Symptoms in Inflammatory Bowel Disease:
pathophysiological aspects and their relation with disease activity

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Symptoms in Inflammatory Bowel Disease; pathophysiological aspects and their relation with disease activity

Symptomen in Inflammatory Bowel Disease; pathofysiologische aspecten en de relatie met ziekteactiviteit.

(met een samenvatting in het Nederlands)

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Introduction

Introduction

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal (GI) tract characterized by a relapsing course. The etiology of both diseases is unknown, but they seem to result from complex interactions among susceptibility genes, the environment, and the immune system. Both entities have a broad spectrum of clinical presentations. IBD patients frequently complain of fatigue, and a wide range of gastrointestinal symptoms among which abdominal pain and diarrhea, during an exacerbation of the disease. These complaints, however, are not always related to disease activity, since a significant proportion of patients experience the symptoms even when the disease is in remission. To date, our knowledge of factors that contribute to these symptoms is still very limited.

IBD and fatigue

In the literature, fatigue is a frequently reported symptom in chronic diseases,[1] as is IBD. Despite its prevalence, the symptom remains poorly understood as biochemical and hematological tests seldom provide an explanation. In the past, many studies have been performed trying to reveal the mystery around chronic fatigue syndrome (CFS). It has been suggested that CFS may be related to abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis, causing a mild adrenal insufficiency in CFS patients.[2-4] In IBD patients, flare–ups are often treated with glucocorticosteroids which may result in HPA axis suppression and secondary hypocortisolism.[5] Hypocortisolism can be accompanied by fatigue. Other studies in CFS patients suggest an immunological basis for fatigue, mediated by cytokines.[6-9] Some of these cytokines also play a role in IBD, especially in active disease (e.g.TNF-α).[10-15] Infliximab, a chimeric antibody against TNF-α, has a direct effect on circulating TNF-α. In the first chapters of this thesis the prevalence and severity of fatigue in IBD patients in remission is assessed, and the role of the HPA axis as well as the role of cytokines (by using infliximab) is explored as potential underlying causes of fatigue in IBD patients in remission.

IBD and IBS-like symptoms

As mentioned above, IBD patients may experience gastrointestinal symptoms even when the disease is in remission and the mucosal damage is restored. The symptoms that remain strongly resemble the symptoms that are encountered in patients with irritable bowel syndrome (IBS).[16; 17] IBS is a functional bowel disorder in which abdominal discomfort or pain is associated with features of a disordered defecation. Nowadays the Rome II criteria are the most applied and accepted criteria for IBS.[18] In none of the previous studies that investigated the prevalence of IBS-like symptoms IBD patients the Rome II criteria were systematically applied to get a

clear view on the frequency and severity of IBS-like symptoms in patients with IBD in remission.

Observations that IBS may be precipitated by a transient inflammatory process in the gut have raised interest for inflammation as the basis for symptom generation in IBS.[19; 20] It is thought that the inflammatory process may leave the bowel irritable and hypersensitive after the inflammatory infiltrate has regressed and normal gut architecture has grossly been restored. Interestingly, increased visceral sensation in IBS patients is found not to be restricted to the colon, but can also be found in the small intestine,[21] and the esophagus.[22] Visceral sensitivity, and motility in, and between the different regions of the gut is accomplished through the enteric nervous system (ENS), epithelial cells, and the mucosal immune system. In IBD, inflammation affects the intestinal mucosa, which can result in structural and/or functional alterations of the ENS in a remote site of the gut.[23] This influences the excitability of neurons and nerve endings, and the distribution of neurotransmitters.[24; 25] Serotonin (5-HT) is a pivotal neurotransmitter in the gut. It plays an important role in visceral sensation,[26] and it initiates peristaltic and secretory reflexes by acting on 5-HT receptors.[27; 28] Studies in humans reveal that 5-HT availability is altered in IBD, as well as in IBS.[29-32]

In the second part of the thesis, the prevalence of IBS-like symptoms in IBD patients in remission is assessed, using the Rome II criteria. Furthermore, the question which mechanisms underlie the generation of IBS-like symptoms in IBD patients in remission is addressed. We explored stimulus-specific antroduodenal motor responses in IBD patients in remission to study the potential alterations in motor and sensory function throughout the gut, in previously never inflamed sites of the gut, which can result in IBS-like symptoms when the disease is in remission. Apart from hypersensitivity, generated by direct damage of the ENS, we focused on the role of an altered 5-HT signaling of the GI tract, secondary to inflammatory mechanisms.

Furthermore, it has been suggested that psychological factors are determinants in the development of persistent bowel symptoms secondary to an inflammatory or infectious processes.[19; 33] Coping, defined as a conscious response to stressful or negative situations, plays a significant role in a person's adaptation to stressful life events.[34] Different coping strategies may determine the outcome of a period of stress with respect to the emergence of IBS-like symptoms. In Chapter 3 we aimed to assess the possible relation between IBS-like symptoms in IBD patients in remission and coping strategies in stressful situations.

Crohn's disease and disease activity

At present, the severity of mucosal inflammation assessed by endoscopy is considered to be the gold standard for disease activity in CD. In daily practice, however, physical examination, inspection of stools, and laboratory parameters are often

used as a surrogate marker for inflammatory activity. Over the last 30 years, a number of disease specific instruments have been created to assess disease activity in CD. These disease activity indices are constituted of clinical and laboratory parameters. In the last part of the thesis the predictive value for mucosal inflammation of clinical disease activity indices, quality of life questionnaires, and biochemical markers is systematically investigated using the endoscopic disease activity indices as gold standard. In most studies and clinical trials the Crohn's Disease Activity Index (CDAI)[35] is used to define clinical remission and/or clinical response. Several reports show that the mucosal inflammation correlates poorly with the CDAI.[36; 37] Therefore, we aimed to design a new CD activity index, based on a combination of clinical characteristics, and readily available laboratory parameters, predicting endoscopic disease activity in patients with both quiescent and active disease (Chapter 7).

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High prevalence of fatigue in quiescent IBD is not related to adrenocortical insufficiency

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Chapter

1

Abstract

Objectives

Inflammatory bowel disease (IBD) patients, with active as well as quiescent disease, frequently complain of fatigue. This often has consequences for patients' work and daily lives. The primary aim of this study was to assess the prevalence and severity of fatigue in IBD patients in remission. Furthermore we studied the correlation between fatigue and disease activity, quality of life, biochemical and hematological test results, and the role of (secondary) hypocortisolism.

Methods

80 subjects with proven IBD were included. Disease activity was assessed using the clinical activity index for ulcerative colitis and the Crohn's disease activity index. Quality of life was measured by the inflammatory bowel disease questionnaire (IBDQ), and fatigue was assessed using the multidimensional fatigue inventory (MFI). Routine biochemical and hematological tests were performed, and basal cortisol was determined. To evaluate adrenocortical reserve in subjects with a cortisol level of <0.4 μmol/l, a low dose ACTH test was performed. Healthy age-and sex-matched subjects (n=67) served as controls.

Results

More than 40% of the IBD patients in remission suffered from fatigue. Mean MFI scores of the IBD patients were comparable to mean MFI scores reported in cancer patients. The IBDQ showed a negative correlation with the MFI (r -.735, $P < 0.001$). No correlation was found between fatigue and basal cortisol levels or other laboratory parameters.

Conclusions

Fatigue is an important feature in IBD in remission, adversely affecting the quality of life. It does not, however, affect all patients, nor does it seam to be the result of hypocortisolism.

Introduction

Inflammatory bowel disease (IBD) comprises ulcerative colitis (UC) and Crohn's disease (CD), which are chronic inflammatory diseases of the GI tract. Like many other chronic, inflammatory or autoimmune disorders, IBD seems to result from complex interactions among susceptibility genes, the environment, and the immune system. Patients with IBD, in active as well as in quiescent disease, frequently complain of fatigue or tiredness. Whereas occasional fatigue is a part of the experiences of every day life, constant or frequent fatigue is not. It can be disabling when it has consequences for patients' work and daily lives. In the literature, fatigue is a frequently reported symptom in chronic diseases.[1] Despite its prevalence, it remains poorly understood as biochemical and hematological tests seldom provide an explanation for its occurrence.

In IBD patients, flare–ups are often treated with glucocorticosteroids, which may result in hypothalamic-pituitary-adrenal (HPA) axis suppression and secondary hypocortisolism (adrenocortical insufficiency).[2] This will particularly be the case in patients who cannot be withdrawn from glucocorticosteroid treatment, leading to a steroid dependency in 36% of the patients.[3] Hypocortisolism can be accompanied by fatigue, vague, non-specific complaints of weakness, weight loss, and anorexia. In the past it has been suggested that the chronic fatigue syndrome (CFS) may be related to abnormalities in the HPA axis, causing a mild adrenal insufficiency in CFS patients.[4-6] Recently, Reinshagen et al. found an impairment of the HPA axis in a subgroup of patients (25%) with Crohns disease.[7] We hypothesize, therefore, that fatigue in IBD patients is related to secondary hypocortisolism.

The primary aim of this study was to assess the prevalence and severity of fatigue in patients with IBD in remission. Furthermore, we studied the correlation between fatigue and disease activity, quality of life and biochemical and hematological test results. Finally, we studied the role of (secondary) hypocortisolism in fatigue.

Materials and methods

Study protocol

All patients with proven IBD visiting the outpatient Department of Gastroenterology at the University Medical Center in Utrecht (UMCU) and meeting the inclusion criteria were invited to participate in this study. After giving their written consent, they were asked to fill out the Clinical Activity Index for Ulcerative Colitis (CAI UC) or the Crohn's Disease Activity Index (CDAI), the Multidimensional Fatigue Inventory (MFI), and the Inflammatory Bowel Disease Questionnaire (IBDQ). Blood was taken between 8.00 a.m. and 9.00 a.m. If the plasma cortisol level was less than

0.4 μmol/L, a low dose (1 μg) ACTH stimulation test was performed. The study was approved by the Medical Ethical Committee of the UMCU.

Subjects

Patients

Patients, older than 18 years, had to be in remission according to the investigator, based on clinical parameters and medical records. Patients were excluded if they were taking steroids within 12 months prior to study entry or if they suffered from anemia (female: hemoglobin < 7.0 mmol/L; male: hemoglobin < 8.0 mmol/L). Comorbidity, possibly resulting in (additional) fatigue was also considered an exclusion criterion (e.g. kidney or adrenal insufficiency, autoimmune diseases, malignancy, depression, a recent viral infection, or psychotrauma). Of 185 patients approached for this study, 99 patients (46 male, 53 female) with proven IBD (56 CD and 43 UC) were willing to participate (response rate of 54%). Data on age, sex, duration and location of the disease, use of medication, the presence of fistula, and extraintestinal manifestations of the disease were collected from the medical records.

Controls

Unpaid, sex and age-matched relatives of hospital employees (n=67) served as controls and were asked to fill out the MFI. Screening procedures to ensure the healthy status of the subjects relied primarily on self-reported answers to standard questions about their medical condition.

Questionnaires

CDAI / CAI UC

Disease activity was assessed using the Clinical Activity Index for Ulcerative Colitis (CAIUC)[8] and the Crohn's Disease Activity Index (CDAI).[9]

The Inflammatory Bowel Disease Questionnaire (IBDQ)

Quality of life was measured by the IBDQ. The IBDQ is a disease-specific, health-related, quality of life questionnaire containing 32 items, with a graded response range of 1 (worst) to 7 (best) and a possible total score of 32 to 224.[10] The 32 items can be divided into 4 dimensional scores, including bowel symptoms (10 items), systemic symptoms complaints (5 items), emotional well-being (12 items), and social function (5 items). The IBDQ has been translated and validated in the Dutch language and proved to be valid, discriminative and reliable.[11]

Multidimensional fatigue inventory (MFI-20)

Fatigue was assessed using the MFI, a 20-item, self-report instrument designed to measure five fatigue dimensions: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue.[12-14] Each scale consists of two items

indicating fatigue and two items contraindicating fatigue, to limit possible influence of answering tendencies. A higher score indicates a higher level of fatigue (range 4-20). In some studies, the scale of general fatigue was preferred to the use of numeri rating scale because of its more favorable psychometric properties, so the MFI scale of general fatigue can be referred to as "fatigue".[12; 14; 15]

Blood tests

In patients, routine biochemical (creatinine, sodium, potassium, albumin, C-reactive protein, ferritin, glucose) and hematological tests (hemoglobin, mean corpuscular volume, platelet count, serum $B_{12,}$ serum folate, and white cell count) were performed. Furthermore, basal cortisol values were assessed between 8.00 a.m.-9.00 a.m.[16] A morning plasma cortisol > 0.4 μmol/L was considered appropriate, excluding hypocortisolism .[17] Plasma cortisol was measured using an immunoassay based on chemiluminescence.

Low dose (1 μg) ACTH stimulation test

To evaluate adrenocortical reserve in subjects with low cortisol levels, all patients with a morning plasma cortisol level below 0.4 μmol/L were invited for an ACTH stimulation test. The low dose (1 μg) ACTH test was used because this test is thought to reflect the adrenocortical reserve in a more physiological way and is more sensitive for the detection of hypocortisolism compared with the high dose (250 μg) test, especially in cases of mild adrenal insufficiency, allowing assessment of pituitary-adrenal suppression after long-term treatment with glucocorticoids.[16; 18; 19] The solution (1 μg tetracosactide dissolved in 1ml saline) was injected i.v. as a bolus after a basal blood sample was taken for measurement of the basal cortisol. Additional blood samples were obtained 20 and 30 minutes after injection of the bolus. Results were considered to be normal if a 0.5 μmol/L peak level cortisol was reached after i.v. injection of 1 μg ACTH.[16]

Statistical Analysis

The distributions of the scores on all scales and items were evaluated by means of descriptive statistics (means, std. error mean). Unpaired Student *t* –tests were performed for comparisons between patients and controls for age and on the different scales of fatigue. Pearson's correlation coefficients were computed between the subscales and the total scores of the MFI and the IBDQ, and between the subscales and total score of the MFI and the hematological and biochemical determinations. A *P* value of less than 0.05 was considered to indicate significance. Statistical analysis was performed with the SPSS version 10.0 for Windows (SPSS, Chicago, IL).

Results

Sample

Patients

Of the 185 patients who were approached in this study, 86 patients declined participation, mainly because they were not able or willing to visit the hospital between 8.00 and 9.00 a.m. for the blood tests. Patients were asked if they suffered from fatigue before enrollment. Thirty percent of the patients who declined participation considered themselves fatigued, 14% considered themselves not fatigued and, in 56% no response was obtained. Ninety-nine IBD patients participated in this study (response rate of 54%; 46 male, 53 female; 56 CD and 43 UC), of whom 26% initially claimed to be fatigued and 29% to be not fatigued. From 45% of the patients who participated, no data on fatigue were obtained before enrollment. Patients with a stoma or pouch (n = 18) were withdrawn from the results because their scores of the CDAI or the CAI UC could not be computed. One patient did not fill out the CAI UC at all and was excluded from the enrollment. In the remaining 80 patients, age (mean ± SEM) was 42.9 ± 1.52 yr, and duration was 13.9 ± 1.13 yr. Remission was defined as a CDAI score of <150 or a CAI UC score for the two consecutive days of less than 10. Ten CD patients were found to have mild disease activity according to the CDAI (CDAI score) was 217 ± 9.82). Data of these patients were separately analyzed. 70 patients (36 CD and 34 UC) were in remission (CDAI score 67 ± 5.81; CAI UC score for the two consecutive days 3 ± 0.23).

Controls

The age of the healthy subjects (40 male, 27 female) was 41.4 ± 1.26 years.

In Table 1, demographic and clinical characteristics of the controls and the 80 participating patients are presented.

