avoidance of € 17-23 mill annually in Sweden as compared to direct referral for colonoscopy”

National Health Service UK 2010, purchasing and supply service.

Economic report

Value of Calprotectin in screening out IBS
February 2010
The use of Calprotectin in the detection of inflammation of the bowel has the potential to improve the management of IBS pts in primary care, reducing the need for referral to Secondary care. Health care recourses utilisation would therefore be reduced.
Case story I

40 year old woman, many years with abd. pain and diarrhea.

Normal colonscopy and gastroscopy including biopsies

Normal Calprotectin

Weaping during both examinations, despite sedation...?

Follow-up consultation, sexually abused by close relative and living in a violent marriage

Needs a psychiatrist not a gastroenterologist
Data from the USA, shows that among women with IBS, as many as 22-50% have been neglected or sexually abused during childhood. When balloon-stimulating the rectum, using 50 ml balloon, they experience strong pain.

Functional MRI of the brain shown enhanced activity in the central region of the brain.
Functional MRI in an abused IBS patients

Figure 1. Response to painful (50 mm Hg) distentions. Greater activation in left MCC (ROI-FDR, $P = .022$, $t = 5.61$, at $-8, 6, 42$) and left PCC (ROI-FDR, $P = .033$, $t = 5.00$, at $-12, -34, 42$) in subjects with a history of abuse compared with subjects without abuse history. The lower activation in the left sACC was not statistically significant after volume correction. Note that the 3 regions presented (sACC, MCC, and PCC) together form the dorsal cingulate cortex.

Figure 3. Response to painful (50 mm Hg) distentions. Greater activation in left MCC (ROI-FDR, $P = .021$, $t = 5.29$, at $-8, 6, 42$) and left PCC (ROI-FDR, $P = .049$, $t = 4.81$, at $-12, -34, 42$), less activation in the left sACC (ROI-FDR, $P = .01$, $t = 4.86$, at $-6, 42, 6$) and right sACC (ROI-FDR, $P = .078$, $t = 3.13$, at $8, 42, 8$), and a trend for greater activation in right PCC (ROI-FDR, $P = .077$, $t = 3.82$, at $2, -52, 20$) in subjects with both IBS and a history of abuse compared with other subjects. Note that the sACC effects that can be seen in Figures 1 and 2 as trends attain significance when comparing those with both IBS and abuse against those with only one or neither condition.
Malabsorption in post-infectious IBS patients

Figure 5. Faecal fat excretion (g/day) in Giardia cured patients (n=23) and healthy controls (n=16). The dotted line represents the upper reference level for faecal fat excretion in health.
32 year old woman meny years troubled with abd. pain and diarrhea.

At age 16 diagnosed with IBS.............?

2010, Calprotectin < 1250mg/kg (n< 50)

Telephone conversation offering colonoscopy next day; ” no thank you, On my way to Tenerife”. It’s only my IBS, I’m used to that.

Colonoscopy 3 weeks later; massive Crohns disease of the colon and T. Ileum

Both parents were physicians.......
Food intolerance and irritable bowel syndrome

116 pts with IBS according to the Rome ll criteria

93 women and 23 men

All pts had a sigmoidoscopy (< 40) or a colonoscopy (>40) w/biopsy

4 weeks diet eliminating;
  - cow milk protein
  - wheat protein
  - egg
  - tomato
  - chocolate

Pts improved, underwent a double blind, placebo controlled food challenge

Carroccio Clin Gastro Hepatol 2010
Food intolerance and IBS, examinations I

DBPC was performed administrating capsules containing
- milk protein
  * casein
  * lactalbumin
  * lactoglobulin
- wheat protein

Serum Total IgE and allergen specific IgE was assessed
- egg
- cow milk
- soy
- peanut
- wheat
- tomato
Food intolerance and IBS, examinations II

Flow Cytometric Allergen Stimulation Test (Flow2Cast)

Patients were tested on the following allergens:
- α- lactalbumin
- β- lactalbumin
- casein
- egg white and yolk
- wheat
- soybean
- fish
- tomato
- plus others if indicated

Intra assay and inter assay variation was 2.5% and 6.8%
Reproducibility, 20 pts and 3 investigators; Kappa value 0.92

Carroccio Clin Gastro Hepatol 2010
Food intolerance and IBS, results

44 of the pts improved during the 4 weeks food elimination period

These underwent DBPC food challenge
- 19 were intolerant for both cow milk and wheat protein
- 3 were intolerant for cow milk only
- 2 were intolerant for wheat only

Other foods causing IBS like symptoms
- egg 16 cases
- tomato 12 cases
- soy protein 5 cases
- yeast, pork, prawns, fish, celery 3 cases each
Food intolerance and IBS, results from BAT

Patients and controls (non-IBS) were tested using
- Flow2Cast vs total IgE, $p < 0.0001$

