Study Protocol
Brachyspira and intestinal allergy-like immune reactions in patients with irritable bowel syndrome (IBS)

Original version: 17-8-2021

This version is based on the original English version. This version included only visit 1, 2, and 3. Visit 4,5, and 6 are added later to the protocol since this was added to the Swedish version of the protocol.

This current English protocol (based on the Swedish originals) is made 29-4-2024

Background

IBS, characterized by abdominal pain and abnormal bowel habits, but normal findings at clinical investigations, is the most common functional GI disorder, and affects 5-10% of the adult population. Few effective treatment alternatives exist, largely due to incomplete understanding of the underlying pathophysiology. Many IBS patients frequently consult different health care providers, report absence from work, and have poor quality of life, leading to profound suffering, and high costs for individuals and society.

Current view on IBS pathophysiology. Today, IBS is considered a disorder of gut-brain interactions, where abnormalities in the gut and the brain interact and explain the hallmark symptoms of IBS, abdominal pain and abnormal bowel habits. However, causes of the abnormal function in the gut and the disturbed interaction with the brain are unknown.

The gut microenvironment and food. Gut microbiota alterations, as well as other factors in the gut microenvironment, such as altered immune and barrier function, are considered to be of relevance in IBS. The current view is that these alterations can interact with our nervous system and generate symptoms in susceptible individuals. However, the exact mechanisms are still not known. To date, the most important factors accepted to trigger the onset or exacerbation of IBS are gastrointestinal infections and psychological factors. In addition, the majority of patients report food-related symptoms. The focus on diet and IBS has so far mainly been on major food groups (e.g., fermentable carbohydrates), rather than on individual nutritional components and potential allergy-resembling mechanisms. However, recent ground-breaking studies indicate that diet-microbiota interactions are responsible for local immune reactions in the gut. A study in mice demonstrated that a bacterial GI infection could promote break of oral tolerance to dietary antigens, and ingestion of these food antigens after the infection was resolved led to mast cell activation and the development of long-standing visceral hypersensitivity. Moreover, local injection of food antigens into the colon in a small group of IBS patients induced local edema and mast cell activation, supporting the involvement of loss of tolerance to dietary antigens in IBS. Another study identified signs of nonclassical food allergy in the small intestine in more than 50% of patients with IBS. These studies have demonstrated novel peripheral mechanisms underlying food-induced symptoms in IBS without systemic signs of classical allergy, induced by diet-microbiota interactions.

This project will focus on the recent discoveries of *Brachyspira* infection and intestinal allergy-like immune reactions as potential causative factors for symptoms in IBS. It will advance this field substantially, by identifying novel mechanisms for symptom generation and treatment targets for large groups of patients, and specifically identify subgroups of IBS patients where treatment options today are absent. To achieve the goals, we will use our unique collaborative set-up with large and well-characterized patient cohorts and state-of-the art methods to study the link between symptom reports and objective findings and test novel treatment options

Aim

To define local immune responses in the GI tract to food antigens in IBS patients with and without Brachyspira infection using advanced imaging.

Hypothesis

We hypothesize that *Brachyspira* infection can cause IBS symptoms by inducing loss of oral tolerance to dietary antigens through development of food-specific intestinal immune reactions and subsequent development of visceral hypersensitivity.

Methods

Subjects

All patients will be carefully characterized at baseline including detailed clinical phenotyping, allergy testing, nutrient intake, detailed information about adverse food reactions, collection of blood and fecal samples, and identification of *Brachyspira* in colonic biopsies. Before and after the endoscopic food challenge, biopsies will be taken. These will be analyzed with a particular focus on the mucosal immune response (e.g. tryptase activity, as a measure of mast cell degranulation; immunohistochemistry to determine IgE/mast cells and other immune cells; mucosal structure, eosinophils, and intraepithelial lymphocytes with Hematoxylin and Eosin histology). Baseline characteristics (e.g. clinical and nutritional profile, gut microbiota composition and function, *Brachsypira* infection, immune activation) associated with a positive food challenge test, i.e. with an intestinal allergy-like reaction, will be determined.