Comparison of the patient groups

No significant differences were found between the two different patient groups regarding age and sex. CD patients in remission had a significantly longer disease duration compared with UC patients. The patient group with mild activity only comprised CD patients. No significant differences existed between the CD patients with mild activity and the CD patients in remission regarding age, sex, and disease duration.

MFI

Table 2 shows the average MFI scores for IBD patients and healthy controls.

In the combined IBD group (remission and active disease) all subscale scores were significantly higher compared with the healthy controls ($P<0.001$). If patients with

Table 1
Demographic and clinical characteristics of the controls and the 80 analyzed patients.

		Patients in remission		Patients with activity
	Controls (n=67)	UC (n=34)	CD (n=36)	UC (n= 0) CD (n=10)
Age (yr) (mean + SEM)	41.4 (± 1.26)	43.6 (± 2.49)	42.5 (± 2.33)	43.5 (± 3.92)
Disease duration (yr) (mean + SEM)		10.5 (± 1.11)	16.2†(± 2.04)	16.6 (± 2.91)
Gender				
male (n)	40 (60%)	20 (59%)	17 (47%)	4 (40%)
female (n)	27 (40%)	14 (41%)	19 (53%)	6 (60%)
Operations				
none (%)		97	44	70
ileocecal resection (%)		0	44	30
ileocecal-and sigmoid resection (%)		0	6	0
(subtotal)colectomy (%)		0	0	0
hemi colectomy (%)		0	6	0
sigmoid resection (%)		3	0	0
Localization				
ileocecal (%)		0	53	40
colon (%)		100	17	50
ileocecal and colon (%)		0	30	10
PSC (%)		6	3	0
Fistula (%)		0	25	40
Medication				
5 –ASA medication (%)		100	94	90
Immunosuppressives (%)		21	31	30

PSC = primary sclerosing cholangitis; 5-ASA = 5-aminosalicylic acid.
CD patients vs. UC patients: † $P < 0.02$.

Table 2
Average MFI scores for IBD patients and healthy controls.

	Patients in remission (n=70)	CD patients in remission (n=36)	UC patients in remission (n=34)	Patients with activity (n=10)	Controls (n=67)
General fatigue	10.97 (± 0.60)**	10.50 (± 0.71)	11.47 (± 0.98)	16.30 (± 1.58)**†	7.01 (± 0.35)
Physical fatigue	9.73 (± 0.53)**	9.03 (± 0.64)	10.47 (± 0.47)	14.20 (± 1.36)**†	6.34 (± 0.29)
Reduced activity	8.99 (± 0.49)**	7.86 (± 0.54)	10.18 (± 0.80)‡‡	11.90 (± 1.19)* ‡	7.01 (± 0.28)
Reduced motivation	8.34 (± 0.53)*	7.33 (± 0.61)	9.41 (± 0.84)‡‡	12.00 (± 1.59)o ‡	6.46 (± 0.27)
Mental score	9.63 (± 0.61)**	8.56 (± 0.76)	10.76 (± 0.93)	12.70 (± 1.33)**	6.96 (± 0.34)

All values represent mean (SEM).
Patients in remission or patients with activity vs. controls: ** $P \leq 0.001$, * $P \leq 0.002$, o $P < 0.01$
Patients with activity vs. patients in remission: † $P \leq 0.004$, ‡ $P < 0.04$, UC patients in remission vs. CD patients in remission: ‡‡ $P \leq 0.05$

mild disease activity were excluded, outcomes still differed significantly for each scale.
If fatigue is defined as the 95 percentile of the score on the general fatigue scale of the healthy control group, 41% of the IBD patients in remission suffered from fatigue (Figure 1).

Correlations with fatigue

All dimensions of IBDQ were inversely correlated with all dimensions of the MFI-20 (range -.237 to -.816; correlation IBDQ total vs. general fatigue: r -.735 $P < 0.001$). No significant correlations were found between the fatigue scores and the cortisol levels, or any other biochemical or hematological test results. A significant positive correlation was found between the plasma cortisol and the number of years patients were off steroids (r 0.364; $P < 0.02$).

Low dose ACTH stimulation test

Of all patients participating in this study, 21 patients had a plasma cortisol level less than 0.4 μmol/L. Of these 21 patients, 9 were excluded for the low dose ACTH test because of protocol violation (steroids administration within 12 months prior to the test, basal cortisol level taken after 9.00 a.m.) and 2 patients were lost to follow up. Ten patients (7 CD, 3 UC; 2 female, 8 male) were invited for the ACTH test, 1 patient (CD, male) declined participation. All patients showed an appropriate response to

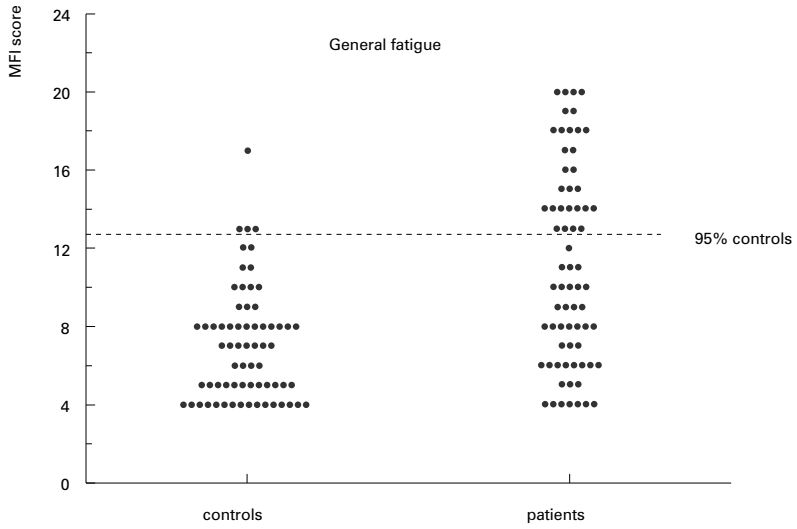

Figure 1
95 percentile of MFI scores of control group applied to MFI scores of IBD patients in remission

iv. administration of ACTH (mean peak level 0.73 μmol/L, range 0.57 μmol/L - 0.87 μmol/L; mean Δ rise compared with basal plasma cortisol 0.29 μmol/L, range 0.10 μmol/L –0.40 μmol/L).

Discussion

In this study, we have shown that the degree of fatigue experienced by a cohort of IBD patients in remission is significantly greater compared with age- and sex-matched controls. If fatigue is defined as the 95 percentile of the score of a healthy control group, 41% of the IBD patients in remission suffered from fatigue.

Although the response rate was not very high (54%), we believe that this study gives a reliable reflection of the prevalence of fatigue in this study population. The patients who participated were found to be representative of the whole study population regarding demographic parameters. The responding patients did not experience fatigue more often compared with those who declined participation.

Recruitment of patients from a referral center, as was carried out in this study, may result in an overestimation of the prevalence of fatigue, as patients with difficult to control problems tend to visit the clinic more frequently. On the other hand, fatigue

is known to be related to negative emotions, in particular depression. In the present study, all patients with a known depression in the preceding 5 years were excluded. The healthy volunteers had to meet the same criteria concerning negative emotions. Excluding patients with a depression or other comorbidities possibly explaining fatigue (e.g. kidney or adrenal insufficiency, autoimmune diseases, malignancy, depression, a recent viral infection, or psychotrauma) can result in an underestimation of the prevalence and severity of fatigue. On the whole, we think this study represents a true reflection of fatigue in Dutch IBD patients in remission.

In the literature, the MFI scores of different patients groups are reported.[15; 20-22] Comparing the results of the IBD patients with these patient groups, we found impressive mean MFI scores, comparable to mean MFI scores reported in cancer patients. In Figure 2, average scores on the general fatigue scale of the MFI for IBD patients and other patient groups are shown. In most studies, the study population appeared to be heterogeneous regarding disease severity and exact diagnosis. We believe that the current study gives a realistic impression of severity of fatigue in patients with IBD in remission.

Data from the present study show a strong negative correlation between fatigue and quality of life in patients with IBD in remission, which supports the thought that fatigue influences the quality of life to a great extend. Although there is some overlap between questions of the MFI and the IBDQ, outcomes still correlated significantly if questions on the IBDQ concerning energy loss and fatigue where left out. No relation was found between the MFI scores and biochemical and hematological test results. This outcome was not unexpected, because of the biochemical and hematological exclusion criteria we used (apparent anemia, recent infection, renal and liver dysfunction).

No correlation between the severity of fatigue and the basal cortisol level was found. We postulated that a disturbance in the HPA axis would contribute to fatigue in these patients, but we could not substantiate this in the present study as measured by the basal cortisol levels or the low dose ACTH test. The latter is a physiological and sensitive test, especially in cases of mild adrenal insufficiency after long-term treatment with glucocorticoids.[16; 18; 19]Although we observed some patients with a basal cortisol levels lower than 0.4 μmol/L, none of them suffered from adrenal insufficiency. Our results are in contrast with those of Reinshagen et al.,[7] who found an impairment of the HPA axis in a subgroup of patients (25%) with CD. In this abstract it is not made clear whether only patients in remission or a heterogeneous group of CD patients were included. The high cutoff point (0.6 μmol/L cortisol after adrenal stimulation) and less strict exclusion criteria regarding steroid use may explain the differences between these two studies. Our finding of a significant positive correlation between the plasma cortisol and the time patients were off steroids supports this hypothesis.

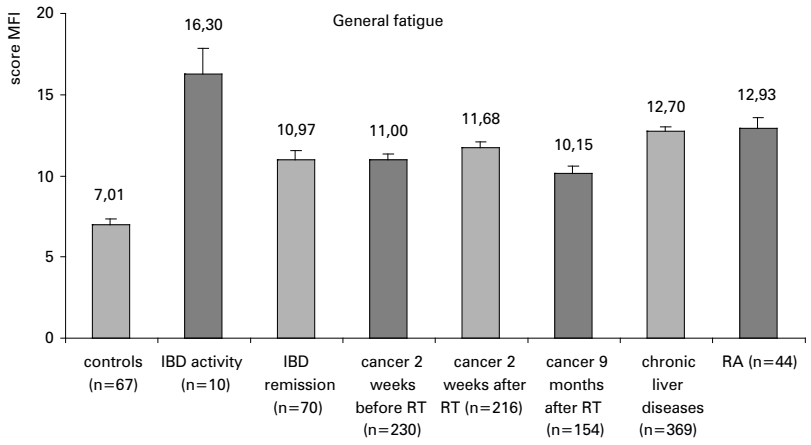

Figure 2
Average scores on the general fatigue scale of the MFI for IBD patients and other patient groups [15; 20-22] (RT= radiotherapy; RA= rheumatoid arthritis).

As fatigue seems not to be the result of hypocortisolism in this group of IBD patients, questions can be raised regarding other possible causes of this symptom. There is a growing interest in several conditions that share the symptoms of persistent disabling fatigue, such as primary biliary cirrhosis, Sjögren's syndrome, and chronic fatigue syndrome.[22; 23] Although their causes are unknown, it has been speculated that CFS and chronic disabling fatigue results from inflammation of immune activation, and that symptoms of these illnesses are mediated bij cytokines.[24-26] Pro-inflammatory cytokines play an important role in IBD, especially in active disease.[27] Possibly, they are also marginally increased in a subset of patients in clinical remission, leading to such a distressing symptom as fatigue.
In conclusion, we have shown that fatigue is an important feature in IBD in remission, adversely affecting the quality of life. It does not, however, affect all patients, nor does it seem to be the result of hypocortisolism. Because fatigue may influence quality of life to a great extend, further research into the pathogenesis of fatigue in IBD patients is needed.

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Crohn's disease, fatigue, and infliximab: is there a role for cytokines in the pathogenesis of fatigue?

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Chapter

2

Abstract

Aim

To study the effect of infliximab on fatigue in relation to cytokine levels in Crohn's disease (CD) patients.

Methods

Fourteen CD patients were blinded for treatment and received placebo at baseline, and infliximab 2 weeks later, with a follow-up of 4 weeks. Blood samples were drawn on a regular basis, and questionnaires on fatigue, depression, quality of life, and clinical disease activity were completed at regular intervals.

Results

After placebo infusion, fatigue scores decreased within 3 days (3.5 points ± 1.1, $P \leq 0.01$), but returned to baseline values 14 days after this infusion. The drop of fatigue scores following infliximab infusion sustained until the end of the study (3.8 points ± 1.4, $P \leq 0.05$). Quality of life was increased at the end of the study compared to baseline values (138.6 ± 9.4 *vs* 179.4 ± 6.7; $P \leq 0.005$), whereas depression scores were decreased (20.4 ± 9.4 *vs* 11.3 ± 2.2; $P \leq 0.01$). No correlation between the severity of fatigue and the level of cytokines was observed.

Conclusion

The reduction of fatigue after infliximab infusion is subjective to a placebo effect. The effect of infliximab on fatigue, however, persists while the placebo effect disappears after a short period of time. A clear role of cytokines could not be substantiated.

Key words
Crohn's disease, fatigue, infliximab, cytokines

Introduction

Fatigue is a frequently reported symptom in inflammatory bowel disease (IBD) patients, even when the disease is in remission. It can be disabling when it has consequences for the patient's work, daily life, and quality of life. Recently, we reported fatigue in 40% of IBD patients with quiescent disease.[1] Our data also indicated that disease activity leads to a higher prevalence and level of fatigue. Despite the high prevalence, fatigue in Crohn's disease (CD) patients remains poorly understood as biochemical and hematological tests seldom provide an explanation. Studies in patients with chronic fatigue syndrome suggest an immunological basis for fatigue, mediated by cytokines.[2-5]Some of these cytokines also play a role in IBD, especially in active disease (tumor necrosis factor (TNF)-α, interleukin (IL)- 6, IL-10, IL-18).[6-11]

The treatment of CD has dramatically changed since the introduction of infliximab, a chimeric antibody against TNF-α. A clinical response was reported in two- thirds of the treated patients and even remission in one-third of the CD patients following treatment with infliximab.[12-15] Infliximab has a direct effect on circulating TNF-α and is thought to induce apoptosis of activated T-cells.[15] Patients frequently experience a rapid reduction of fatigue after treatment with infliximab. A previous, placebo-controlled study has shown a significant decrease of fatigue in CD patients treated with infliximab, based on a disease-specific health related quality of life questionnaire.[16] This finding has been confirmed by Persoons et al,[17] although not in a placebo-controlled study.

The primary aim of this pilot study was to assess, in a placebo-controlled way, the role of cytokines in the pathogenesis of fatigue in CD patients, using infliximab. Furthermore, we measured the effect of infliximab on fatigue, clinical disease activity, and depression scores, using validated questionnaires developed for these purposes.

Materials and Methods

Patients

Fourteen consecutive CD patients from the Outpatient Department of Gastroenterology of the University Medical Center in Utrecht were enrolled in the study. The diagnosis of CD was based on clinical, radiological, endoscopic and histological features.[18] Comorbidity, possibly resulting in fatigue (e.g. kidney or adrenal insufficiency, autoimmune diseases, malignancy), was considered an exclusion criterion. Demographic and clinical characteristics of the included patients are presented in Table 1. Four patients, including 3 patients with fistulizing disease, had a stoma (3 ileostoma, 1 colostoma). In 4 of the patients who used corticosteroids, the dose was tapered two weeks after infliximab infusion. Doses of other medications were stable during the whole study period. None of the subjects used infliximab within 3 months prior to study entry. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, and written informed consent was obtained from all subjects.

