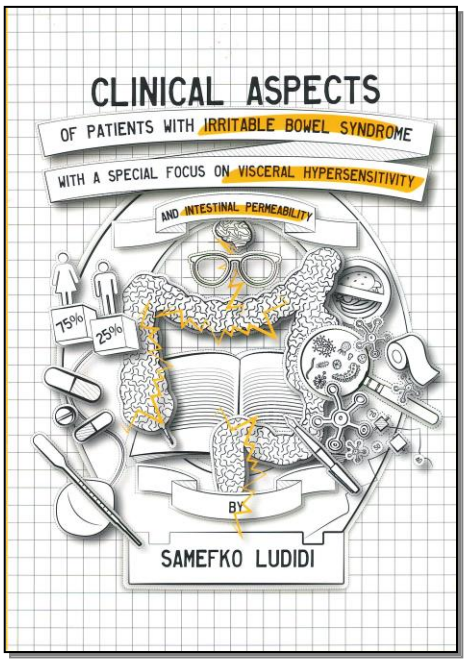

Samenvatting van het proefschrift
Samefko Ludidi

"Clinical aspects of patients with irritable bowel syndrome, with a special focus on visceral hypersensitivity and intestinal permeability"

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Irritable bowel syndrome (IBS) is a frequently occurring gastrointestinal disorder in Western society with a heterogeneous disease phenotype. Its pathophysiology is incompletely understood and involvement of a multitude of mechanisms involved in the aetiology of this disorder has been proposed. Among these mechanisms, visceral hypersensitivity plays a prominent role as it is present in up to 60% of IBS patients and therefore considered a hallmark of IBS. In Chapter 1 we briefly reviewed the recent literature with respect to IBS, its epidemiology and proposed pathophysiology and proposed the aims and outline of investigations.
Mechanical or chemical stimuli to the GI tract have been reported to trigger symptoms in patients with IBS. Especially food intake has been related to exacerbation of symptoms, such as abdominal pain. In Chapter 2 we investigated food-induced symptom aggravation, by measuring visceral hypersensitivity under fasted and postprandial circumstances. Aim was to optimise the rectal barostat procedure for the assessment of visceral hypersensitivity (in patients with IBS, compared to healthy control subjects (HC). Seventy-one IBS patients and 30 HC underwent a rectal barostat procedure under fasting and postprandial conditions (liquid meal; 368 kCal; 19 gr fat) and sensations of urge, discomfort and pain were scored on a visual analogue scale (VAS) over a pressure range of 0 – 50 mmHg. Cut-off for hypersensitivity was based on previously defined criteria, i.e. mean pain threshold in HC minus 2SD (VAS > 10 mm at pressure 23 mmHg). Post-prandially, IBS patients showed significantly increased VAS scores for al sensations. HC showed significantly increased postprandial scores for urge and pain only. Although intake of the liquid meal significantly increased pain perception, the number of patients with visceral hypersensitivity did not significantly differ before versus after meal intake (40.9% vs. 39.4%; P = 1). Meal intake therefore, did not increase the yield for the detection of visceral hypersensitivity. However, optimising the cut-off based on a large group of IBS pa-

tients may increase the accuracy to detect visceral hypersensitivity in these patients.
Hypersensitivity rates have been reported in literature to vary between 30% - 90% and results are hard comparable between studies due to different barostat protocols applied and lack of clear cut-off values to appoint visceral hypersensitivity. To further optimise the barostat procedure in our research center, in Chapter 3 three cut-off criteria were compared based on their sensitivity and specificity to detect visceral hypersensitivity in 126 patients with IBS versus 30 HC. All patients and HC underwent a single barostat according to a semirandomised staircase distension protocol and scored urge, discomfort and pain on a VAS. Cut-off was based either on 1) mean threshold for first pain sensation in HC minus 2 SD (i.e. VAS > 10 at 23 mmHg) 2) 10th percentile of the threshold for first pain sensation in HC or 3) maximum discriminative optimum between HC and IBS patients based on ROC curves for pain perception. Using these cut-offs resulted in 34.9%, 64.3% and 63.5% of all IBS patients being hypersensitive, respectively. The latter cut-off criterion had sensitivity of 63% and specificity of 90% and a positive predictive value of 96% hereby showing the best discriminative capacity to differentiate between patients with IBS and HC, based on rectal barostat distensions. Using this criterion, 63.5% of the IBS patients were found to be hypersensitive versus 10.0% of the HC.
In the Chapter 4. we aimed to unravel clinical and psychosocial characteristics associated with having visceral hypersensitivity. Using several validated questionnaires and a symptom diary, we examined clinical and demographic patient characteristics, psychological comorbidity (by HADS), the use of medication, symptoms (by GSRS) and quality of life (by RAND-36) . The study population consisted of 188 IBS patients with (N = 93) and without (N = 95) visceral hypersensitivity, according to previously the defined criteria defined for our research institute (Chapter 3). Compared to the normosensitive patiens, we found that having visceral hypersensitivity was significantly associated with younger age (mean ± SEM: 36.9 ± 1.62 vs. 45.8 ± 1.71 years; $P < 0.001$), female sex (81.7% vs. 66.0%; $P < 0.05$) and use of SSRI mediation (16.1% vs. 6.3%; $P < 0.05$). However, in the multivariate analysis only age remained significantly associated with having visceral hypersensitivity with an Odds Ratio of 0.97 (95% CI: 0.94; 0.99). In Apart of being significantly younger, the patients with hypersensitivity showed significantly increased scores for GSRS abdominal pain indigestion, reflux and constipation syndrome. IBS-related symptom intensity and discomfort as well as mean symptom composite score were significantly increased in the hypersensitive patients. Future mechanistically oriented studies within these subgroups of patients may provide biological markers or predictors for patients with and without visceral hypersensitivity.
Chapter 5 explored intestinal barrier function, as being one of the mechanisms potentially involved in the aetiology of IBS and visceral hypersensitivity. Ninety-one patients with IBS and 94 HC underwent a multi-sugar test for the assessment of intestinal barrier function per intestinal segment. Findings were corrected for potential confounding demographic factors (i.e. age, sex and BMI), psychological symptomatology (anxiety and