Cow milk allergy
- Flow2Cast vs sp-IgE $p < 0.01$
- Flow2Cast vs total IgE $p < 0.0001$

Wheat allergy
- Flow2cast vs sp-IgE $p < 0.01$

Carroccio  Clin Gastro Hepatol 2010
In conclusion, in patients with IBS, this method is feasible for making a diagnosis for food hypersensitivity. It may replace routine allergy tests such as skin prick test and specific IgE assays.
Gluten Causes Gastrointestinal Symptoms in Subjects Without Celiac Disease: A Double-Blind Randomized Placebo-Controlled Trial

Jessica R. Biesiekierski, B Appl Sci¹, Evan D. Newnham, MD, FRACP¹, Peter M. Irving, MD, MRCP¹, Jacqueline S. Barrett, PhD, BSc, MND¹, Melissa Haines, MD¹, James D. Doecke, BSc, PhD², Susan J. Shepherd, B Appl Sci, PhD¹, Jane G. Muir, PhD, PGrad Dip(Dietetics)¹ and Peter R. Gibson, MD, FRACP¹
Screened n=103

Celiac disease not excluded (34)
Unwillingness to participate (23)
Symptomatic on gluten-free diet (7)

Randomised n=39

Gluten n=20

Inadequate symptom control during baseline (1)

Per protocol Gluten n=19

Placebo n=19

Inadequate symptom control during baseline (3)
Acute psychiatric illness (1)
Results from Gluten vs placebo

VAS score 0= none, 100 worst

Gluten

Placebo
Flow2 CAST® Principle

- **positive control anti FcεR**
- **anti CD 63 FITC**
- **anti CCR3 PE**

- **Allergen**

- **pseudo allergic reaction (non IgE mediated)**
CCR3 as Basophil Selector

Basophils
Eosinophils
Neutrophils
Lymphocytes
Monocytes

Cellular Allergy / MC

October 2009
In conclusion,

IBS is a heterogeneous patient group, were organic aetiology cannot be ruled out, but colonoscopy is NOT necessary the thing to do.
Cow milk allergy in children

Affects between 2 – 3 % (7%) of children < 1 year of age

Requires complete exclusion of milk protein in diet

50% of the children outgrows the hypersensitivity by 1 year, 80 – 90 % by age 5.
Symptoms of cow milk allergy in children

Often simultaneous symptoms from many organs;
- atopic dermatitis 50 – 70 %
- gastrointestinal symptom 50 – 60%
  * vomiting
  * diarrhoea
  * malabsorption
  * failure to thrive
- respiratory tract 20-30%
Diagnosis of cow milk allergy in children

Dietary elimination of cow milk protein, also from mother in case of breast feeding

In case of improvement, reintroduction of CMP. If resumption of symptoms, further testing.

- Specific IgE testing (Rast), sens approx 67 % (cutoff 2kUA/l)

- Prick test, may be dangerous

- Basophile Activation Test (BAT) Flow2Cast

Rubio Allergy 2010
Results from a comparison study

Sensitivity and specificity for detection of CMP allergy
- IgE; 67% / 67%

- SPT; 100% / 21%

- BAT; 91% / 90%

Kappa statistics for oral CMP challenge
- IgE 0.35

- SPT N A

- BAT 0.752
Figure 1 Receiver operating characteristic (ROC) curve for the basophil activation test (BAT) with cow’s milk. Receiver operating characteristic curve for the percentage of basophils activated by cow’s milk proteins (CMP). The area under ROC curve (AUC) = 0.866.

Figure 3 Receiver operating characteristic (ROC) curves for specific IgE (sIgE) and skin prick tests (SPT) with cow’s milk. (A) ROC curve for cow’s milk sIgE. The area under ROC curve (AUC) = 0.758. (B) ROC curve for SPT with cow’s milk. AUC = 0.809.
Table 1. Patient characteristics according to the dietary treatment group

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Gluten</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Median age (range) in years</td>
<td>40 (29–55)</td>
<td>49 (33–51)</td>
</tr>
<tr>
<td>Men</td>
<td>16%</td>
<td>7%</td>
</tr>
<tr>
<td>Median body mass index range (range)</td>
<td>23 (18–41)</td>
<td>22 (18–33)</td>
</tr>
</tbody>
</table>

**Number with predominant bowel habit**

<table>
<thead>
<tr>
<th></th>
<th>Gluten</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipation in percentage</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Diarrhea in percentage</td>
<td>58</td>
<td>33</td>
</tr>
<tr>
<td>Alternating percentage</td>
<td>26</td>
<td>47</td>
</tr>
</tbody>
</table>

**HLA type**

<table>
<thead>
<tr>
<th></th>
<th>Gluten</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQ2 or DQ8 positive in percentage</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td>DQ negative in percentage</td>
<td>47</td>
<td>40</td>
</tr>
</tbody>
</table>