Study protocol

The study was designed as a single blinded study. All patients received placebo (i. e. saline) at baseline, followed by infliximab (5 mg/kg) at 2 weeks. Patients with fistulae received one extra dose of infliximab, 4 weeks after baseline. All patients were followed up for 4 weeks after the last infliximab infusion. Fatigue scores were obtained every week (plus three days after each infusion), using the Multidimensional Fatigue Inventory (MFI). Depression scores were assessed every two weeks by the Center for Epidemiological Studies Depression scale (CES-D). Quality of life was measured at baseline and at the end of the study, using the Inflammatory Bowel Disease Questionnaire (IBDQ). Clinical disease activity was determined by the Crohn's disease activity index (CDAI), while in patients with fistulizing disease the perianal Crohn's disease activity index (PCDAI) was employed as well. Blood samples were drawn every two weeks.

Questionnaires

Multidimensional Fatigue Inventory (MFI-20) - Fatigue was assessed using the MFI-20, a 20-item self-report instrument designed to measure five fatigue dimensions: general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue.[19-21] Each scale consists of two items indicating fatigue and two items contraindicating fatigue, to limit possible influences of answering tendencies. A higher score indicates a higher level of fatigue (range 4-20). The questionnaire was developed and validated in the Netherlands.[20] In some studies the scale of general fatigue was preferred to the use of numeric rating scale because of its more favor-

Table 1
Demographic and clinical characteristics of the patients

	Included CD patients (*n* = 14)
Age (yr) (mean ± SD)	32.2 (± 8.6)
Disease duration (yr) (median; range)	7.5 (1-28)
Gender male (*n*)	5
Refractory, clinical active luminal disease (*n*)	9
Active (draining) fistulae (*n*)	5
Medication use	
Mesalamine (*n*) (range)	9 (1.5-3 g/day)
Azathioprine (*n*) (range)	12 (25-175 mg/day)
Prednisone (*n*) (range)	5 (10-50 mg/day)
Infliximab naïve (*n*)	8

able psychometric properties. Therefore, the general fatigue scale can be referred to as "fatigue".[19; 22] In the present study, we only used this scale. If fatigue is defined as the 95 percentile of the score on the general fatigue scale of a healthy control group, a score of 13 or higher indicates "fatigued".[1]

Inflammatory Bowel Disease Questionnaire (IBDQ) - Quality of life was measured by IBDQ. The IBDQ is a disease-specific health related quality of life questionnaire, containing 32 items, with a graded response range of 1 (worst) to 7 (best) and a total score of 32 to 224.[23] The 32 items can be divided into 4 dimensional scores, including bowel symptoms (10 items), systemic symptoms complaints (5 items), emotional well-being (12 items), and social function (5 items). The subscale "systemic symptoms" contains two questions evaluating fatigue/energy loss. The IBDQ has been translated into and validated in the Dutch language and proven to be valid, discriminative and reliable.[24]

Center for Epidemiological Studies Depression scale (CES-D) - Depression scores were assessed every two weeks by the CES-D. The CES-D consists of 20 questions, 16 questions are related to feelings of mental depression (negative questions) and 4 questions are related to the absence of feelings of mental depression (positive questions). All questions refer to the situation during the last week, with a graded response range of 0 (<1 day) to 3 (5 to 7 days). The total CES-D score ranges from 0-60. A high score reflects more feelings of mental depression and reduced psychological wellbeing. The CES-D has been translated into and validated in the Dutch language.[25; 26]

Clinical disease activity

Crohn's disease activity index (CDAI) - Disease activity was assessed using the CDAI,[27] the most frequently used and accepted clinical activity score for Crohn's disease worldwide. A clinical response was defined as a reduction of 70 or more points of the CDAI score.[13; 15] The CDAI was not computed for patients with a stoma (n = 4).

Perianal Crohn's disease activity index (PCDAI) - The PCDAI was used to measure the severity of perianal Crohn's disease in patients with fistulizing disease.[28] The PCDAI incorporates 5 items: discharge, pain, restriction of sexual activity, type of perianal disease, and degree of induration. Each category is graded on a 5-point Likert scale ranging from no symptoms (score of 0) to severe symptoms (score of 4). A higher score indicates more severe disease.

Cytokine, biochemical and hematological tests

The cytokines TNF-α, interleukin (IL)-6, IL-10, and IL-18 were assessed before, two weeks after each infusion, and 4 weeks after the last infliximab infusion, using the Luminex Multiplex system which is described in detail previously.[29; 30] Biochemical and hematological tests (e.g. hemoglobin, C-reactive protein (CRP) were performed at the same time as the cytokines were assessed. A CRP under 7 mg/L was considered normal.

Statistical analysis

The distributions of the data were evaluated by means of descriptive statistics (mean or median; SEM or range). Paired Student *t* tests or Wilcoxon Signed Ranks tests were performed for comparisons before and after the different infusions. Dichotomous variables were expressed as frequency (%), and differences between frequencies were analyzed using the McNemar test. Partial correlation coefficients were computed between general fatigue and the IBDQ, the CES-D, the decrease in clinical activity scores, cytokines, and hematological tests. $P < 0.05$ was considered to be significant. Statistical analysis was performed with the SPSS version 11.5 for Windows.

Results

Questionnaires

Fatigue scores- Table 2 shows the average decrease of fatigue score (subscale "general fatigue") after placebo and infliximab infusion. At baseline the mean general fatigue score (± SEM) was 15.8 (± 1.00). Three and 7 days after placebo infusion, a significant decrease in fatigue was measured compared to the baseline values. Fourteen days after placebo infusion, the fatigue scores had returned to baseline values. After infliximab infusion, the fatigue score dropped significantly after 7 days, and this effect sustained until the end of the study (4 weeks after the last infliximab infusion).

If fatigue is defined as a score on the general fatigue scale of 13 or higher,[1] 86% of the patients was fatigued at baseline. Of these patients, 33% responded to the placebo infusion, leaving 57% of all participating patients fatigued. After infliximab administration, this percentage reduced to 36%and 21% of all participating patients after 2 and 4 weeks. Analyzing infliximab naïve patients separately did not change the outcomes.

Quality of life - Quality of life was significantly increased 4 weeks after the last infliximab infusion compared to baseline values. Table 3 shows the average of the total IBDQ score and the subscale "systemic symptoms".

Depression scale - Placebo infusion did not interfere with feelings of mental depression or reduced psychological well-being. A significant effect of infliximab infusion on feelings of depression was found 4 weeks after the last infusion (Table 3).

Clinical disease activity

CDAI - At baseline the mean CDAI score (± SEM) was 222.3 (± 25.7). After the infliximab infusion, as well as after the placebo infusion, CDAI scores showed a significant decrease (Table 2). If clinical response is defined as a reduction of 70 or more points of the CDAI score, 50% of the patients clinically responded after infliximab infusion, compared to 20% after placebo infusion. Excluding the patients with fistulizing disease ($n=2$), the same results were obtained, with a more explicit, although not significant, difference in CDAI score decrease after both infusions.

PCDAI - No significant decrease in the PCDAI score in the five patients with fistulizing disease was measured at the end of the study, compared to the baseline values. No differences were found in the effect of the two infusions.

Cytokine, biochemical and hematological tests

IL-18 - All patients had detectable levels of IL-18. A significant decrease was found 2 weeks after infliximab infusion, which still existed 4 weeks after the last infliximab infusion. Placebo infusion did not influence the levels of IL-18 expression (Table 3).

Table 2
Decrease in general fatigue score and CDAI score after infusion (mean ± SEM)

	General fatigue		CDAI score	
	Placebo	Infliximab	Placebo	Infliximab
D 3	3.5 ± 1.1 [b]	2.4 ± 1.5		
D 7	3.5 ± 1.1 [b]	3.5 ± 1.6 [a]	40 ± 10.6 [b]	63 ± 18.9 [b]
D 14	1.9 ± 1.1	2.6 ± 1.2 [a]	39 ± 13.3 [a]	65 ± 27.1 [a]
D 21	.	3.6 ± 1.4 [a]		72 ± 28.9 [a]
D 28	.	3.8 ± 1.4[a]		68± 36.2

D: day; [a] $P \leq 0.05$, [b] $P \leq 0.01$ *vs* baseline. CDAI: Crohn's disease activity index

Table 3
Effect of infliximab on IBDQ and CES-D scores, and IL-18, CRP (mean ± SEM)

	Placebo infusion		Infliximab infusion		
	Baseline	D 14	Baseline	D 14	D 28
IBDQ total (range 32-224)	138.6±9.4	.	.	.	179.4 ±6.7[a]
Systemic symptoms (range 5-35)	19.9 ±5.9	.	.	.	25.1 ±1.6[b]
CES-D (range 0-60)	20.4 ±9.4	17.0 ±2.9	17.0 ±2.9	14.0 ±2.4	11.3 ±2.2 [b]
IL- 18 (ng/L)	37.1± 6.4	37.3± 6.5	37.3 ± 6.5	27.4 ± 5.2[c]	26.6 ± 3.8[d]
CRP (mg/L)	21.4 ± 6.2	21.9 ± 6.3	21.9 ± 6.3	9.1 ± 1.4[d]	8.7 ± 0.8[d]

D: day; IBDQ: Inflammatory Bowel Disease Questionnaire; CES-D: Center for Epidemiological Studies Depression scale; IL-18: interleukin 18; CRP: C-reactive protein.
[a] $P \leq 0.005$; [b] $P \leq 0.01$ between baseline (before infliximab infusion) and 4 weeks after infliximab infusion;
[c] $P \leq 0.005$; [d] $P \leq 0.05$ between baseline and 2 or 4 weeks after infliximab infusion.

IL-6, IL-10, TNF-α - These cytokines were detected in half of the patients or less, at different measure points. The circulating levels of IL-6, IL-10 andTNF-α were not affected by infliximab or placebo.
Hemoglobin, CRP **-** No differences in levels of hemoglobin were found after both infusions. At baseline, 6 patients had a CRP above 7 mg/L. A significant decrease in CRP was found 2 wk after infliximab infusion, which still existed 4 weeks after infusion. Placebo infusion did not influence the CRP level (Table 3).

Partial correlation

The general fatigue score was positively related with the total CES-D score ($r = 0.781$, $P < 0.001$), as was the decrease in general fatigue levels with the decrease in CDAI score ($r = 0.642$, $P < 0.001$). The total IBDQ score, and the subscale systemic symptoms were both inversely correlated with the fatigue scores ($r = 0.765$, and $r = 0.791$; both $P < 0.001$). No significant correlations were found between fatigue scores and CRP and hemoglobin, or with any of the tested cytokines.

Discussion

The present study is the first placebo-controlled study that was conducted to examine the effect of infliximab on fatigue in CD patients, and the role of cytokines in this respect. Our results show that fatigue is significantly reduced by administration of infliximab, although it is important to bear in mind that complaints like fatigue are susceptible to a placebo effect, as was clearly demonstrated in the present study. Furthermore, infliximab reduces depression scores, and improves the quality of life. This particular study design enabled us to use patients as their own controls, restricting the sample size. A cross-over design was not thought to be appropriate since we anticipated a long lasting carry-over effect of infliximab.
Although the number of subjects studied was relatively small, we believe that this study truly reflects the influence of infliximab on fatigue in patients with clinically active CD. In daily practice, patients frequently experience a rapid reduction of fatigue after treatment with infliximab. In the present study, fatigue scores rapidly decreased after infusion with placebo, but returned to baseline values within 14 days. In contrast, the decrease of fatigue scores after infliximab infusion persisted until the end of the study, suggesting a real pharmacological effect of infliximab.
The effect of infliximab on fatigue has been described previously,[16; 17; 31] but this has never been studied in a placebo-controlled study, using dedicated and validated instruments. Lichtenstein et al.[16] reported a significant increase in the subscale systemic symptoms of IBDQ in infliximab-treated CD patients. The IBDQ, however, is not designed to study fatigue. As mentioned before, the subscale "systemic

symptoms" contains only two questions evaluating fatigue/energy loss. Persoons et al. reported a significant improvement of fatigue levels in CD patients after treatment with infliximab within two weeks. In this uncontrolled study, a dedicated instrument designed to measure multiple fatigue dimensions was used.[17]

We also demonstrated a beneficial effect of infliximab on feelings of mental depression or psychological well-being, as did Persoons et al. recently.[32] Fatigue is known to be related to negative emotions, in particular depression,[17; 22] which was confirmed in the present study by a positive relation between fatigue and feelings of depression. The impact of fatigue on the quality of life was demonstrated by the inverse correlation between both questionnaires.

A clinical response was found after infliximab infusion as well as after placebo infusion, indicating the presence of a placebo effect. This is in line with the findings of Targan et al. who demonstrated a clinical response in patients treated with infliximab (50%-81%), as well as in the placebo group (17%).[13] Su et al. [33]reported in their meta-analysis, placebo rates of remission up to 50%.

Studies of human and experimental models indicate that IL-18 plays an important role in CD.[6; 7; 11] IL-18 is up-regulated in colonic specimens of CD patients, especially in the mucosal samples taken from areas with disease activity.[7] In the present study, IL-18 was expressed in all patients, showing a significant decrease of IL-18 levels after infliximab infusion. Simultaneously with the decrease in IL-18 levels, a decrease in fatigue scores was detected after infliximab infusion, suggesting a relation between both parameters. However, this could not be demonstrated statistically.

In conclusion, we confirmed the impact of fatigue on the quality of life, and psychological well-being. Furthermore, we have shown that the reported rapid reduction of fatigue after treatment with infliximab in daily practice is subjective to a placebo effect. The effect of infliximab on fatigue, however, persists while the placebo effect extinguishes after a short period of time. This implicates that infliximab can interfere in the pathogenesis of fatigue in CD patients. An evident role of cytokines in the generation of fatigue could not be substantiated.

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IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior

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Chapter

3

Abstract

The aim of this study was to assess the prevalence of Irritable Bowel Syndrome-like symptoms in healthy controls and Inflammatory Bowel Disease patients in remission using the Rome II criteria. Furthermore, the possible relation of Irritable Bowel Syndrome-like symptoms with the quality of life and coping behavior was studied. Seventy-three ulcerative colitis patients in remission, 34 Crohn's disease patients in remission, and 66 healthy controls completed questionnaires on Irritable Bowel Syndrome, quality of life and coping. Using the Rome II criteria, Irritable Bowel Syndrome-like symptoms were found in one-third of ulcerative colitis patients and in 42% of Crohn's disease patients in remission. The presence of Irritable Bowel Syndrome-like symptoms impaired the quality of life of patients, while no relation was found between the presence of symptoms and coping strategies.

Keywords
IBS-like symptoms, Rome II criteria, inflammatory bowel disease, remission, coping, quality of life

Introduction

Irritable Bowel Syndrome (IBS) is a functional bowel disorder in which abdominal discomfort or pain is associated with features of a disordered defecation. Surveys of Western populations have revealed IBS in 15-20% of adolescents and adults, with a higher prevalence in women.[1] Nowadays the Rome II criteria are the most applied and accepted criteria for IBS.[1] Talley's group showed that the more restrictive Rome II criteria identified fewer subjects with IBS than the Manning criteria.[2; 3]

Observations that IBS may be precipitated by a transient inflammatory process in the gut have raised interest in inflammation as the basis for symptom generation in IBS.[4; 5] It is thought that the inflammatory process may leave the bowel irritable and hypersensitive after the inflammatory infiltrate has regressed and normal gut architecture has grossly been restored. The observation that a substantial number of patients with inflammatory bowel disease (IBD) in remission demonstrate bowel symptoms resembling IBS supports this hypothesis.[6; 7] IBD, comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disease of the gastrointestinal tract, characterized by a relapsing course. The reported prevalence of symptoms suggestive of IBS varies from 33% in UC patients in remission to 57% in CD patients in remission.[6; 7] In none of the previous studies the Rome II criteria for IBS were used.

