**Psychological distress and intestinal microbiome composition in post-infectious irritable bowel syndrome – is there an association?** By J. Sundin[1], L. Östlund-Lagerström[1], J. König[1], S. Fuentes[2], WM. de Vos[2] and RJ. Brummer[1]. *[1]Nutrition Gut Brain Interactions Research Centre, School of Health and Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden. [2]Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands.*

For our health maintenance we rely on a flawlessly working immune system efficiently preventing harmful organisms from causing infection and disease. The gut represents the body's largest repository of immune cells (1) readily mobilising controlled immune/inflammatory responses to combat antigens and microbes breeching the epithelial barrier. By definition, acute inflammation is an effective host defence strategy, however, if the response is prolonged and disproportionate to the threat, the outcome can be immunopathology and inflammatory disease.

Irritable bowel syndrome (IBS) is a functional gastrointestinal disease, with poor options for clinical treatment, rising to become a very common disorder in Europe and North America with a prevalence of 10–15% (2). The symptoms portraying IBS are usually pain, gastrointestinal discomfort and altered bowel habits, typically of chronic nature. Patients with IBS are characterised based on their symptoms according to the ROM III criteria (3).

A subset of individuals diagnosed with IBS particularly develops symptoms after exposure to an enteric infection and is thus characterised as post-infectious IBS (PI-IBS) patients. The reported incidence of PI-IBS currently varies between 3% and 35% (4). The microbial composition of the colon microbiome differs in PI-IBS patients in comparison to non-PI-IBS patients and healthy controls (5). Suggestively, one of the causes behind the gastrointestinal discomfort experienced by these patients is an aberrant host–microbe response, triggering persistent immune activation, inflammation and altered intestinal barrier function (6). Evidently, distinct abnormalities in the gut mucosa, such as continuous low-grade mucosal inflammation, have been observed in PI-IBS patients (6, 7).

The gastrointestinal system is closely related to the brain via various afferent and efferent signalling pathways, commonly referred to as the gut-brain axis (8). IBS has previously been recognised as a disorder of partially psychosomatic origin and has been found closely linked to psychological symptoms such as anxiety and depression (9). Of note, affective disorders are commonly associated with an elevated inflammatory activity, and vice versa (10). Mast cells - which are destabilised by hypothalamic release of corticotropin-releasing hormone - are important mediators in both inflammation and bidirectional gut-brain signalling, via e.g. the enteric nervous system and by their secretion of various potent chemical substances such as proteases (11). The typical reaction to excessive chemical release from mast cells, i.e. diarrhoea and abdominal discomfort and cramps, is very similar to the symptomatology of IBS (12). Interestingly, earlier studies have found augmented numbers and atypical location of mast cells in the intestinal mucosa of IBS patients (7). Experience of stress can trigger increased release of mast cell proteases into the circulation (13) and thus, there is reason to suspect that psychological distress can partake in the exaggeration and maintenance of the gastrointestinal symptoms in persons suffering from IBS, including PI-IBS patients.

Based on the notion that psychological distress, inflammation and changes in intestinal microbial composition are important factors in IBS aetiology in general and the pathophysiology of PI-IBS in particular, this study was conducted to investigate the potential association between psychological distress and intestinal microbiome composition in patients with PI-IBS.

**Method**

Faecal samples and colonic mucosal tissue samples were obtained from thirteen PI-IBS patients and sixteen healthy controls, respectively. A *Hospital anxiety and depression scale (HADS)* was completed by all subjects in order to evaluate psychological distress.

The *Hospital anxiety and depression scale (HADS)* scale was originally developed by Zigmond and Snaith (14) and is a well-used instrument for the evaluation of psychological distress in medical settings. The instrument consists of 14 items, which either can be condensed into two subscales measuring anxiety (seven items) and depression (seven items) or be used together generating a total score of psychological distress. The respondent rated each item on a four-point scale, where higher scores indicate the presence of problems. In the current study the total HADS score was used for the evaluation of psychological distress. The validity and reliability of HADS have been reported in several studies (15, 16).

The microbial composition of the faecal as well as the colonic mucosa samples was assessed using the Human Intestinal Tract Chip (HITChip) technology. The HITChip method is a validated method used for the characterisation of the human gut microbiome based on the identification of different bacterial strains through their variation in the 16s ribosomal RNA genes (17). Faecal samples were collected by each study participant according to standardised protocol. The faeces was instantly frozen in -20 $^{o}$C and subsequently delivered to the investigators for storage at -80 $^{o}$C, without intermediate thawing, until analysis. Distal colonoscopy was performed in the morning after an overnight fast and without prior bowel cleansing. Mucosal biopsy specimens were obtained at a standardised location in the sigmoid colon, approximately 20–25 cm from the anal verge at the crossing with the arteria iliaca communis. Out of the ten biopsies (with a mean weight of 0.1 g) collected, one was instantly snap-frozen in liquid nitrogen and used for analysis of the composition of the mucosal microbiome composition.

Data analysis was carried out with the latest version of R. Multivarate statistical analyses were performed with Canco 5. The microbial profile separating the study groups was identified with redundancy analysis (RDA) on log transformed probe signal intensity profiles derived from the HITChip phylogenetic microarray. Monte Carlo Permutation Testing (MCPT) determined significance of parameters. Between-group comparisons of microbial abundance and clinical data were analysed using ANOVA with Tukey's Honest Significant Differences (HSD) post-hoc analysis. The diversity of the microbial profiles was computed on probe-level data by Simpson's reciprocal index of diversity (1/D) on probe-level data, consisting of richness and evenness. All correlations between two datasets were analysed using Spearman's coefficient ($\rho$) and pairwise-comparisons.

## Results

Analyses of psychological distress and microbiome composition in faecal and intestinal tissue samples showed a significant association between total HADS scores and the microbial profile in both the faecal ($p < 0.02$) and mucosal microbiome ($p < 0.05$). In addition, a significant inverse correlation was found between the combined faecal and mucosal microbial diversity and HADS score ($\rho = - 0.37$, $p < 0.05$).

## Conclusion

This study revealed a significant association between measures of psychological distress and microbial composition in faecal samples and the colonic mucosa in PI-IBS patients and healthy controls, two groups previously shown to have significantly different microbiome composition (5). Furthermore, the study displayed an inverse relationship between psychological distress and microbial diversity. Both these findings suggest the importance of the microbe-gut-brain axis in the pathophysiology of PI-IBS, and strongly encourage further investigation of such interactions.

**References**


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