**Elevated serum celiac antibodies (percentage of patients (mean (s.e.m.) Units/ml))**

<table>
<thead>
<tr>
<th></th>
<th>Gluten</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue transglutaminase (IgA)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tissue transglutaminase (IgG)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endomysium (IgA)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Whole gliadin (IgA)</td>
<td>27 (39 (9))</td>
<td>30 (33 (9))</td>
</tr>
<tr>
<td>DQ2/8 positive</td>
<td>5 (33 (0))</td>
<td>7 (24 (0))</td>
</tr>
<tr>
<td>Whole gliadin (IgG)</td>
<td>25 (25 (1))</td>
<td>0</td>
</tr>
<tr>
<td>DQ2/8 positive</td>
<td>5 (23 (0))</td>
<td>0</td>
</tr>
</tbody>
</table>

HLA, human leukocyte antigen.

There were no significant differences between dietary groups for any index (independent samples t-test and $\chi^2$ test).
Table 2. Celiac serology, intestinal permeability, and C-reactive protein results before and during therapy with gluten or placebo, shown as median (range), and changes in those indices, shown as mean (s.e.m.)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Glutin (n=19)</th>
<th>Placebo (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (Unit/ml)</td>
<td>With therapy (Unit/ml)</td>
</tr>
<tr>
<td>Tissue transglutaminase (IgA)</td>
<td>3.0 (2.0–7.0)</td>
<td>4.5 (2.0–7.0)</td>
</tr>
<tr>
<td>Whole gliadin (IgA)</td>
<td>10.8 (3.5–241.5)</td>
<td>4.6 (0.1–51.3)</td>
</tr>
<tr>
<td>Whole gliadin (IgG)</td>
<td>14.6 (12.1–31.5)</td>
<td>15.5 (11.4–50.6)</td>
</tr>
<tr>
<td>Intestinal permeability (L:R ratio)</td>
<td>0.02 (0.01–0.6)</td>
<td>0.01 (0.01–2.4)</td>
</tr>
<tr>
<td>Highly sensitive C-reactive protein (mg/l)</td>
<td>1.4 (0.3–5.3)</td>
<td>0.3 (0.4–19.8)</td>
</tr>
</tbody>
</table>

ANCOVA, analysis of covariance; L:R ratio, lactulose-to-rhamnose ratio

There were no statistically significant differences within each dietary group (paired t-test on log-transformed data) or between treatment groups whether evaluated using baseline and treatment data (ANCOVA) or the changes in indices (independent samples t-test; all P>0.1).
1. **Preparation of tubes**
   - Add 50 µl Allergen/Control (in Stimulation Buffer)
   - Add 100 µl Stimulation Buffer (contains IL-3, Ca2+ and Heparin)
   - Add 50 µl Whole Blood (EDTA)
   - Add 20 µl Staining Reagent (contains CCR3-PE/CD63-FITC)

2. **Stimulation** Incubate 15’ / 30’ at 37°C in water bath

3. **Lyse** Add 2 ml Lysing Solution 5’ at RT

4. **Spin** 5’ at 500 x g

5. **Resuspend** cells with 300 µl Wash Buffer

6. **Measure Acquire data**

7. **Analyze**
Samples to Measure

Patient Background
Stimulation buffer
+Blood
+Staining

Positive Control
Stimulation control
+Blood
+Staining

Allergen
Allergen X
+Blood
+Staining

PB
PC
A1
Lysing of red blood cells → Centrifugation → Analysis

Results within 45 min
• Choice between technology
• evaluated and adapted allergens
• efficient tools for non IgE mediated drug allergy diagnosis
• provide additional benefit to sIgE
Advantages of the new protocol

Fast protocol
- Short incubation times
- Time to result 1h!
- Incubation times down to 25 minutes

Whole blood
- Easier handling
- no cell isolation
- More reproducible (leucocytes recovery)

Basophil selection with CCR3
- Easier gating and data analysis
- Two color assay (FITC and PE)

Test suitable for routine Laboratories
A major concern is the seriousness of allergic reactions

Around 30% of the western population is affected by allergies during their lifetime

A Swiss study estimates the incidence of life threatening anaphylaxis caused by Allergy at 8-10 cases per 100000.