The impact of IBS symptoms on the quality of life has been shown to be clinically significant in IBS patients.[8] Fewer data on the impact of these symptoms in the general population are available, but O'Keefe et al. have reported that among 520 elderly community subjects, those with IBS had significantly lower quality of life scores.[9] Simrén et al. have studied quality of life of IBD patients in remission. IBD patients with IBS-like symptoms had higher levels of anxiety and depression and lower well-being scores than those without IBS-like symptoms.

Not all IBD patients in remission suffer from IBS-like symptoms, indicating that, apart from inflammation other factors play a role. It has been suggested that psychological factors are determinants in the development of persistent bowel symptoms secondary to an inflammatory or infectious process.[4; 10] In 1992 Whitehead et al. found that IBS patients had significantly higher levels of stress compared to healthy controls, even when adjusted for individual differences in somatisation.[11] The results also suggested that IBS patients showed a greater reactivity to stress than asymptomatic controls. Coping, defined as a conscious response to stressful or negative situations, plays a significant role in a person's adaptation to stressful life events.[12] Different coping strategies may determine the outcome of a period of stress with respect to the emergence of IBS-like symptoms. We hypothesized that IBD patients in remission who suffer from IBS-like symptoms have coping strategies that are different from those without these symptoms.

The aims of the present study were therefore (a) to compare the prevalence of IBS-like symptoms according to the Rome II and Manning criteria in patients with UC and CD in remission and in healthy subjects, (b) to assess the possible relation between IBS-like symptoms in IBD patients in remission and coping strategies in stressful situations, (c) to assess the impact of the presence of IBS-like symptoms on quality of life of IBD patients in remission.

Materials and methods

Study protocol

Patients with IBD who visited the outpatient clinic of the Department of Gastroenterology at the University Medical Center of Utrecht, and who were in remission, were asked to participate in the study. If patients agreed, they received information about the study and questionnaires by mail. After having given their written consent, patients were asked to fill out a questionnaire for determination of the clinical activity of the disease: the Clinical Activity Index for Ulcerative Colitis (CAI UC) or the Crohn's Disease Activity Index (CDAI), the Manning and the Rome II criteria questionnaire, the Coping in Stressful Situations questionnaire (CISS), and the Inflammatory Bowel Disease Questionnaire (IBDQ). Furthermore, subjects were questioned about smoking habits, medication use, and education level. Partners or relatives living in the same house as the patients served as controls. They had to fill out the CISS, the Manning and the Rome II questionnaires. Questionnaires were returned by mail. At 2-month intervals, attempts were made to contact patients who had not responded by telephone. In addition to the usual demographic data, details of duration of disease were obtained from the medical records of the patients. The study was approved by the Medical Ethical Committee of the University Medical Center of Utrecht.

Subjects

Patients were eligible for the study when they were older than 18 years of age, had proven IBD for at least 3 years, and were in remission according to their physician, based on clinical parameters and medical records. The diagnosis of IBD was based on the usual clinical, radiological, endoscopic and histological features.

Patients were excluded if they were on steroids within 3 months prior to study entry or if their inflammatory markers were not within normal range (Hb, C-reactive protein, platelet count, leukocyte count, and serum albumin). Comorbidity, possibly resulting in IBS-like symptoms, was considered an exclusion criterion (e.g. malignancy, diabetes, HIV, celiac disease, major gastrointestinal surgery in the past),

as was inability to read or understand the questionnaires. Subjects who had less than one bowel movement per week were also excluded.
Partners and relatives of patients were asked by mail to serve as controls and were included in this study after having given their written consent (n=66). A series of questions about their medical history served to check the health status of the control subjects.

Questionnaires

CDAI / CAI UC- Disease activity was assessed using the Crohn's disease activity index (CDAI)[13] and the clinical activity index for ulcerative colitis (CAI UC).[14] Remission was defined as a CDAI score of <150 or a CAI UC score for 2 consecutive days of less than 10.
Inflammatory Bowel Disease Questionnaire (IBDQ) - Quality of life was measured by the IBDQ, a disease-specific health-related quality of life questionnaire, containing 32 items, with a graded response range of 1 (worst) to 7 (best) and a total score of 32 to 224.[15] The 32 items can be divided in 4 dimensional scores, including bowel symptoms (e.g. stool frequency and abdominal pain or cramps; 10 items), systemic symptoms (e.g. fatigue and energy loss; 5 items), emotional well-being (e.g. depressed feelings; 12 items), and social function (e.g. limited sexual activity; 5 items). The IBDQ has been translated and validated in the Dutch language and proved to be valid, discriminative and reliable.[16]
Coping in Stressful Situations (CISS) - Coping was measured by the CISS, containing 48 items, with a graded response range of 1 (not at all) to 5 (very much).[12] The 48 items are divided into three 16-item independent scales, assessing task-, emotion-, and avoidance-oriented coping. Coping by altering the situation is reflected by higher scores for task-oriented coping, coping by regulating emotional distress is reflected by higher scores for emotion-oriented coping, and coping by seeking other people's company or distraction is reflected by higher scores for avoidance-oriented coping.[17] The CISS proved to be reliable and valid for assessment of coping in healthy subjects as well as in patients[12; 18] and has been translated and validated in the Dutch language.[19; 20]
Manning criteria - The Manning criteria were established by Manning to distinguish IBS from organic disease.[2] Subjects were considered to have IBS if they had abdominal pain that kept recurring over a period of more than 3 months in the previous year and if they fulfilled two or more of the six Manning criteria (distension, relief of pain with a bowel movement, more frequent and looser stools at the onset of pain, mucus per rectum, feeling of incomplete evacuation).[21; 22]
Rome II criteria - The Rome II diagnostic criteria for IBS were developed through a committee consensus approach and are considered to be stricter than the Manning criteria.[1; 3] Subjects were diagnosed as having IBS according the Rome II criteria, if

they had, for at least 12 weeks in the preceding 12 months abdominal discomfort or pain with two of the three following features: (i) relieved with defecation, (ii) onset associated with a change in stool frequency of stool, and (iii) onset associated with a change in form (appearance) of stool.

Statistical Analysis

The distributions of the scores on all scales and items of the CISS, the IBDQ, the CAI UC or CDAI were evaluated by means of descriptive statistics (means, SEM). One-way ANOVA was used for comparisons between both patient groups and controls for age and the different scales of the CISS. To compare age, disease duration, IBDQ and the different scales of the CISS between patients with IBS-like symptoms versus patients without these symptoms unpaired Student *t* –tests were computed. To compare the prevalence of IBS-like symptoms in the group of healthy controls to the group of IBD patients in remission, a chi square test was used. A *P* value of less than 0.05 was considered to indicate significance. All calculations were performed with the SPSS version 10.0 for Windows.

Results

Sample

Patients and control subjects

Of 132 patients approached for this study, 10 patients declined participation, while 9 patients did not return the questionnaires and could not be contacted. Finally, 113 IBD patients participated in this study (response rate of 81%). Six CD patients were found to have mild disease activity according to the CDAI (mean CDAI score ± SEM, 176 ± 16.3) and were not analyzed further. Consequently, data of 107 patients (34 CD and 73 UC; 51 male, 56 female) were analyzed. The mean CDAI score was 38 ± 8.7; the mean CAI UC score for the two consecutive days was 2 ± 0.20. The mean age of the 66 healthy subjects (34 male, 32 female) was 45.5 ± 1.61 years.

In Table 1, demographic and clinical characteristics of the controls and the 107 analyzed patients are presented.

Comparison of the groups

Healthy controls had had significantly more surgery in the past, in particular, appendectomy, compared to both patients groups. No statistically differences were found between the two different patient groups regarding age and sex. Significantly more CD patients in remission used immunosuppressives (P=0.02) compared with UC patients in remission.

Table 1
Demographic and clinical characteristics of the controls and the 107 analyzed patients.

	Controls	Patients in remission (n=107)	
	(n=66)	UC (n=73)	CD (n=34)
Age (yr)(mean ± SEM)	45.5 ± 1.61	44.3 ± 1.47	41.3 ± 2.28
Disease duration (yr)(mean ± SEM)	.	15.3 ± 1.16	12.3 ± 1.29
Gender			
male (n)	52% (34)	51% (37)	41% (14)
Operations (n)			
none	71%† (47)	95% (69)	91% (31)
appendectomy	18% (12)	0% (0)	3% (1)
other[1]	11% (7)	5% (4)	6% (2)
Medication (n)			
5 –ASA medication	0% † (0)	89% (65)	82% (28)
Immunosuppressives	0% † (0)	32% (23)	56% (19) ††
Laxatives	0% † (0)	40% (29)	29% (10)
Smoking (n)	23% (15)	22% (16)	32% (11)

† $P \leq 0.001$; controls *vs*. UC and CD patients, †† $P \leq 0.02$; CD patients *vs*. UC patients
[1]other operations = caesarean section, hysterectomy, cholecystectomy, fistulectomy;
5-ASA=5-aminosalicyclic acid

IBS symptoms

Table 2 shows the proportions of patients and controls fulfilling the Manning criteria and/or the Rome II criteria. Of the 55 subjects classified as IBS, 26 were classified as IBS by both definitions. Table 3 shows demographic and clinical characteristics of the IBD patients meeting the Rome II criteria and the ones who did not. Patients who fulfilled the Rome II criteria had a significantly lower mean disease duration compared with the patients who did not fulfill the criteria (11.8 yr ±1.39 vs. 15.7 yr ±1.13, P=0.039). No difference in education level was found between the two groups (not shown in table 3).

Table 2
Prevalence of IBS according to the Rome II and the Manning criteria in controls and patients in remission.

	Controls (n=66)	Patients in remission (n=107)	
		UC (n=73)	CD (n=34)
IBS according to Rome II criteria (n)	7.6% † (5)	31.5% (23)	41.7% (14)
IBS according to Manning criteria (n)	9.1% †† (6)	34.2% (25)	23.5% (8)

† $P \leq 0.001$; controls *vs*. UC and CD patients, †† $P \leq 0.001$; controls *vs*. UC patients

CISS and IBDQ

The average CISS scores of all subjects (IBD patients and healthy controls) fulfilling the Rome II criteria vs. all subjects who did not fulfill the criteria were not significantly different. Table 4 shows the average CISS scores and the average IBDQ scores on the various subscales of the combined IBD group (CD and UC), leaving out the healthy volunteers. Patients who fulfilled the Rome II criteria had a significantly lower total IBDQ score ($P < 0.001$).

Table 3
Demographic and clinical characteristics of IBD patients who met the Rome II criteria and those who did not.

	Patients in remission (n=107)	
	IBS according to Rome II criteria (n=37)	No IBS according to Rome II criteria (n=70)
Age (yr)(mean ± SEM)	43.3 ± 2.14	43.3 ± 1.53
Disease duration (yr)(mean ± SEM)	11.8 ± 1.39*	15.7 ± 1.13
Gender		
male (n)	35% (13)	54% (38)
female (n)	65% (24)	46% (32)
Country of birth (n)		
the Netherlands	95% (35)	99% (69)
Europe	5% (2)	0% (0)
Africa	0% (0)	1% (1)
Operations (n)		
none	86% (32)	97% (68)
appendectomy	3% (1)	0% (0)
other[1]	11% (4)	3% (2)
Medication (n)		
5 –ASA medication	96% (35)	83% (58)
Immunosuppressives	38% (14)	40% (28)
Laxatives	43% (16)	33% (23)
Smoking (n)	22% (8)	27% (19)

* P=0.039
[1]other operations = caesarean section, hysterectomy, cholecystectomy, fistulectomy
5-ASA=5-aminosalicyclic acid

Table 4
The average CISS scores and IBDQ scores for IBD patients fulfilling the Rome II criteria and those who did not.

	Patients in remission (n=107)	
	IBS according to Rome II criteria (n=37)	No IBS according to Rome II criteria (n=70)
CISS		
Task orientated coping (range 16-80)	51.6 (1.86)	52.9 (1.33)
Emotion orientated coping (range 16-80)	36.3 (1.73)	36.9 (1.35)
Avoidance orientated coping (range 16-80)	40.5 (1.67)	40.4 (1.44)
IBDQ		
IBDQ total (32 items; range 32-224)	174.5 (3.41)**	191.3 (2.78)
Bowel symptoms (10 items; range 10-70)	55.0 (1.22)**	61.5 (0.85)
Systemic symptoms complaints (5 items; range 5-35)	23.5 (0.79)**	27.6 (0.66)
Emotional well being (12 items; range 12-84)	65.0 (1.67)*	69.5 (1.30)
Social function (5 items; range 5-35)	31.1 (0.66)*	32.6 (0.43)

All values represent mean (SEM). ** $P < 0.001$,* $P < 0.05$; patients with IBS-like symptoms *vs*. patients without IBS-like symptoms

Discussion

In this study, we have shown that 32% of the UC patients and 42% of the CD patients in remission fulfilled the Rome II criteria for IBS, whereas only 8 % of the controls did. Using previously validated questionnaires, it was found that the presence of IBS-like symptoms affected the quality of life of patients significantly, while IBS-like symptoms do not seem to result from different coping strategies.

Since the prevalence of IBS is dependent on gender, age and social status,[1; 23 24; 25] partners or housemates were chosen to serve as controls in the present study to minimize bias concerning social and economical status and diet. We appreciate that this may introduce other bias, but we consider this design superior to others. Still, we found a remarkable difference in number of appendectomies between the healthy volunteers and the UC patients. This finding is in line with previous studies in which appendectomies were found to protect against the development of UC.[26; 27]

Using the Rome II criteria, we found an IBS prevalence of 7.6 % in the control group. This number is higher than previously reported in the Dutch population, but more in accordance with data in the literature.[1] In 2001 Boekema et al. noted an incidence of IBS symptoms of 5.8% in an apparently healthy Dutch population using the Manning criteria in a telephone survey.[21] In the present study, using two of the Manning criteria combined with pain, a prevalence of 9 % in the healthy population was observed. The different recruitment and interview methods used may explain the difference in outcome of the two studies.

Concerning IBD patients, we found that 32% of the UC patients and 42% of the CD patients in remission fulfilled the Rome II criteria for IBS. In previous studies similar prevalences of IBS-like symptoms have been found in UC patients, although different criteria were used in these studies. In 1983 Isgar et al. noted symptoms of IBS, defined as abdominal pain combined with three or more of the Manning criteria, in 33% of the UC patients in remission.[7] Remission was confirmed using sigmoidoscopy. Although endoscopy was not performed to confirm remission in the present study, we are confident that use of inflammation parameters, judgment by the physician and clinical activity scores effectively excluded patients with active disease. Recently, Simrén et al. also found that one-third of their UC patients in long-standing remission suffered from IBS-like symptoms.[6] In this study, IBS-like symptoms were defined by the presence of at least one sensory symptom (i.e. pain, bloating, or feeling of incomplete rectal evacuation) in combination with at least one motility-related symptom (i.e. diarrhea or constipation). The symptoms had to be of at least moderate severity during the preceding week.

With regard to the results in the CD patients, Simrén et al. found more CD patients suffering from IBS-like symptoms (57%), compared to our results using the Rome

II criteria (42%). The difference might be explained by the inclusion of patients with a history of major intestinal surgery in Simrén's study; 52% of Simrén's CD patients had undergone ileocaecal resection or limited small bowel resection, compared to 0 % in our study population. Patients who underwent major gastrointestinal surgery for CD are probably more prone to develop IBS-like symptoms, as suggested by Simrén's observation that 67% of the CD patients with a history of intestinal surgery suffered from IBS-like symptoms. Leaving out IBD patients with a history of major intestinal surgery limits the possibility to extrapolate the results to the entire CD population, since there is a high rate of surgery in CD patients. However, we believe that the current study gives a realistic impression of the prevalence of IBD-generated functional symptoms, without interference by symptoms resulting from intestinal surgery.

