Clinical Audit
on food adverse reactions
and elimination diet
- retrospective study

Background
IgG food antibodies may have a role
in IBS and in GERD

AIMS
assess the therapeutic potential of
elimination diet based on IgG4 antibodies
Intestinal Mucosal Barrier

Proposed mechanism of tolerance induction in the Gut-Associated lymphoid tissue (GALT)

1. Antigen sampling: Dietary Antigens are (a) sampled by dendritic cells (DC) that extende between epithelial cells into the lumen (b) trasported by microfold (M) cells or (c) pass directly between epithelial cells and taken up by DCs

2. Antigen presentation: DCs travel to mesenteric lymph nodes and Peyer's patches and present antigen to naive T cells. Tolerogenic DCs secrete cytokines that induce differentiation of naive T cells into antigen-specific T regulatory (Treg) cells (FoxP3; Th3; Tr1)

3. Suppression of the allergic response: Treg cells inhibit Th1 and Th2 differentiation, suppress mast cells and induce B cell switching from IgE to IgG4 via cell contact or cytokines TGF-beta and IL-10

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Intestinal Mucosal Barrier

- Cronicity of antigen exposure
- Increased permeability to macromolecules
- Hyperactive mucosal immune system

IgG antibodies blocking or sensitizing

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Parish demonstrated the presence of anaphylactic IgG antibodies in human sera

Lancet, 2:591, 1970

IgG anaphylactic antibody activity could not be removed through precipitation with anti-IgE but only by precipitation with anti-IgG, clearly indicating a novel mechanism for mast cell recruitment into inflammation

J Allergy Clin Immunology, 56:417, 1975
Clin Allergy, 9:645, 1979

IgG anaphylactic antibody activity is a subtype of IgG4

Annals of Allergy, 58:14-27, 1987


**IgG4 antibodies may act as sensitizing as well as blocking antibodies**

*Clinical Allergy, 9:263-270, 1979*

*Clin Rev Allergy, 1:213-224, 1983*

*N Engl Reg Allergy Proc, 9(1): 63-6, 1988*

*Int Archs Allergy Appl. Immun, 78:81-85, 1985*

*Allergy Asthma Proc, 23-(6): 373-6, 2002*

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**This dual role of IgG4 lends weight to defining IgG4 subtypes 4a and 4b**

*Allergy. Annals of Allergy, 58:14-27, 1987*

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**IgG4 RSD (Related Systemic Disease) Clinical disorders unified by elevated IgG4 levels and specific histopathologic findings**

*Curr. Opin. Rheumatol. 2011 Jan;23(1):119-24*
**IBS**

Food elimination diet based on IgG involves a significant decrease in symptoms, compared with dietary restrictions not guided by those antibodies

Mast cells and their mediators have been found increased in the proximity of terminal nerves of intestinal mucosa


*Barbara G. Gut 2002;51: 141-144 doi:10.1136/Gut.51 suppl 1.i41*
TOTAL MUCOSAL CELLULARITY

% CELLS/AREA

- HC (n=17)
- IBS-D (n=14)
- IBS-C (n=14)

*p<0.01

Barbara et al. DDW 2000

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Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome

Barbara et al, Gastroenterology 2004

Only mast cells in close proximity to nerves were significantly correlated with severity and frequency of abdominal pain/discomfort.
REDUCED GENOTYPE OF INTERLEUKIN IN PATIENTS WITH IBS

Gonsalkorale et al, Gut 2003

% pz con genotipo G/G (high producer) IL-10

IBS (n=230)  HC (n=450)

* p=0.003

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Potential non IgE-mediated food allergies:
comparison of open food challenge (OFC) and
double blind placebo controlled food challenge
(DBPCFC)

Otolaryngology-head and Neck Surgery 2007-137,803-809

- Evidence of non IgE food reactions because most were negative to food specific SPT and in vitro testing for sIGE