depression, lifestyle factors (smoking and alcohol use) and the use of medication. Compared to HC, patients with IBS, unspecified for disease subtype, had significantly increased excretion of sucrose. However, after correction for confounders the sucrose excretion, indicating gastroduodenal permeability was no longer different between IBS patients and controls. With respect to small intestinal permeability we found that the patients with IBS-D had a significantly increased lactulose-rhamnose excretion versus HC (0.023 [0.0020 – 0.0999] vs. 0.014 [0.0002 – 0.1512]; $P < 0.05$), that remained statistically significant after correction for confounding factors. Colonic permeability was not different between IBS and controls. None of the IBS subtypes did show significant alterations in colon permeability. It was concluded that small intestinal permeability is significantly increased in patients with IBS-D, irrespective of confounding factors. The mechanisms underlying this increased permeability in IBS-D need to be further explored.. Our findings also demonstrated that potential confounders should be taken into account in future studies on intestinal permeability in IBS. We further investigated potential local and systemic factors contributing to altered intestinal barrier function in patients with IBS (Chapter 6). Factors that have previously been shown to be able to modulate barrier function are tryptase and LPS. In order to assess the involvement of these mediators after basolateral exposure, intestinal barrier function was assessed in vitro using a 3D Caco-2 cell culture. Caco-2 spheroids were exposed to different concentrations of tryptase (10 – 50 mU) and LPS (1 – 50 ng/mL), after which permeability was assessed using FITC dextran 4KD (FD4) permeation. Both tryptase (20 and 50 mU) and LPS (6.25 – 50 ng/mL) were found to significantly increase permeability of the 3D model (all $P < 0.05$). Inhibition of these mediators using nafamostat mesylate for tryptase and polymyxin B for LPS significantly attenuated this effect ($P < 0.01$). Subsequently, the model was exposed to plasma samples of patients with IBS-D, IBS-C and HC from the basolateral side. Plasma of patients with IBS-D showed significantly increased levels of tryptase versus HC. Moreover, the plasma of IBS-D patients induced a significant increase in paracellular permeability, compared to IBS-C and HC. Selectively inhibiting tryptase and LPS significantly reduced the observed effect. These findings point to a role of systemic tryptase and LPS in epithelial barrier alterations in patients with IBS-D.

Targeting specific mechanisms in subgroups of IBS patients may improve treatment success. Accordingly, in Chapter 7, we performed a double blind randomised controlled intervention study in a group of 40 IBS patients characterised by a common pathophysiological mechanism i.e. visceral hypersensitivity. Patients were randomly assigned to either a specifically designed multispecies probiotic ($N = 21$) or a placebo ($N = 19$). Patients took their probiotic or placebo daily for a period of 6 weeks. Before and after the intervention, all patients underwent a rectal barostat procedure for the assessment of visceral pain perception. Secondary, they kept a symptom diary 2 weeks prior each barostat procedure. Although the percentage of patients with visceral hypersensitivity decreased significantly in the probiotic group (to 76.5%; $P < 0.05$), it also did in the placebo group (to 71.4%; $P < 0.05$) and did not differ between both groups. Improvement in pain scores and

mean symptom scores also did not differ significantly between probiotic and placebo treated patients. We concluded that in our group of hypersensitive IBS patients, no significant effect of the multispecies probiotic could be observed. For future studies, targeting specific mechanisms using probiotics, treatment duration and probiotic product should be taken into account.