In the CD group, use of the Rome II criteria resulted in a two times higher prevalence of IBS-like symptoms compared with the Manning criteria. This is in contrast with what can be expected, since the Rome II criteria are thought to be more restrictive than the Manning criteria [3]. We can not explain this remarkable discrepancy.

There is considerable evidence that the quality of life in IBD patients is impaired,[28] more so with more severe disease.[29] Data from the present study showed that IBD patients in remission who have IBS-like symptoms have a more reduced quality of life than IBD patients without such symptoms. Although the method used to measure quality of life was different, this study confirms the findings of Simrén et al. It is also in line with the observation that quality of life is significantly lower in IBS patients compared with asymptomatic controls.[8]

The instruments used in this study have some overlapping domains; for example the IBS-like symptoms could increase the CDAI score and decrease the total IBDQ score. Despite this disadvantage, we have chosen to work with validated and international accepted questionnaires and instruments. Moreover, the reduced quality of life found in our study is not only due to a decrease in the bowel dimension of the IBDQ, but also to systemic symptoms, impaired emotional well-being, and impaired social function.

Finally, we did not find any conformation concerning the hypothesis regarding coping. Coping strategies do not seem to play a major role in the development of IBS-like symptoms.

In conclusion, this study has shown that about one-third of UC patients and 40% of CD patients in remission suffer from IBS-like symptoms, affecting their quality of life. The development of IBS-like symptoms does not seem to result from insufficient coping strategies. In future, when IBS-like symptoms in IBD are studied, well defined and generally accepted criteria, such as the Rome II, have to be used, since especially in CD patients the prevalence found seems to be dependent on the criteria used.

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A pilot study on chemospecific duodenal visceral sensitivity in inflammatory bowel disease in remission

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Chapter

4

Abstract

Background

Patients with inflammatory bowel disease (IBD) in remission frequently experience symptoms resembling irritable bowel syndrome (IBS). In IBS altered motility and visceral sensitivity are found throughout the whole gastrointestinal tract. We aimed to study chemospecific antroduodenal sensitivity in IBD patients.

Methods

Antroduodenal manometry was performed in 10 IBD patients in remission and 13 controls. Small volumes of nutrients and acid were administered intraduodenally. Motility variables and sensation scores were compared before and after each infusion.

Results

Acid- and lipid infusion decreased the number of antral pressure waves in both groups ($P < 0.05$). After acid infusion the number of duodenal pressure waves in the sideholes just distal to the infusion port increased in IBD patients compared to the controls ($P < 0.05$). Lipid infusion increased the number of duodenal propagated pressure waves in both groups, but in controls they were also increased over longer distances ($P < 0.005$). None of the infusions significantly affected the sensation scores.

Conclusion

Subtle alterations in chemospecific responses to lipids and acid in IBD patients in remission were observed, affecting duodenal motor activity but not duodenal perception. These changes are indicative for changes at the level of chemoreceptors in the duodenal wall in this patient group.

Keywords
antroduodenal manometry, chemospecific visceral sensitivity, inflammatory bowel disease, colonic inflammation

Introduction

Patients with inflammatory bowel disease (IBD), in active as well as in quiescent disease, frequently complain of a wide range of gastrointestinal symptoms. A substantial proportion of patients in remission experience bowel symptoms resembling irritable bowel syndrome (IBS),[1-3] while other symptoms are more likely to be related to the proximal part of the intestine. Visceral hypersensitivity is widely regarded as an important pathophysiological factor in the development of functional gastrointestinal disorders, such as IBS and functional dyspepsia (FD).[4; 5] Interestingly, increased visceral sensation in IBS patients is found not to be restricted to the colon, but can also be found in the small intestine,[6] and the esophagus.[7] These findings suggest hypersensitivity throughout the whole gastrointestinal tract in this functional gastrointestinal disorder.

Work carried out in animal studies demonstrated that inflammation at one site in the gut can produce altered motor function at a remote non-inflamed site.[8-10] Furthermore, Bercik et al.[11] reported long-lasting gut dysmotility and hyperalgesia in mice after transient intestinal inflammation, suggesting that alterations in motility and sensitivity are prolonged after mucosal inflammation has disappeared.

IBD comprises ulcerative colitis (UC) and Crohn's disease (CD). Studies evaluating rectal visceral sensitivity in UC patients (with disease activity as well as patients in remission) have yielded conflicting results.[12-17] Bernstein et al. showed that CD patients with inflammation confined to the small bowel have rectal hypoalgesia,[18] while Andersson et al.[19] found increased rectal sensitivity in CD patients without macroscopic proctitis. The findings of the latter study are in line with the results of Galleazzi et al,[20] who showed esophageal hyperalgesia in patients with UC, indicating the existence of diffuse hyperalgesia in intestinal inflammatory processes such as CD and UC.

Altered visceral sensitivity to stimuli can be revealed in several ways: (i) abnormalities in the local motor responses close to the site of the stimulus; (ii) altered feedback mechanisms, and/or (iii) by altered visceroperception. To study these mechanisms different stimuli including stimulation of chemo-, thermo-, and mechanoreceptors can be applied. Of these, only stimulation of mechanoreceptors has been studied in depth. Recently, our group has demonstrated that physiological amounts of different nutrients and acid induce chemospecific motor responses when infused intraduodenally.[21] These motor responses are mediated by chemoreceptors in the duodenal wall and involve the enteric nerve system (ENS). When compared to healthy controls, Schwartz et al.[22] observed altered chemospecific responses to nutrients and acids in FD patients, affecting both duodenal motor activity and duodenal perception. This suggests an abnormality at the level of visceral afferents and/or mucosal chemoreceptors in these patients. In IBD,

inflammation affects the intestinal mucosa which can result in structural and/or functional alterations of the ENS in a remote site of the gut.[23] We hypothesized that IBD patients in remission, with prior disease activity restricted to the colon, are burdened with visceral hypersensitivity throughout the whole gastrointestinal tract, resulting in altered chemosensitivity in a remote, previously never inflamed site of the gut. Therefore, in this pilot study our first aim was to investigate whether stimulus-specific antroduodenal motor responses are altered in IBD patients in remission when compared to healthy controls. The second aim was to assess whether duodenal hypersensitivity (viscero-perception) in IBD patients in remission exists, and if so whether this hypersensitivity depends on the chemical composition of the stimulus.

Materials and Methods

Subjects

Patients

Ten consecutive patients with IBD in remission from the outpatient Department of Gastroenterology of the University Medical Center in Utrecht were enrolled in the study (6 women and 4 men; mean age ± SD: 46.5 ± 12.1 years). The diagnosis of IBD was based on the usual clinical, radiological, endoscopic and histological features.[24] In all 10 patients previous inflammation was restricted to the colon (3 CD patients, 7 UC patients; mean disease duration 18 ± 10.0 yr). At least one-third of the colon had to be involved in the past. Remission was confirmed by colonoscopy prior to the manometric study. None of the CD patients was known with fistulizing or stenotic disease behavior, or with ileal or duodenal disease activity. All had had an upper GI endoscopy in the past to exclude duodenal involvement. Furthermore, none of the CD patients suffered from symptoms indicating disease activity in the upper gastrointestinal tract.

All patients used mesalamine preparations (1.5 g- 3.0 g per day), 5 patients used azathioprine (75 mg –150 mg per day) and 3 patients used laxatives (macrogol, magnesiumoxide, and psyllium fibers). The laxatives were stopped two days prior to the study.

Four patients had a history of abdominal surgery (appendectomy, cholecystectomy, hysterectomy, sterilization). Of all patients, two fulfilled the Rome II criteria for FD and two patients suffered from IBS and FD according to the Rome II criteria.

Healthy controls

Thirteen healthy controls (6 women, 7 men; mean age: 30.8 ± 10.6 years) participated in this study. None of them suffered from gastrointestinal complaints. A se-

ries of questions about their medical history served to check the health status of the control subjects. Two of the 13 healthy controls had had abdominal surgery in the past (appendectomy, adhesiolysis).
None of the subjects (patients and healthy controls) used medication known to influence gastrointestinal motor activity (e.g., erythromycin, loperamide, morphine, codeine, anti-cholinergic medication) or steroids within 3 months prior to study entry. Comorbidity possibly resulting in gastrointestinal symptoms was considered an exclusion criterion (e.g. diabetes, neuropathy, celiac disease). The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, and written informed consent was obtained from all subjects. All actions were performed in accordance with the Declaration of Helsinki.

Study protocol

After an overnight fast the manometric catheter was introduced transnasally and positioned across the pylorus, using fluoroscopic control if necessary. The position of the catheter was monitored by measurement of the antroduodenal transmucosal potential difference (TMPD). Subjects were in a supine position with the head of the bed tilted at an angle of 30° during the experiment. After an accommodation period of at least 20 minutes, the experiment started during a well-defined phase II. Infusion of 5 ml of saline (NaCl 0.9%), 5 ml acid (0.1 M HCl solution), 5 ml dextrose 25 % (1 kcal/ml), and 5 ml lipid emulsion (Intralipid 10 %, 1.1 kcal/ml) was administered via an infusion port in the duodenal bulb at a rate of 5 ml/min and followed by a washout period of at least 15 min. The sensations of nausea, abdominal pain, bloating, urgency and fullness were scored on a visual analogue scale (VAS), before, 1 min after, and 5 min after each infusion. Patients were blinded for all infusions; acid and saline were administered in a randomized order, followed by dextrose and lipid infusion.

Manometry assembly

Antropyloroduodenal manometric recordings were obtained using a 20-channel water-perfused silicone rubber catheter with a length of 220 cm and a total diameter of 4 mm, incorporating 20 lumina with a diameter of 0.4 mm diameter, and one central lumen with a diameter of 1.0 mm for intraduodenal infusion (Dentsleeve International, Wayville, South Australia; Figure 1).[21; 25-27] The assembly incorporated a 4-cm long transpyloric sleeve sensor with 3 sideholes spaced along the sleeve, 4 antral sideholes, and 12 duodenal sideholes. The duodenal sideholes were spaced at 1.5 cm-intervals (D1-D12). The most proximal duodenal sidehole (D1) was located 2 cm from the mid-pyloric region; the most distal sidehole (D12) was located 18.5 cm from the pylorus. The infusion port was located at 4.25 cm from the mid-pyloric region, between D2 and D3. Manometric sideholes were perfused with

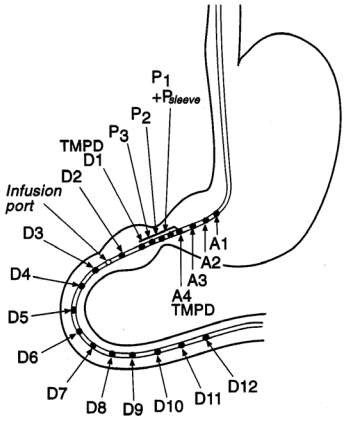

Figure 1
Schematic drawing of the manometric assembly (see text for details)

degassed distilled water at a rate of 0.08 ml/min, using a pneumohydraulic perfusion system (Dentsleeve Pty.Ltd.). TMPD was measured via two sideholes (A4 and D1, located at each end of the sleeve sensor), which were perfused with degassed saline from separate reservoirs at a rate of 0.08 ml/min. A disposable Ag/AgCl electrode attached to the infraclavicular region was used as reference electrode.
Pressures from all perfused sideholes were recorded via external transducers (Abbott, Chicago, USA). Pressure and TMPD data were stored in digital format in two 12-channel data loggers (Medical Measurement Systems, Enschede, The Netherlands) with a memory capacity of 4 MB each, using a sample frequency of 8 Hz for pressure and 1 Hz for TMPD signals. At the end of each manometric study the data was transferred the hard disk of a computer for subsequent analysis.

Analysis of pressure data

Manometric recordings were analyzed only when the difference between antral and duodenal TMPD was at least –15 mV. The phases of the interdigestive migrating motor complex (MMC) were determined visually by applying the following definitions: phase I = motor quiescence starting immediately after completion of phase III; phase II = pressure waves ≥ 1.4 kPa occurring at a rate higher than 2 per 10 min and less than the maximum frequency of the antrum (3 contractions/min) or the duodenum (10-12 contractions/min), and phase III = rhythmic contractile activity at the maximum frequency (3 contractions/min) in the antrum for at least 1 min and in the duodenum (10-12 contractions/min) for at least 2 min. Phase III has to be antegradely propagated over at least four duodenal recording sites and followed by motor quiescence.[28] Antral pressure waves (i.e. sideholes A1-A3) and duodenal

pressure waves (i.e. sideholes D3-D11) were evaluated in 5-min intervals.
Analysis was performed on 5-min recording periods pre- and postinfusion. The infusion period of 1 min was included in the 5- min postinfusion period. Data from three preinfusion periods were summed and averaged for each subject.
The number of pressure waves of amplitude ≥ 1.4 kPa, their mean amplitude and their spatiotemporal characteristics were analyzed in an automated fashion using previously described software.[21; 22] For each duodenal sidehole, the number of antegradely propagated pressure waves originating from this location, was calculated. All antegradely propagated pressure waves originating from D3 –D11 were summed to calculate the total number of antegradely propagated pressure waves. A sequence of six sideholes distal to D2 (i.e. sideholes D3-D8) was selected for detailed analysis of propagation. Sidehole 8 was chosen because a statistically analysis distal to D8 according to propagation distance was not feasible, because of the limited length of the catheter.[21] An antegradely propagated pressure wave was defined as a pressure wave propagating over at least two recording sites (i.e. a distance of at least 1.5 cm). Pressure waves were considered temporally associated when the intervals corresponded with a propagation velocity was between 0.9 and 4.0 cm/s.[25] Short –traveling pressure waves were defined as antegradely propagated duodenal pressure waves over 1.5 cm to 4.5 cm (one to three sideholes), originating from D3 –D8.

Assessment of gastrointestinal sensations

During the study, the sensations of nausea, abdominal pain, bloating, fullness and urgency were scored on a 100 mm visual analogue scale (VAS) ranging from "no sensations" to sensations "as bad as can be". All these sensations were scored before, 1 minute after, and 5 minutes after each infusion.[22]

Statistical analysis

For each time interval of 5 minutes, the number of antral pressure waves was averaged over the three antral sideholes, analyzed and the mean amplitude was calculated. Repeated measures analysis of variance (RM-ANOVA) was performed to test for infusion-induced differences in manometric variables obtained from 9 duodenal sideholes (D3-D11). Identification of spatial differences in infusion-induced duodenal motor responses could lead to division of sideholes in blocks and subsequent separate analysis of these blocks using RM-ANOVA. RM-ANOVA was also used for comparisons between patients and controls, by defining a group variable. Depending on distribution, the Student *t* test or Mann-Whitney test was used for unpaired comparisons, and the paired sample *t* test or Wilcoxon signed rank test for paired comparisons in the other motility parameters. Comparisons between the different infusions were not performed. The sensation scores, which were not normally dis-

tributed, were compared using non-parametric tests. The Wilcoxon signed rank test was used for paired intraindividual comparisons and the Mann-Whitney *U* test for comparisons between patients and controls. $P < 0.05$ was considered to be statistically significant in all analyses. Data are presented as mean ± SEM, unless stated otherwise. Statistical analysis was performed with the SPSS version 11.5 for Windows.

Results

Comparison of the groups

Patients were significantly older than the healthy controls (46.5 ±12.2 years vs. 30.8 ± 10.6 years; $P < 0.005$). Furthermore, they used significantly more medication ($P < 0.001$) and suffered more frequently from gastrointestinal symptoms ($P < 0.05$).

