SERUM CYTOKINES ARE ELEVATED IN PATIENTS WITH IRRITABLE BOWEL SYNDROME (IBS) BUT LARGELY UNRELATED TO SYMPTOM CHARACTERISTICS

SEAN M. P. BENNET 1, OLAFUR PALSSON 2, WILLIAM E WHITEHEAD 2, DAVID A BARROW 2, HANS TÖRNBLOM 1, LENA ÖHMAN 1, MAGNUS SIMRÉN 1, MIRANDA A.L. VAN TILBURG 2.

1. INTERNAL MEDICINE AND CLINICAL NUTRITION, MEDICINE, GOTHENBURG, SWEDEN.
2. CENTER FOR FUNCTIONAL GI AND MOTILITY DISORDERS, UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, NC, UNITED STATES.
Irritable Bowel Syndrome (IBS)

Understanding the pathophysiology of irritable bowel syndrome is challenging:

- Heterogeneity among the patients
- Large overlap with healthy profile

ROME III subgroups based on bowel habits

- IBS-Constipation
- IBS-Diarrhea
- IBS-Mixed

Few differences in the underlying pathophysiological mechanisms between subgroups have been identified.
Low grade inflammation in IBS

- Altered levels of immune mediators indicative of immune system activation is proposed to be relevant in IBS pathophysiology

Cytokines

Messenger signal proteins affecting behavior of secreting cell or another cells

Among other roles, important in coordinating an immune response

Markers of immune activity and inflammation

- Slight increase in systemic concentrations of cytokines IL-6, IL-8 and TNF in IBS
Prior findings:

• Recently our group demonstrated that serum levels of IL-6 and IL-8 tended to be increased in IBS patients.

• A small cluster of IBS patients with an immuno-active profile was identified.

• Still, the link between altered cytokine levels and IBS symptoms is unclear.

(Bennet et al. AJG 2016)
Examination of serum cytokine profiles of healthy and IBS subjects through multivariate analysis

Hypothesis

• Within each IBS cohort a cluster of patients is present which have an increase in immune activity compared to other IBS patients and healthy subjects

• An increased immune activity is associated with symptoms of IBS

Aim

• To determine the serum cytokine levels in IBS and healthy subjects

• To establish if cytokine levels are linked to IBS symptoms and other pathophysiological factors
Study cohort

- **246** ROME III IBS patients were enrolled at the University of North Carolina at Chapel Hill

  IBS-C = 35  
  IBS-D = 51  
  IBS-M = 160

**Exclusion factors:**

- Inflammation or other GI disease
- Being pregnant at the time of the study

- **21** Healthy volunteers with no prior history of GI disorders within prior seven days of study

- No study subjects were taking any medication known to affect the immune system or gastrointestinal tract
Materials and Methods

Serum obtained from 246 IBS 21 healthy

Bio-Plex 200, Bio-Rad immunoassay for detection of

Pro-inflammatory cytokines IL-6, IL-8, TNF

and

Anti-inflammatory IL-10 in serum
Principle Component Analysis (PCA)

- Allows for the unsupervised definition of groups through cluster analysis of a data set with multiple variables
- Uses all variables simultaneously
- Shows which variables are responsible for cluster discrimination
Healthy subjects have similar serum cytokine profiles while IBS are more heterogenous.
Serum levels of cytokines increased in IBS patients compared to healthy controls.
Four clusters identified by unsupervised hierarchical analysis

Prominent cluster cut off
Four clusters identified by unsupervised hierarchical analysis

Cluster 1: 7 healthy and 18 IBS
Cluster 2: 11 healthy and 105 IBS
Cluster 3: 2 healthy and 72 IBS
Cluster 4: 0 healthy and 49 IBS
Altered symptoms between clusters

**Pain Threshold (z-Score)**

- Cluster 1 (14 IBS)
- Cluster 2 (76 IBS)
- Cluster 3 (51 IBS)
- Cluster 4 (39 IBS)

**Catastrophe Score**

- Cluster 1 (7 healthy and 18 IBS)
- Cluster 2 (11 healthy and 105 IBS)
- Cluster 3 (2 healthy and 72 IBS)
- Cluster 4 (0 healthy and 49 IBS)

$p=0.01$

$p=0.02$

$p=0.01$
No difference between clusters regarding other measured symptoms

- **IBS - Symptom Severity Score (Total)**
  - Pain Severity
  - Bloating
  - Satisfaction with bowel movements
  - Interferance of symptoms with daily life

- **Motility**
  - Baseline
  - Distention
  - Post Meal
  - Resting

- **RSPQ - Somatizisation**

- **BSI - Depression**

- **BSI - Anxiety**

- **Onset of IBS symptoms**
Orthogonal partial least squares-discriminant analysis (OPLS-DA)

- Supervised multiple regression analysis
- Identifies discrimination between different datasets:
  X Variables (Cytokine serum level)
  Y Variables (IBS and healthy subjects)
- Uses all variables simultaneously
- Shows variables responsible for class discrimination

SIMCA
Multivariate analysis allows for identification of patients with an enhanced immune activity.
Serum levels of cytokines increased in Immuno-active patients compared to immuno-norm patients

No difference in symptoms between the two patient groups
Immuno-active cluster comprised some subjects from some from unsupervised Clusters 2 and 3 but all from Cluster 4

Cluster 2
Cluster 3
Cluster 4

Cluster 4
(0 healthy and 49 IBS)
Summary

• In this study we identified clusters among IBS patients and healthy subjects based on serum cytokine profiles

• Serum cytokines did not seem to correlate with IBS symptoms
Conclusion

• Serum cytokines are elevated in IBS patients compared to healthy subjects

• Immune activation characterizes 45% of IBS patients, but the modest associations between the cytokine profile and IBS symptoms suggests immune activity does not play a direct role in symptom generation in IBS
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