Inclusion criteria

- IBS patients aged between 18 and 70 years.
- Patients with IBS diagnosis according to their treating physician.
- Association between intake of food and GI symptoms.
- Witnessed written informed consent prior to any study procedures.
- Patients who are capable to understand the study and the questionnaires, and to comply with the study requirements.

Exclusion criteria

- Patients with relevant concurrent organic GI disease (inflammatory bowel disease, abdominal cancer), or a major disease such as diabetes, uncontrolled thyroid disease, heart disease, kidney disease, liver disease, and active malignant disease (not those that were in remission at least 5 years).
- Patients with a history of bowel surgery (not appendectomy or cholecystectomy) that affects GI motility.
- Patients with systemic food allergy as evidenced by positive allergy tests (blood, prick test).
- Clinical history of severe allergic reactions.
- Patients with concurrent major confounding condition(s) based on the clinician's judgement, e.g. DOMINANT psychiatric disorder, vital depression, alcohol or substance abuse in the last 2 year.
- Female patients who are pregnant or lactating (females of fertile age are requested to use a safe contraceptive) at the time of inclusion.
- Patients who use or used new medications that affect the GI functioning within 1 month before the start of the study.

Questionnaires

- Baseline questionnaires; demographic, symptoms, symptom/medication/diet history, co-morbid medical conditions
- IBS symptoms: IBS-Symptom Severity Scale (IBS-SSS) and Gastrointestinal Symptom Rating Scale (GSRS)-IBS
- Psychological distress: Hospital Anxiety and Depression Scale (HADS), Patient Health Questionnaire (PHQ)-9 (both visit 1,4,5,6, generalized Anxiety Disorder 7-item scale (GAD-7)
- Somatization: PHQ-15 for the number and severity of bodily symptoms.
- Gastrointestinal specific anxiety: Visceral Sensitivity Index (VSI).
- Sensitivity: Central Sensitization Inventory (CSI).
- Food avoidance and restriction: (ARFID).

- Stool habits and GI symptoms: 14-day GI symptom diary based on Bristol Stool Form Scale (BSFS).
- Quality of life: IBS-Quality of Life (QOL).
- Food intake: 4-days food diary, MealQ.

Measurements/tests

Colonoscopic allergen provocation test, COLAP:

• A local allergen provocation test, where dietary antigens (soy, wheat, egg, gluten and milk), with saline and histamine as negative and positive controls, respectively are injected in the sigmoid mucosa (similar to skin prick test used for clinical allergy testing).
• The intestinal reaction is determined visually ("wheal and flare reaction"), and biopsies are taken to characterize the immune response.
• Bowel preparation before the investigation follows the normal clinical routine for colonoscopy, and i.v. sedatives and opioids are given during the investigation according to clinical routines at the endoscopy unit

Confocal laser endomicroscopy, CLE:

• A probe-based endoscopic technique to study intestinal food reactions after iv injection of fluorescein.
• Disruption of the small intestinal barrier in duodenum upon exposure to food antigens (soy, wheat, egg, gluten, milk, and control) can be determined.
• Can detect and quantify changes in intestinal tissues and cells, including increases in intraepithelial lymphocytes and fluid extravasation through epithelial leaks in real time.

Visceral sensitivity:

•Rectal barostat sensitivity measurement: With the rectal barostat we can measure the rectal sensitivity. A balloon is inserted and inflated in the rectum in a controlled setting. The patient indicates when defined sensory thresholds are reached (first feeling of the balloon, urge to empty bowel, discomfort or pain). When the patient indicates discomfort or pain, or another reason to stop, the balloon inflation will be stopped.

Sigmoidoscopy:

•Flexible sigmoidoscopy without bowel preparation, to interfere as little as possible with the normal gut microenvironment; fresh biopsies for specific analyses and biopsies stored for subsequent analyses.