Effects of infusion on motility

Antral pressure waves

Preinfusion. The amplitude and the number of pressure waves per 5 min averaged over the 3 antral sideholes analyzed were comparable during the preinfusion recordings in patients and controls (Table 1).

Saline infusion. After infusion of 5 ml saline, no differences were recorded in antral motility variables when compared with the preinfusion period (Table 1).

Dextrose infusion. Dextrose infusion did not affect any of the parameters of motility. No difference between the groups was found in the mean amplitude or number of antral pressure waves (Table 1).

Acid infusion. In both patients and healthy controls, acid infusion induced a significant decrease in the number of antral pressure waves when compared to preinfusion. The mean amplitude of the antral pressure decreased significantly after acid infusion in patients, but not in the control group. Consequently, patients were found to have lower mean amplitudes of antral pressure waves after infusion compared to healthy controls. No difference was found in the mean number of antral pressure waves between both groups.

Lipids infusion. In both groups, lipids induced a significant decrease in the number of antral pressure waves and in mean amplitude. Between the groups, no differences in antral motility variables were found (Table 1).

Table 1
Effect of each infusion on motility parameters in IBD patients in remission and healthy controls

	preinfusion		saline		dextrose		acid		lipids	
	patients	controls	patients	controls	patients	controls	patients	controls	patients	controls
averaged number of APW	1.8 ± 0.4	2.3 ± 0.4	1.7 ± 0.5	2.3 ± 0.6	1.4 ± 0.6	1.3 ± 0.3	0.5 ± 0.4‡	1.2 ± 0.4¶	0.2 ± 0.1‡	0.5 ± 0.2‡
mean amplitude of APW (kPa)	5.5 ± 1.3	9.2 ± 1.8	7.3 ± 2.6	8.6 ± 2.3	6.4 ± 3.6	6.2 ± 2.2	0.4 ± 0.3‡ ¶¶	7.5 ± 2.2	0.4 ± 0.2‡	3.0 ± 2.1¶
total number of APDW	9.9 ± 2.3	11.8 ± 1.5	11.2 ± 3.1	13.2 ± 2.7	19.7 ± 5.6	18.2 ± 3.5 ¶	17.5 ± 5.4	17.4 ± 2.6	27.7 ± 8.7¶	37.1 ± 5.8‡

Data are expressed as mean ± SEM
APW=antral pressure waves (sideholes A1-A3); APDW= antegradely propagated duodenal pressure waves originating from D3 to D11.
¶ $P < 0.05$ compared with preinfusion; ‡ $P \leq 0.005$ compared with preinfusion; ¶¶ $P \leq 0.01$ compared with healthy controls

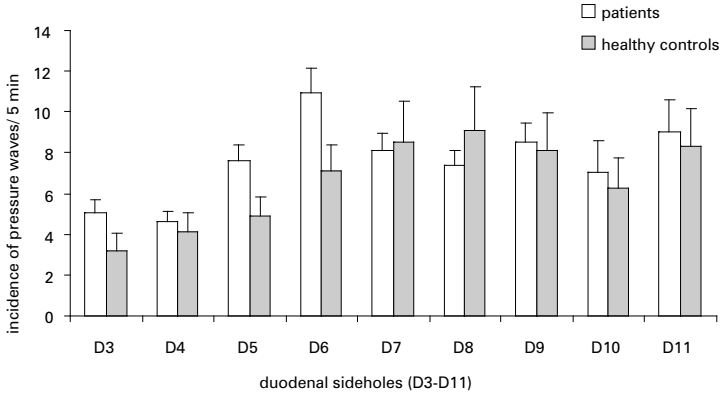

Figure 2
Incidence of duodenal pressure waves during the preinfusion 5-min period in IBD patients in remission and healthy controls.

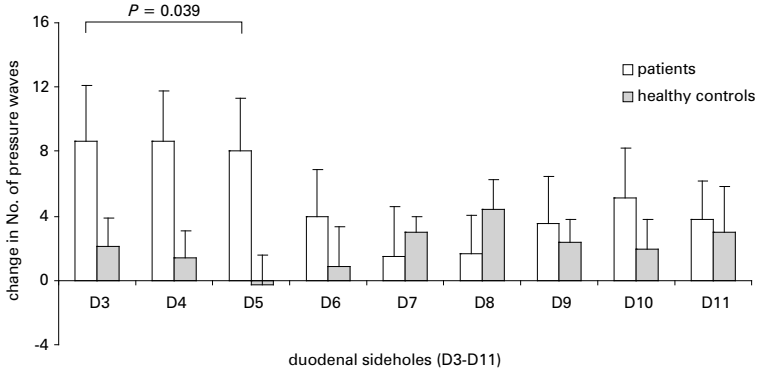

Figure 3
Acid-induced change in number of duodenal pressure waves in IBD patients in remission and healthy controls

Duodenal pressure waves

Preinfusion. The incidence and mean amplitude of duodenal pressure waves did not differ between both groups during the preinfusion recordings (Figure 2).

Saline infusion. Infusion of 5 ml saline did not affect the incidence or mean amplitude of duodenal pressure waves in both groups.

Dextrose infusion. The number of duodenal pressure waves was not affected by infusion of dextrose in either of the groups, nor was the mean amplitude of the pressure waves.

Acid infusion. Acid infusion induced a non-significant overall increase in duodenal

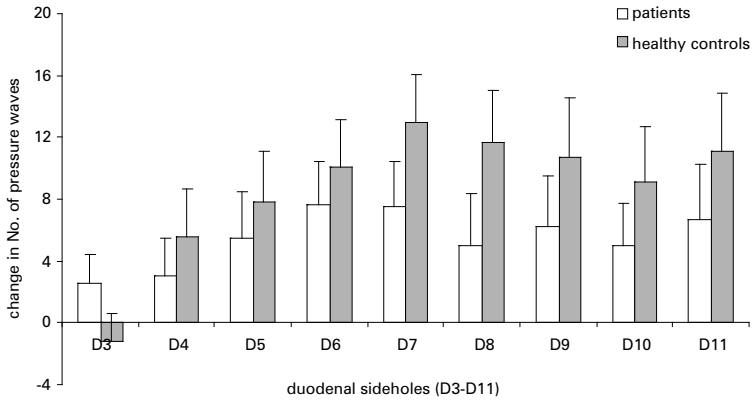

Figure 4
Lipids-induced change in number of duodenal pressure waves in IBD patients in remission and healthy controls

pressure waves in healthy controls as well as in patients (Figure 3). However, in patients the incidence and amplitude of pressure waves in the sideholes just distal to the infusion port (D3-D5) were significantly increased when compared to the preinfusion period (respectively RM-ANOVA; $P < 0.05$ and $P < 0.01$). Consequently, the increase in the number of pressure waves after acid infusion was significantly greater in patients than in healthy controls in sidehole D3 to D5 (RM-ANOVA; $P < 0.04$; Figure 3). No difference in amplitude was found between both groups.
Lipids infusion. Distal to the infusion site the number of duodenal pressure waves increased compared to preinfusion values in both patients and healthy controls after lipid infusion (Figure 4). In healthy controls the increase in duodenal pressure waves from D3 to D11 reached significance (RM-ANOVA; $P < 0.005$), whereas in patients no significant difference was observed ($P = 0.092$). No difference could be found comparing healthy controls and patients. Analysis of pressure waves recorded from sidehole D5 to D11 separately, yielded similar results.

Propagated duodenal pressure waves

Preinfusion. The total number of antegradely propagated pressure waves was comparable in both groups during the preinfusion recordings (Table 1). Most propagated waves were propagated over short distances in both groups (Figure 5).
Saline infusion. Infusion of 5 ml saline did not affect the total number of antegradely propagated pressure waves in either of the groups (Table 1).
Dextrose infusion. Dextrose infusion increased the total number of antegradely propagated pressure waves in healthy controls (Table 1).

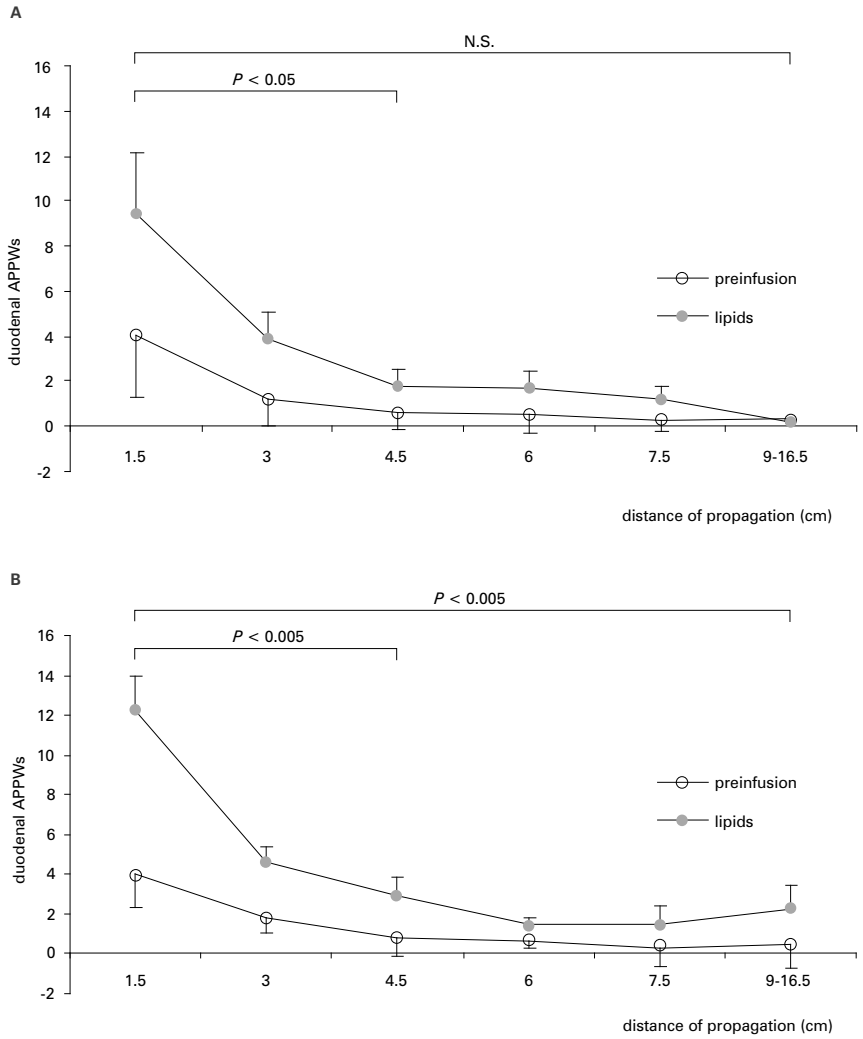

Figure 5
Effects of lipids on the mean cumulative numbers of duodenal antegradely propagated pressure waves (APPW) recorded in sideholes D3 to D8, in IBD patients in remission (A) and healthy controls (B).

Acid infusion. Acid infusion did not lead to an increase in total number of antegradely propagated pressure waves in either group.
Lipids infusion. The total number of duodenal propagated waves was significantly increased in both groups after lipid infusion (Table 1). An increase in short-traveling propagated pressure waves was found in both groups (RM-ANOVA; healthy controls, $P < 0.005$; patients, $P < 0.05$), whereas in controls the duodenal propagated waves were also increased over longer distances (RM-ANOVA; $P < 0.005$) (Figure 5).

Effects of infusions on sensations

The baseline scores for all sensations were similar in both groups. None of the infusions significantly affected the sensation of nausea, fullness, abdominal pain, bloating or urge in healthy controls or patients. Between the 2 groups, no differences were found. Analyzing patients with symptoms of functional dyspepsia separately, again, no differences were found.

Discussion

In the present study we explored duodenal chemosensitivity in IBD patients in remission in order to study duodenal visceral sensitivity.
As mentioned before, duodenal motor responses to nutrients or acid can roughly be divided into two types: a local motor response close to the site of infusion, and a motor response that involves duodenogastric feedback loops. We demonstrated that the inhibitory feedback loops were intact in patients as well as in healthy controls. Saline and dextrose infusion did not result in an antral motor response, while infusion of acid and lipids induced suppression of the number of antral pressure waves. The data are in line with the results of Schwartz et al. and Mueller [22; 29] who reported similar antral motor responses in healthy controls and FD patients after infusion of these nutrients and acid. A significant difference in the mean amplitude of antral pressure waves in IBD patients was observed compared to healthy controls during acid infusion. This may indicate a more intensified duodenogastric neural reflex in these patients. With respect to the duodenal motility variables, we found that saline and dextrose infusion did not affect any of the variables in IBD patients, while infusion of acid and lipid induced coordinated motor responses. Acid increased the incidence and amplitude of pressure waves in the sideholes just distal to the infusion port (D3-D5) in patients when compared to the preinfusion period. The increase in number of pressure waves was significantly greater in patients than in healthy controls. Lipid also induced an increase in the number of duodenal pressure waves compared to preinfusion in both patients and healthy

controls. This increase of duodenal pressure waves partly consisted of an increased number of short-traveling propagated pressure waves. However, only in the healthy controls the number of duodenal propagated waves also increased over longer distances. Although the number of subjects in this pilot study was relatively small, we have shown subtle differences in chemospecific responses to lipids and acid in IBD patients in remission. In our opinion these changes are indicative for changes at the level of chemoreceptors in the duodenal wall in this group of patients. Still, one can question the clinical relevance of these findings since none of the infusions induced altered perception of nausea, abdominal pain, bloating, urgency or fullness.

In the past Schonveld et al. showed that healthy controls experienced nausea while drinking large quantities of 10% Intralipid.[30] Furthermore, Bjornsson et al.[31] found that the occurrence of nausea during a 1- hour duodenal lipid infusion was more common in FD patients than in healthy controls. In this last study the infusion rate was (2 kcal/min). In the present study Intralipid 5.5 kcal/min was infused for 1 min. Perhaps we might have found altered sensation scores if the infusion duration had been extended or the infusion volume had been enlarged. We believe that by infusing small quantities of nutrients with low caloric value only differences in chemical composition could account for the observed differences in responses.

Whereas our motility group previous showed that FD patients had a significant increase of nausea scores after acid infusion,[22] analyzing IBD patients with symptoms of FD separately did not change the outcome. This can of course be due to the small sample size (n=4), but it can also be hypothesized that there is different mechanism underling functional gastrointestinal symptoms in patients with IBD in remission. Heightened visceral perception in remote, non-inflamed sites have been found in IBD patients in the past, but previous studies on this topic have focused on mechanosensitivity. In these studies some patients with mild disease activity were included [20] and disease activity was clinically defined.[19; 20] In the patients studied, however, more severe disease activity might have been present and the reported altered visceral perception could have been generated by unrecognized inflammatory activity. While most studies reported hypersensitivity in IBD patients, suggesting a possible central sensitization effect secondary to their chronic inflammation, Bernstein et al. found rectal hypoalgesia. In this study, remission or mild activity was confirmed by endoscopy.[18]

As mentioned earlier, duodenal chemospecific visceral perception has not been studied before in this particular patient group.

In the present study, duration of remission could also have affected the outcome of the perception scores. All patients included were in remission for a longer period of time. Perhaps visceral sensitivity is heightened during and directly after an exacerbation of the disease, after which the effect of the inflammation gradually extinguishes. Furthermore, to measure altered visceral sensitivity, it is possible that the

site of previous inflammation (colon) should be in closer proximity to the site studied, although this is not in line with the findings of Galeazzi et al.[20] who found esophageal hyperalgesia in UC patients. However, in their study mechanosensitivity instead of chemosensitivity was assessed in the esophagus in a patient group including UC patients with mild disease activity. Finally, the severity, extend, and other aspects of disease behavior in the past might be relevant considering the generation of altered motility or hypersensitivity in IBD. It cannot be ruled out that in patients with a more aggressive phenotype than enrolled in the present study, abnormalities in chemosensitivity can be found more easily.