- OFC is a useful method for identifying or confirming food allergy and is nearly as efficient as DBPCFC
Treating irritable bowel syndrome with a food elimination diet followed by controlled food challenge and probiotics


**Significant improvement**
- after IgG elimination diet (p<0.05)
- stool frequency (p<0.05)
- pain (P<0.05)
- IBS-QOL scores (p < 0.0001)

The 1-year follow up demonstrated:
- Significant continued adherence food rotation diet (4.00 +/- 1.45)
- Minimal Symptomatic problems with IBS (4.00 +/-1.17)
- Perception of control over IBS (4.15 +/-1.23)
Food specific serum IgG4 and IgE titers to common food antigens in IBS

Zar e coll Scand Journal Gastroenterology 2005 July 40 (7) : 800-7

- 108 IBS (52 D-IBS, 32 C-IBS & 24 A-IBS) & 43 controls
- IgE & IgG4 titres to 16 common foods included milk, eggs, cheese, wheat, red meats, chicken, fish, potatoes, rice, tomatoes & shrimps
- No significant difference in IgE titres in IBS vs controls
- IgG4 titres to common foods are significantly elevated in IBS vs controls

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Significant improvement after IgG4 elimination diet

- Pain severity: $p < 0.001$
- Pain frequency: $p = 0.034$
- Bloating severity: $p = 0.001$
- Satisfaction with bowel habits: $p = 0.004$

Effect of IBS on life in general at 3 months: $p = 0.008$

Symptoms improvement was maintained at 6 months

Effect of exclusion diet in IBS

Zarecoll Scand Journal Gastroenterology 2005 July 40 (7) : 800-7
IgG4 guided exclusion diet in IBS

Food elimination based on IgG in IBS: a randomised controlled trial


- Double blind, randomised controlled trial
- Specific IgG4 ab titres to a panel of 29 foods tested
- Patients were randomised to ‘true’ diet or ‘sham’ diet x 12 weeks
- Symptom severity was assessed using visual analogue scale

Atkinson et al, Gut 2004; 53: 1459-1464
Mean change in symptoms scores severity at 12 weeks according to the DEGREE OF ADHERENCE

Difference between the groups with high adherence: 101 (95% confidence interval 54, 147) ***p<0.001
Food elimination based on IgG in IBS: a randomised controlled trial


**A)** - MEAN CHANGE in the secondary outcome measures of NON COLONIC SYMPTOMS AND QUALITY OF LIFE for whole group and full adherence group

**B)** - MEDIAN CHANGE in the secondary outcome measures of ANXIETY AND DEPRESSION for whole group and full adherence group
GERD - Eosinophilic esophagitis

Eosinophilic esophagitis (EE) is an increasingly common diagnosis in patients with refractory GERD without esophagitis

Less than 10% of patients with refractory GERD (PPIs twice daily) have an abnormal esophageal reflux profile

Food or aeroallergen hypersensitivity is involved in ~50% of EE

World J Gastroenterol 2006; 12(15): 2328-2334
Am J Gastroenterol 2003; 98: 777-782
Mayo Clin Proc 2003; 78: 830-835
Gut 2003; 52: 181-185

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**Methods**

Retrospective study:
24 months period between February 2010 and March 2012 was chosen among the patients of General Practice

50 patients between 18 and 75 yrs

- 25 IBS by Rome II Criteria (diarrhea predominant) who had failed standard medical therapies - IBD excluded by colonoscopy and histological colonic biopsies

- 25 refractory GERD - PPI b.i.d - HP negative
  Typical Symptoms +
  Dyspepsia
  OGD: NERD subgroup;
  no Hiatus Hernia;
  Microscopic Esophagitis;
  No Eosinophilic Esophagitis

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Test Protocol

General Practice

- Routine Blood tests / ESR-CRP
- Electrolytes
- Thyroid function tests
- Total IgA/IgE
- Aga IgA IgG
- TTG IgA IgG
- Abdominal ultrasound
- Lactose Hydrogen Breath Test
- Comprehensive stool analysis
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Test Protocol
General Practice