Biological samples:

• Blood – and plasma samples: Fasting blood samples are taken for routine blood tests (exclusion of organic diseases) and to determine the metabolic profile and genetic and immunological markers of relevance to intestinal function and nerve function. Serum samples will be analyzed with nuclear magnetic resonance (NMR) for metabolomic profile and microbial composition.
• Fecal – and urine sample: We will characterize the composition and function of the metabolome in detail via 16S analysis, metagenomics, transcriptomics, metabolomics, cell culture and cell count. Urine and fecal samples will also be analyzed with nuclear magnetic resonance (NMR) for metabolomic profile and microbial composition.
• Biopsies from the stomach or the colon (location based on the performed endoscopic examination): We will conduct a detailed analysis of immune cells, proteins, nerve cells, and intestinal bacteria which will allow detailed mapping of intestinal function with respect to nerve, immune and barrier function.

Study visits

Visit 1 *– screening visit – 2 hours*
1. Screening. The subjects come fasting (8 hours) to our lab and the researcher obtains informed consent, confirms the IBS diagnosis and study criteria. Clinical routine blood tests for diagnostic purposes (blood cell count, liver, kidney, thyroid gland status, electrolytes, fasting glucose, C-reactive protein, transglutaminase (gluten) antibodies, and faecal calprotectin. The subjects fill in the baseline questionnaires. Height and weight are measured.
2. Rectal barostat. The researcher explains the rectal barostat procedure and the subjects undergo the rectal barostat. Thereafter, they will obtain information about the screening period including questionnaires, tubes for fecal and urine samples. Visit 2 and 3 will be planned.
Period before visit 2 – 14 days
The subjects register their dietary intake (food diary) for 4 days before visit 2. GI symptoms and bowel habits (BSFS) are registered during 14 days (GI diary), as well as IBS symptoms according to 2 questionnaires (IBS-SSS and GSRS-IBS) during the preceding week.

Allergologist: Through visits to the allergology clinic, (food) allergies will be excluded according to usual clinical routine (blood tests and prick tests)

Visit 2 *– Sigmoidoscopy – 2 hours*
1. Blood test, microbiota, immunological markers and metabolites. The subjects come fasting (8 hours) to our lab, where a nurse will take fasting blood samples (20 ml). The researcher will freeze fecal (10g), urine (3ml) and serum (5ml) samples in minus 80 degrees for later analyses. Pregnancy test (urine) is taken.
2. Questionnaires. The researcher checks the filled in questionnaires (screening period) and the subjects fill in the web-based questionnaires.
3. Sigmoidoscopy. The researcher and gastroenterologist explain the procedure. No bowel preparation, fresh biopsies will be taken for specific analyses and biopsies will be stored (a.o. -80 degrees Celsius) for subsequent analyses.

Visit 3 *– CLE or COLAP test – 5 hours*
1. Blood test, microbiota, immunological markers and metabolites. The subjects come fasting (8 hours: CLE , or according to laxatives protocol: COLAP) to our lab. The researcher will freeze fecal (10g) samples in minus 80 degrees for later analyses.
2. A) CLE. The researcher and gastroenterologist explain the procedure. Check if patient complied with dietary guidelines. The subjects undergo the CLE. Biopsies will be frozen. B) COLAP. The researcher and gastroenterologist explain the procedure. Check if patient complied with bowel preparation and dietary guidelines. The subjects undergo the COLAP. Biopsies will be frozen.

Visit 4-6: only if COLAP or CLE were positive for 1 or more food items.

Visit 4
Questionnaires (IBS-SSS, GSRS-IBS, HAD, PHQ-15). Instructions and advice from the dietician which food item(s) to exclude from the diet, based on CLE or COLAP. Period of exclusion: 4 weeks.

Visit 5 (digital, 4 weeks after visit 4)
Questionnaires (IBS-SSS, GSRS-IBS, HAD, PHQ-15). Instruction to re-introduce the CLE or COLAP positive food item(s) for 4 weeks.

Visit 6 (digital, 4 weeks after visit 5)
Questionnaires (IBS-SSS, GSRS-IBS, HAD, PHQ-15).