In summary, this pilot study shows subtle alterations in the chemospecific responses to lipids and acid in IBD patients in remission, affecting duodenal motor activity but not duodenal perception. The existence of these abnormalities is indicative of a defect either on local mucosal (chemoreceptor) level or in neural reflex controls in (a subset of) these patients. This outcome warrants further research, but in our opinion, the use of manometry will not provide the desired answers. To study changes at the level of the duodenal wall, immunological and/or histological assessments will probable be more appropriate.

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Serotonin synthesis and uptake in symptomatic patients with Crohn's disease in remission

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Chapter 5

Abstract

Background and aims

Symptoms resembling irritable bowel syndrome (IBS) are reported frequently in Crohn's disease (CD) patients in remission. Studies of the mucosal content of serotonin, which is a pivotal neurotransmitter in the gut, suggest that serotonin availability is altered in IBS patients. We aimed to study the role of serotonin in the generation of IBS-like symptoms in CD patients in remission.

Methods

Ileal- and colonic biopsy specimens were obtained from 20 CD patients in remission, 10 with and 10 without IBS-like symptoms, and 11 healthy controls. Enterochromaffin cells were counted, and messenger RNA expression levels of tryptophan hydroxylase (TpH)-1 and serotonin reuptake transporter were determined.

Results

The levels of mucosal serotonin reuptake transporter expression were significantly higher in the ileum than in the colon, in all groups studied ($P < 0.02$). When ileum and colon were analyzed separately, TpH-1 expression in the colon of CD patients with IBS-like symptoms was found to be significantly higher compared with the 2 other groups studied (controls: $P < 0.005$; CD patients without IBS-like symptoms: $P < 0.01$). The number of enterochromaffin cells per gland was comparable for the patient groups in ileum and colon.

Conclusions

CD patients in remission who experience IBS-like symptoms have increased mucosal TpH-1 levels in the colon, suggesting that increased serotonin biosynthesis in the colon plays a role in the generation of the symptoms.

Keywords

serotonin reuptake transporter, tryptophan hydroxylase –1, inflammation, Crohn's disease, irritable bowel syndrome.

Introduction

Patients with inflammatory bowel disease (IBD), which comprises ulcerative colitis (UC) and Crohn's disease (CD), often experience bowel symptoms resembling irritable bowel syndrome (IBS) such as abdominal discomfort/pain, diarrhea, and features of disordered defecation.[1-3] The reported prevalence of symptoms suggestive of IBS in CD patients in remission varies from 42% to 57%.[2; 3] In IBS patients altered sensitivity and motility are found throughout the whole gastrointestinal (GI) tract.[4; 5] In IBD, altered motility of the gut has been reported as well.[6-8] Several studies evaluating visceral sensitivity in CD patients have shown altered rectal sensitivity in patients with active disease, as well as in remission.[9; 10]Visceral sensitivity, motility, and the coordination between other functions (e.g. secretory reflexes) in, and between, the different regions of the gut is accomplished through the enteric nervous system (ENS), epithelial cells, and the mucosal immune system. The pivotal role of the ENS in these processes is reflected in its complex circuitry, which receives inputs from the cephalic brain and the autonomic nervous system, as well as in its ability to respond to signals from the immune system and epithelial cells of the gut.

In IBD, inflammation affects the intestinal mucosa, which can result in structural and/or functional alterations of the mucosal immune system and abnormalities of the ENS.[11] This influences the excitability of neurons and nerve endings, and the distribution of neurotransmitters.[12; 13] Serotonin (5-HT) is a pivotal neurotransmitter and paracrine signaling molecule in the gut. It plays an important role in visceral sensation,[14] and it initiates peristaltic and secretory reflexes by acting on 5-HT receptors.[15; 16] Therefore alterations in biosynthesis, release, or reuptake of 5-HT may contribute to disordered GI function. It is also known that 5-HT in the brain is involved in mood control. For that reason it is even conceivable that the recently reported relation of depressive disorders with a lack of clinical efficacy of infliximab in CD patients generates from abnormalities in the serotonergic pathways in the brain.[17] In the GI tract, 5-HT is synthesized, stored and secreted by enterochromaffin (EC) cells, which are located in the epithelial layer. The rate-limiting enzyme in the biosynthesis of 5-HT is tryptophan hydroxylase (TpH)-1. To control 5-HT actions in the gut and limit 5-HT receptor desensitization, enterocytes express the serotonin reuptake transporter (SERT),[18] which terminates the action of 5-HT by removing it from the interstitial space. Recently, Linden et al. reported increased 5-HT availability at the mucosal level in different animal models of colitis.[19; 20] Moreover, studies in human beings have shown that 5-HT availability is altered in IBD, as well as in IBS.[21-24] These studies make it conceivable that inflammation of the gut affects 5-HT signaling in the GI tract. Therefore, we hypothesized that IBS-like symptoms in CD patients in remission are associated with a persistent imbalance of mucosal 5-HT availability caused by altered 5-HT synthesis and/or uptake.

Materials and methods

Subjects

Patients

CD patients with a clinical indication for ileocolonoscopy were recruited from the outpatient Department of Gastroenterology of the University Medical Center in Utrecht. The diagnosis of CD was based on the usual clinical, radiological, endoscopic and histological features.[25] Ten consecutive CD patients in remission with IBS-like symptoms according to the Rome II criteria (5 women; 5 men; mean age (± SD): 46.6 years (± 16.4) and 10 consecutive CD patients in remission without IBS-like symptoms (6 women; 4 men; mean age (± SD): 46.3 years (± 11.5) were enrolled in this study. Three of all 20 CD patients had undergone a right hemicolectomy, one patient with IBS-like symptoms, and 2 without these symptoms. Two of the CD patients with, and 3 of the CD patients without IBS-like symptoms had undergone an ileocecal resection in the past. Eight of the 10 patients with IBS-like symptoms suffered from predominant-diarrhea IBS according to the Rome II criteria, whereas the other 2 patients both had 3 symptoms indicative for the diarrhea variant and 1 symptom for the constipation variant.

Disease activity was assessed endoscopically using the Crohn's disease Endoscopic Index of Severity (CDEIS).[26] Patients with endoscopically minimal disease activity (e.g. 1 aphthoid ulceration in the ileum or a segment of less than 2 cm. of frank erythema in the transverse colon) were considered eligible as well.

Healthy controls

Eleven healthy controls (3 women; 8 men; mean age (± SD): 51.6 years (± 16.7), scheduled for screening ileocolonoscopy or for follow-up evaluation for colonic polyps, participated in this study. During endoscopy no signs of inflammation or other abnormalities were seen. None of the healthy controls suffered from gastrointestinal complaints. A series of questions about their medical history served to check the health status of these individuals.

In Table 1 the demographic and clinical characteristics of the controls and the participating patients are presented. None of the subjects used medication known to influence serotonergic signaling (selective serotonin reuptake inhibitors, 5-HT receptor agonists/antagonists). Comorbidity, possibly resulting in gastrointestinal symptoms, was considered an exclusion criterion as well (e.g. diabetes, neuropathy, celiac disease).

Table 1
Demographic and clinical characteristics of healthy controls and CD patients.

	Controls	CD with IBS-like symptoms	CD without IBS-like symptoms
	(n=11)	(n=10)	(n=10)
Disease duration (mean ± SEM)		15.0 (± 3.2)	18.4 (± 2.6)
Fistulizing disease behavior (n)		2	3
Stenotic disease behavior (n)		4	5
Localization			
colon (n)		4	3
ileocecal and colon (n)		6	7
Medication			
Mesalamine (n) (range)	0	6 (3 –6 g/day)	6 (1.5-3 g/day)
Azathioprine (n) (range)	0	6 (100-150 mg/day)	4 (100-150 mg/day)
Steroids (n) (range)	0	4 (9 mg/day Budesonide; 2,5-5 mg/day Prednison)	3 (6-9 mg/day Budesonide)
Methotrexate (n)	0	1	0

Study protocol

Ileocolonoscopy was performed in all subjects by the same endoscopist (B.O.). During endoscopy of CD patients, the CDEIS was filled out. Four biopsy samples, 2 from ileum, and 2 from transverse colon (or from the left colon if patients had undergone right hemicolectomy) were taken from endoscopically normal mucosa for assessment of messenger RNA (mRNA) expression. Biopsy specimens were obtained using a standard-capacity biopsy forceps. The tissue samples of all subjects (11 healthy controls, 10 CD patients with IBS-like symptoms, and 10 CD patients without these symptoms) were snap frozen in liquid nitrogen, and subsequently stored at -80°C until extraction of RNA. To evaluate the histopathological disease activity and the number of EC cells, 2 additional biopsy specimens of the most affected part of the colonic and ileal mucosa were taken from all patients and 4 healthy controls. All subjects were asked to fill out a questionnaire to assess the Rome II criteria for IBS.[27] Furthermore, patients filled out the Crohn's Disease

Activity Index (CDAI).[28] In addition to the usual demographic data, the details of duration of disease, medication use, and previous surgery were obtained from the medical records of the patients. Routine biochemical and hematological tests were performed in patients (e.g. C-reactive protein, white cell count, erythrocyte sedimentation rate). The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, and written informed consent was obtained from all subjects.

Disease activity

Endoscopic disease activity - Endoscopic disease activity in patients was assessed by the Crohn's disease Endoscopic Index of Severity (CDEIS).[26] This index is based on a number of reproducible mucosal (skip) lesions; erythema, edema, pseudopolyps, aphthoid ulcers, (longitudinal) ulcers, and stenosis.[29] At present, the CDEIS represents the gold standard for evaluation of endoscopic disease activity in CD. A specific cut-off value, which defines remission has not yet been determined. Table 2 shows the CDEIS scores of both patient groups.

Histopathological disease activity – Histopathological disease activity in the patients' biopsy specimens was scored by a pathologist, blinded for the endoscopic results.

Clinical disease activity - Clinical disease activity in patients was assessed using the Crohn's disease activity index (CDAI),[28] which is the most used and accepted clinical activity score worldwide. A score of 150 or less indicates remission. Table 2 shows the CDAI scores of both patient groups.

Measurement of mRNA encoding TpH-1 and SERT

Total RNA was extracted from the biopsy specimens using the RNeasy micro kit (Qiagen, Hilden, Germany). Subsequently, first-strand complementary DNA (cDNA) was synthesized from 1 μg of total RNA using the iScript cDNA synthesis kit (BioRad, Hercules, CA, USA) in a volume of 20 μl. For TpH-1 a 114 bp fragment was amplified by polymerase chain reaction (PCR) using the forward primer, 5'-tgcaaaggagaagatgagagaatttac-3', and the reverse primer, 5'-ctggttatgctcttggtgtctttc-3'. The primer set designed for SERT was forward, 5'-tggttctatggcatcactcagttc-3', and reverse 5'-gttgtggcgggctcatcag-3', thereby amplifying a fragment of 148 bp. The forward primers for TpH-1 and SERT span an intron-exon boundary to avoid detection of contaminating genomic DNA. Before real-time PCR analysis, all cDNA samples were diluted 1:10 with RNAse free water. The PCR reactions were set up in a volume of 25 μl, containing 5 μl of the diluted cDNA, 12.5 μl 2$\times$ iQ SYBR Green Supermix (BioRad, Hercules, CA, USA), and gene-specific forward and reverse primers both at a final concentration of 300 nmol/L. The PCR thermal cycling protocol applied consisted of a 3- minute 95°C initial denaturation and enzyme activation step,

Table 2
Disease activity characteristics of CD patients.

	CD with IBS-like symptoms (n=10)	CD without IBS-like symptoms (n=10)
CDEIS[1] (median; range)	0.31 (0.0-5.1)	0.99 (0.0-6.3)
CDAI[2] (mean ± SEM)	156.3 (± 25.7)	119.8 (± 22.9)
White cell count (median; range)	5.5 (3.7-11.5)	5.0 (3.1-10.9)
ESR[3] (median; range)	8 (2 –26)	14.5 (2-31)
C-reactive protein (median; range)	7 (0-7)	7 (0-48)

[1]CDEIS = Crohn's disease Endoscopic Index of Severity, [2]CDAI = Clinical Disease Activity Index, [3]ESR= erythrocyte sedimentation rate

followed by 40 cycles of a 15-second 95°C denaturation step, a 30-second 60°C annealing step, and a 30-second extension step at 72°C. Next, a melting curve analysis was performed by increasing the temperature in 0.5°C increments from 55°C to 95°C and measuring fluorescence at each temperature for a period of 10 seconds. For both genes amplification yielded a single product of a size equivalent to that predicted from the relevant sequence. The housekeeping genes, β-actin (ACTB) and porphobilinogen deaminase (PBGD), were used as a reference for quantification. PCR reactions for these genes were carried out using 5 μl of the diluted cDNA, 12.5 μl 2× iQ Supermix (BioRad, Hercules, CA, USA), and 1.25 μl 20× Assays-on-Demand gene expression assay mix (433762F (ACTB) or Hs00609297 (PBGD); Applied Biosystems, Foster City, CA, USA) in a final volume of 25 μl with a $MgCl_2$ concentration of 4 mmol/L. The PCR thermal cycling conditions were as follows: 95°C for 3 minutes, and 40 cycles of amplification comprising 95°C for 15 seconds and 60°C for 1 minute. All PCR reactions were run in triplicate using the iCycler iQ system (BioRad, Hercules, CA, USA). cDNA synthesized from total RNA extracted from full thickness jejunum resection material was used to generate a relative standard curve. In each run serial dilutions of this cDNA were amplified. The use of a relative standard curve allows comparison of expression levels across runs and takes differences in PCR efficiency into account for the mRNAs analyzed. Expression levels in the various biopsy specimens were quantified by calculating initial target concentrations using the obtained threshold cycle values and the relative standard curve. Subsequently, for each sample the levels of TpH-1 and SERT were

divided by the level of one of the reference genes to obtain the normalized expression level.

Enteroendocrine and enterochromaffin cell counts

To evaluate the number of chromogranin (enteroendocrine) cells and 5-HT (EC) cells per crypt/villous complex (gland), immunostaining procedures were performed on 4-mu paraffin sections using a polyclonal rabbit antibody to human chromogranin (DAKO:A0430, 1:500 dilution, DakoCytomation, Glostrup, Denmark) and a rabbit polyclonal antibody (PSE,1:200 dilution, Eurodiagnostica, Malmo, Sweden) to 5-HT.

Only complete crypt/villous complexes were analyzed. All immunoreactive cells, regardless of the amount of immunoreactivity (slightly, normal or strong staining), were counted and quantified as the number of cells per crypt/villous complex by a pathologist, blinded for the different groups. Ileal and colonic mucosal biopsy specimens, taken from all 20 CD patients and from 4 healthy controls, were available for enteroendocrine and EC cells count. Therefore, data for the healthy controls were left out the statistical analysis of the enteroendocrine and EC cells count. We depict the descriptive data of the healthy controls in Figure 2 to give an idea of the numbers found in this group.

Statistical Analysis

The distributions of the data were evaluated by descriptive statistics (mean or median; SEM or range). Comparisons between ileum and colon mRNA expression within each group were performed with a Wilcoxon Signed Ranks test. Group differences for nonparametric data were assessed using the Kruskal-Wallis test, followed by a post-hoc test, corrected for multiple testing. Bonferroni correction was applied for analysis of the level of expression of mRNA; consequently a *P* value of less than 0.0125 was considered to be significant. All calculations were performed with the SPSS version 11.5 (SPSS Science, Inc, Chicago, Illinois, USA) for Windows (Microsoft Corporation, Redmond, Washington, USA).