Restrictive Diet
Positive Response

Food-specific
serum Antibody titers

Panel food
wheat, rice, corn, gliadine, milk, beef, chicken, pork, lamb,
egg, fish, potatoes, tomatoes, shrimps, yeast, cheese,
soybean, dried fruit

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Food-specific serum IgG1 Antibody titers

We believe is not reliable because IgG1 antibodies tend can bind less selectively to antigens, leading to a greater chance of cross-reactivity and false-positives.
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**Food-specific serum IgG4 Test**

**Top left:** schematic of allergen component microarray layout

**Top right:** schematic of assay principle. Allergen components are covalently coupled to the solid phase. Allergen specific IgG4 antibodies are detected by fluorescence labeled anti IgG4

**Bottom Left:** scan image of fluorescence laser scanning microscope showing different intensities from black (negative) to white (strong positive) on false color scale

**Bottom Right:** Schematic of IgG4 assay reporting
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<table>
<thead>
<tr>
<th>Test Titers</th>
<th>Allowed Food</th>
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<tbody>
<tr>
<td>&lt; 2000 UI</td>
<td>Allowed Food</td>
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<tr>
<td>&gt;2000 UI</td>
<td>Allowed Food moderation</td>
</tr>
<tr>
<td>&gt;5000 UI</td>
<td>Forbidden Food</td>
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Food-specific serum IgG4

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Number of subjects in IBS with elevated IgG4 Food antibody titers at cut-off value >2000 UI and 5000 UI

<table>
<thead>
<tr>
<th>Food</th>
<th>IgG4 &gt; 2000</th>
<th>%</th>
<th>IgG4 &gt; 5000</th>
<th>%</th>
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<td></td>
</tr>
<tr>
<td>gliadine</td>
<td></td>
<td></td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>milk</td>
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<td>23</td>
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<td></td>
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Number of subjects in GERD with elevated IgG4 food antibody titers at cut-off value >2000 UI and 5000 UI

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<th>IgG4 &gt; 5000</th>
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IgG4 Antibodies Titer >5000  IBS - GERD

- Wheat
- Milk
- Tomatoes
- Eggs
- Yeast
- Chicken
- Fish

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Lactose Breath Test H2

IBS Group 7 pts positive 28%

GERD Group 4 pts positive 16%
Elimination Diet

Lactose free diet was started before IgG4 food elimination diet without good improvement

The elimination diet was formulated taking into consideration the Taxonomy of food.

A replacement diet was guaranteed for three months

IgG4 Gliadine >5000

Before starting gluten free diet, Coeliac disease was excluded by oriented duodenal biopsies and where necessary looking for Genetic Predisposition (DQ2 - DQ8)

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IBS Group 25 pts

BOWEL MOVEMENTS

IBS 70% improved having 1 bowel movement each day

Free Diet
Exclusion diet

IBS 70%

improved

having 1 bowel

movement each
day

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**GERD Group 25 pts**

- Free Diet
- Free diet
- mild
- moderate
- severe

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<tr>
<th>Condition</th>
<th>Free diet</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
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GERD Group 25 pt

Symptomatology evaluation two months after beginning of exclusion diet

GERD typical symptoms and dyspepsia disappeared in 56% of cases.

GERD symptoms and dyspepsia became mild in about 50% of the remaining cases.

Exclusion diet
mild
moderate
severe

GERD Group 25 pt

Symptomatology evaluation two months after beginning of exclusion diet

GERD typical symptoms and dyspepsia disappeared in 56% of cases.

GERD symptoms and dyspepsia became mild in about 50% of the remaining cases.
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GERD
Control Group 13 Pts

Sham Diet

Free Diet

No different symptomatology in the control group following the sham diet

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Conclusions

- IgG4 food exclusion diet might be of great help in IBS and refractory GERD treatment.
- IgG4 food test should be requested when there is a reason to suspect food adverse reaction and food allergy test is unremarkable.
- We hope further retrospective and prospectives studies.