60% of anaphylactic shocks are caused by Hymenoptera stings
20% of anaphylactic shocks are caused by reactions to drugs.
10% of anaphylactic shocks are caused by food allergies
Bee & Wasp Venoms

1. Clarification of ambiguous results:
2. Fewer false positive and false negative results
3. Optimised differentiation between bee wasp venom allergy
4. Successful monitoring of specific immunotherapy
Why CCR3 as Basophils detector

Total IgE: 1.9 to 2.3 IU/ml

17 to 21 IU/ml

Donor 1

Donor 2

Donor 3

Cellular Allergy / MC

October 2009
BASOPHIL

Before stimulation

D’après M Dvorak et al. JACI 1994
After stimulation

D’après M Dvorak et al. JACI 1994
Allergy is a chronic disease with high morbidity, causing high costs and has become a significant socio-economic topic in society.
Total Health cost estimates reach a level of 30 Billion Euros for Western Europe, 1.5-2 Billion CHF in Switzerland alone.
Which diagnostic measures are usually taken?

Skin tests

sIgE measurements in the patients serum
1) Bee and Wasp Venoms

- **CLEAR DIAGNOSIS**
  - Skin Test ++
  - IgE ++
  - 70-80%

- **UNCLEAR DIAGNOSIS**
  - Skin Test +/-
  - IgE +/-
  - ~20%

- **NEGATIVE DIAGNOSIS**
  - Skin Test -
  - IgE -
  - 10%

Double positive results due to VENOM CROSS-REACTIVITY.

- CAST®-2000 Flow-CAST® allows for clearer diagnosis!

A very high number of double positivities can be resolved by CAST®-Assays!

75% of undiagnosed cases can be solved by CAST®-Assays!
1) Bee and Wasp Venoms

- 70-80% CLEAR DIAGNOSIS
  - Skin Test ++
  - IgE ++

- ~20% UNCLEAR DIAGNOSIS
  - Skin Test +/-
  - IgE +/-

- 10% NEGATIVE DIAGNOSIS
  - Skin Test -
  - IgE -

Double positive results due to VENOM CROSS-REACTIVITY.

A very high number of double positivities can be resolved by CAST®-Assays!

CAST®-2000 Flow-CAST® allows for clearer diagnosis!

75% of undiagnosed cases can be solved by CAST®-Assays!
1) Bee and Wasp Venoms

... AND – no other assay can identify the culprit venom so well, when double positive results occur with serology and in vivo tests are applied!

Relative Specificity of in vivo and in vitro methods in cases of double positivity:

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>CAST 2000</th>
<th>Flow CAST</th>
<th>CAP FEIA</th>
<th>Skin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee+ / Wasp -</td>
<td></td>
<td>93%</td>
<td>83%</td>
<td>57%</td>
</tr>
<tr>
<td>Wasp+ / Bee -</td>
<td></td>
<td>99%</td>
<td>90%</td>
<td>51%</td>
</tr>
</tbody>
</table>

(Scherer et al.2008)
References

5. O. Hausmann et al.: Double positivity in insect venom allergy – diagnostic approach with basophil activation test Poster, SGAI, 2009
7. O. Hausmann et al.: Usefulness of the basophil activation tests in monitoring the immun response to bee venom immunotherapy controlled by sting challenge - pilot phase results, Poster, EAACI, 2008
Food additives

• Cellular Allergy Assays are reliable tools for the diagnosis of non-IgE mediated food additive intolerances
• Excellent sensitivities for commonly used food additives
• To optimise sensitivity the recommended allergen combination to be tested is Benzoate, Nitrite and Salicylate
Recent publication on Flow CAST® application in pediatrics:

**Poster symposium: gastroenterology**

**BENEFIT OF THE BASOPHIL ACTIVATION TEST IN DECIDING TO REINTRODUCE COW’S MILK FOR AN ALLERGIC CHILD**

1A Rubio, 2,3M Vivinus-Nebot, 1T Bourrier, 1M Albertini, 2,3A Bernard. 1Service de Pediatrie, CHU de Nice, Nice, France; 2Laboratoire D’Immunologie, CHU de Nice, Nice, France; 3Unite INSERM 568, Nice, France

**Conclusion:** The BAT could be a very valuable tool in the management of cow’s milk allergy by contributing to determining whether an oral challenge can safely be undertaken. The BAT could thereby help to avoid the danger, stress and expense of repeated positive challenges.
Advantages of the new protocol

Fast protocol
- Short incubation times
- Time to result 1h!
- Incubation times down to 25 minutes

Whole blood
- Easier handling
- no cell isolation
- More reproducible (leucocytes recovery)

Basophil selection with CCR3
- Easier gating and data analysis
- Two color assay (FITC and PE)

Test suitable for routine Laboratories
IgE -mediated Allergy

Non IgE -mediated Allergy

Basophil characterization marker

Basophil activation marker

Allergy Mediators
Patient with Allergy to peanut

Child with anamnesis for peanut, previous eczema, asthma. Challenge pos. for peanut. sIgE unspecifically positive for 3 more allergens additionally to peanut.

Flow2 CAST clear cut signal specifically for peanut. 46-49% of the basophil population expose CD63 on their outer membrane surface.

Negative control below 15%.
New Marker Combination CCR3/CD63