Results

No difference was observed in disease duration, localization of disease, and medication use between the 2 CD patient groups (Table 1). In addition, none of the CD patients had active histopathological disease activity, and the objective disease activity characteristics of the 2 patient groups (CDEIS score, C-reactive protein, white cell count, and erythrocyte sedimentation rate; Table 2) did not differ as well, all indicating remission in both patient groups. Age was comparable in the 3 groups studied, as was the ratio of women to men.

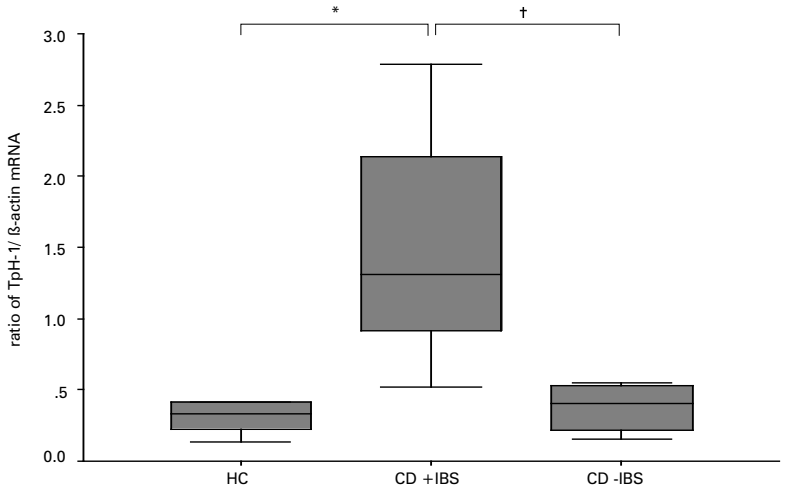

Figure 1
TpH-1 mRNA expression in the colon. CD patients in remission with IBS-like (CD + IBS) symptoms show a higher TpH-1 level compared to controls (HC) (* $P \leq 0.005$) and compared to CD patients in remission without IBS-like symptoms (CD – IBS; † $P \leq 0.01$).

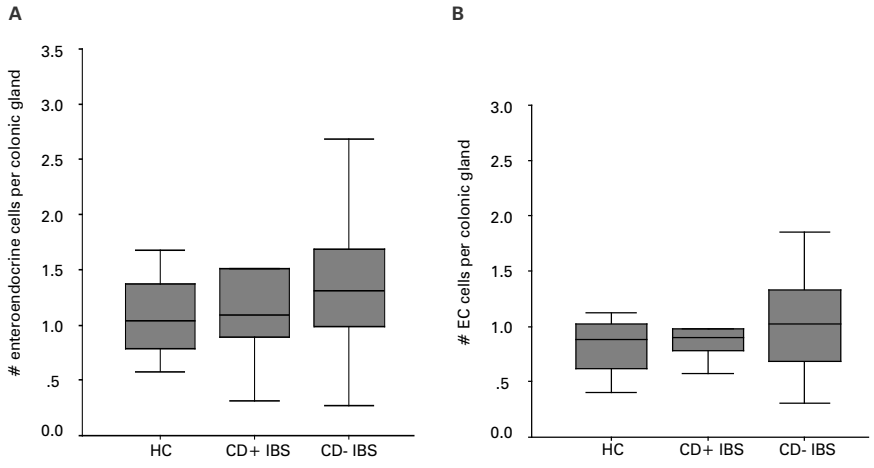

Figure 2
The number (#) of enteroendocrine cells (A) and EC cells (B) per colonic gland in healthy controls (HC), CD patients in remission with IBS-like symptoms (CD + IBS), and CD patients in remission without IBS-like symptoms(CD – IBS). Values represent median and range.

Table 3
TpH-1 and SERT mRNA expression normalized to β-actin.

	TpH-1		SERT	
	ileum	colon	ileum	colon
Controls	0.8 (0.5-1.0)	0.3 (0.2-0.5)	6.7 (4.7-7.8)	0.2 (0.1-0.3)[a]
CD +	1.4 (0.9-2.0)	1.3 (0.8-2.2)[b c]	8.8 (7.2-13.3)	0.4 (0.1-0.8)[a]
CD –	0.8 (0.5-1.1)	0.4 (0.2-0.5)	8.1 (6.1-12.1)	0.1 (0.06-0.4)[d]

All values represent median (25^{th} –75^{th} percentiles).
CD +, CD patients in remission with IBS-like symptoms; CD -, CD patients in remission without IBS-like symptoms;
Ileum vs. colon, [a] $P \leq 0.005$, [d] $P \leq 0.0125$;
CD patients in remission with IBS-like symptoms vs. controls: [b] $P \leq 0.005$;
CD patients in remission with IBS-like symptoms vs. CD patients in remission without IBS-like symptoms; [c] $P \leq 0.01$.

Regional differences in messenger RNA expression

Tryptophan hydroxylase-1 messenger RNA expression – In the 3 groups studied no different levels of TpH-1 expression were found between the ileal and the colonic mucosa (Table 3).

Serotonin reuptake transporter messenger RNA expression - Ileal SERT mRNA levels in controls and both CD patient groups were much higher than those found in colonic mucosa (Table 3), irrespective of the housekeeping gene used.

Group differences in messenger RNA expression

Tryptophan hydroxylase-1 messenger RNA expression - TpH-1 mRNA expression levels in the ileal mucosa were comparable in the 3 groups studied (Table 3). In contrast, TpH-1 expression relative to β-actin in the colon was significantly higher in CD patients with IBS-like symptoms when compared with controls ($P< 0.005$) and CD patients without IBS-like symptoms ($P< 0.01$) (Figure 1; Table 3). These results were confirmed using PBGD as housekeeping gene.

Serotonin reuptake transporter messenger RNA expression - No differences were found between the 3 groups in SERT mRNA expression when ileum and colon were analyzed separately (Table 3).

Enteroendocrine and Enterochromaffin cell count

The number of enteroendocrine cells and EC cells per gland at the level of the ileum and colon were comparable in both patient groups, although patients without IBS-like symptoms showed a large variation in number of enteroendocrine and EC cells.

Figure 2 shows the median of enteroendocrine and EC cells per colonic gland in CD patients in remission, and healthy controls.

Discussion

This study showed molecular alterations in the colon of CD patients in remission who met the Rome II criteria for IBS. We found significantly increased levels of TpH-1 mRNA in the colonic mucosa of CD patients with IBS-like symptoms, compared with the subjects without symptoms. Furthermore, this study showed large regional differences of SERT mRNA expression in the gut. The mRNA levels of SERT found in the colon were lower than the levels measured in the ileum (in controls and CD patients), whereas expression of TpH-1 in ileum and colon was comparable.

We studied 2 well-defined CD patient groups with negligible disease activity, comparable disease behavior, and comparable medication use. Therefore it is highly unlikely that one of these variables caused the observed difference. Almost all patients with IBS-like symptoms had the predominant-diarrhea variant of IBS, whereas the 2 patients with alternating IBS predominantly had symptoms indicative for the diarrhea subtype of IBS. The mean CDAI score of the CD patients with IBS-like symptoms could indicate that this group suffered (very) mild disease activity, but the higher scores can be attributed to the subjective items "abdominal pain" and "general well-being", both scored in the CDAI.

Several studies have been performed to explore the role of inflammation on 5-HT signaling. Linden et al. reported increased 5-HT content, an increased number of EC cells, and an increased amount of 5-HT released under basal and stimulated conditions, with reduced levels of SERT expression in mucosa of guinea pigs with trinitrobenzene sulphonic acid (TNBS)- colitis.[19] In a murine colitis model decreased 5-HT uptake by mucosal epithelial cells was shown.[20] The net result in both models is increased 5-HT availability. In line with these studies O'Hara et al.[30] detected a reduction of epithelial SERT immunoreactivity in the mucosa of guinea pigs with TNBS ileitis. Whether these changes persist in quiescent disease is not known, because this has not been explored in animal models. In human beings, the effect of inflammation was reported in studies evaluating 5-HT signaling in IBS and IBD patients. EL-Salhy et al.[21] found increased numbers of serotonin-immunoreactive

cells in the ileal mucosa of CD patients with slight to severe inflammation in the ileum. Spiller et al. showed a significant increase in the number of EC cells in rectal biopsy specimens following *Campylobacter jejuni* enterocolitis, which could persist for more than a year.[24] These data were confirmed by Dunlop et al. who showed that increased EC cell counts are an important predictor for developing postinfectious IBS.[23] Other studies in postinfectious IBS patients implicated an enhanced peripheral inflammatory response with low-grade inflammation and immune activation in the intestine of these patients because increased levels of interleukin-1β were measured in the intestinal mucosa of these patients.[31; 32] Although interleukin-1β is known to be involved in the inflammatory response in CD, we believe that increase levels of interleukin-1β do not explain the differences found in the present study. The parameters of disease activity did not differ in the patient groups studied (CD patients with IBS-like symptoms and CD patients without IBS like symptoms), and histopathological evaluation of the most affected parts of colonic and ileal mucosa showed that none of the CD patients had inflammatory activity.

The previously mentioned studies exploring the role of inflammation on 5-HT signaling show that inflammation affects the serotonergic pathway, and that some of the alterations persist in IBS patients when mucosal inflammation is not longer detectable. An important role of the serotonergic pathway in IBS also was highlighted in pharmacological studies in which therapeutic benefit of treatment of IBS patients with 5-HT- receptor antagonists was shown.[33] Recently, Coates et al.[22] studied rectal 5-HT signaling in UC patients, IBS patients with constipation, and IBS patients with diarrhea. A distinction was made between severely inflamed UC and nonsevere UC (based on a histopathological scoring system), but UC patients were not classified as having IBS-like symptoms or not. The data of Coates et al.[22] showed that SERT mRNA expression was reduced in all patient groups relative to healthy controls, as was 5-HT content and TpH-1 expression. The reduced TpH-1 expression may be explained by a feedback mechanism in response to higher levels of 5-HT in the interstitial space in UC and IBS patients as a consequence of the reduced SERT expression. The net result is increased 5-HT availability. In comparison with our study, Coates et al.[22] studied mucosal tissue from the distal GI tract with active inflammation in UC patients. UC has a different immunological background, and is not thought to be a Th-1 mediated disease like CD. T cells might induce enteroendocrine cell hyperplasia after inflammation, which in turn can result in increased 5-HT availability.[34; 35]In addition, O'Hara et al.[36] showed in an animal model that in different immune systems, changes in enteroendocrine cells, SERT expression and 5-HT release differ in response to an acquired infection. Translated to human beings, this could mean that in CD patients, UC patients, and (postinfectious) IBS patients, changes in the mucosal serotonergic signaling secondary to inflammation may result in different phenotypes.

In the present study, we aimed to study the serotonin metabolism in CD patients with IBS-like symptoms. Thus, we chose to study tissue from the transverse colon instead of tissue from the rectum because rectal sparing is encountered frequently in CD. We found higher TpH-1 expression in colonic mucosa of CD patients in remission with IBS-like symptoms when compared with subjects without these symptoms (controls and CD patients in remission), although the number of 5-HT producing cells was similar. It is conceivable that this will result in an increased mucosal availability of 5-HT in CD patients with IBS-like symptoms. Because 5-HT influences motility and visceral sensation,[14; 16] this increase in mucosal availability of 5-HT is likely to play a role in the symptom generation of IBS in CD patients. Assuming that during inflammation the serotonergic pathway in all CD patients is affected,[13; 21] we hypothesize that in a subset of patients these alterations are irreversible and result in IBS-like symptoms, even when the disease is in remission. Whether this is related to the inflammatory process itself or to other factors such as the bacterial flora composition, genetic variation, or other co morbidity (such as pre-existing psychiatric disorders) warrants further studies. It is intriguing that we only found a difference in TpH-1 expression in the colon, and not in the ileum. This might be owing to differences in disease activity in the past. Although the 2 studied patient groups were comparable in terms of localization of disease, it cannot be excluded that in both groups the colon was previously more severe inflamed compared with the ileum, possibly leading to the observed results.

In addition, we showed a differential distribution of SERT mRNA expression in the ileum and colon in all groups, while expression of TpH-1 was comparable. This is in line with the results of Van Lelyveld et al.[37] who studied the serotonergic pathway in fundus, antrum, and duodenum in 11 healthy volunteers. They found that the 5-HT synthesis varies only marginally between the three regions while the uptake capacity of 5-HT differs tremendously. The Gershon group studied the serotonin reuptake transporter in the distal colon and small intestine of mice and guinea pigs.[18; 38; 39] In both segments expression of SERT was shown, but no quantitative comparisons between the 2 segments could be made. With regard to mucosal 5-HT concentration, Miwa et al[40] found that the lower GI tract shows an ascending cephalocaudal gradient in mucosal 5-HT concentration. The high rectal 5-HT concentration can be attributed to the peak of EC cell density in the rectum.[41] The ascending cephalocaudal gradient in 5-HT concentration also can be explained by regional differences in 5-HT synthesis, release or uptake. Our data suggest that regional differences in uptake, caused by a higher SERT expression in the ileum, might play a role. The different uptake capacity probably is related to the distinctive functions of the terminal ileum and the colon. A potential unequal distribution of inhibitory and excitatory 5-HT receptors in ileum and colon further differentiates between the roles of serotonin signaling in both regions. The observed difference

in uptake capacity in the present study was not affected by the presence of IBS-like symptoms or former inflammation.

In conclusion, we have shown higher levels of TpH-1 expression in the colonic mucosa of CD patients in remission with IBS-like symptoms when compared with subjects (patients and healthy controls) without these symptoms, with similar numbers of 5-HT producing cells in all groups. Up-regulated 5-HT synthesis might lead to increased 5-HT availability. Increased 5-HT availability has been associated with symptom generation in IBS. Therefore, elevated TpH-1 levels may contribute to IBS-like symptom generation in CD patients in remission. Because it is known that inflammation affects the serotonergic pathway, our data implicate that some of these changes persist in the subset of CD patients in remission with IBS-like symptoms.

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Review: What predicts mucosal inflammation in Crohn's disease patients?

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Chapter

6

Abstract

A number of disease-specific instruments have been created over the last 30 years to assess disease activity in Crohn's disease (CD). These disease activity indices are constituted of clinical and laboratory parameters and their role in predicting disease activity and the course of disease have been reviewed various times. Currently, the severity of mucosal inflammation, assessed by endoscopy, is considered the gold standard for disease activity in CD. In the present review, the most frequently used endoscopic disease activity indices and the correlation between mucosal inflammation and clinical disease activity indices, quality of life questionnaires, and biochemical markers is critically appraised. We conclude that no clinical disease activity index or single laboratory parameter of inflammation reliably predicts the mucosal inflammatory disease activity. A new, easy-to-use and robust activity index predicting mucosal inflammation is highly needed to assess the response to investigational drugs in trials and the effect of therapeutical interventions in clinical practice.

Key words
Crohn's disease, clinical disease activity, mucosal inflammation, endoscopy, laboratory markers

Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal (GI) tract, characterized by a relapsing course. It may affect any part of the GI tract with a broad spectrum of clinical presentations. The disease seems to result from complex interactions among susceptibility genes, the environment, and the immune system, finally leading to an imbalance of the immune system. This imbalance, or disease activity, results in deregulated mucosal immune responses to antigens of enteric bacteria, and is revealed by mucosal inflammation of the GI tract and/or extraintestinal manifestations.

At present, the severity of mucosal inflammation assessed by endoscopy is considered the gold standard for disease activity in CD. In clinical practice, however, physical examination, inspection of stools, and laboratory parameters are usually employed to assess inflammatory activity. These parameters do not reliably reflect the extent and intensity of the mucosal inflammation, since mucosal inflammation is not always accompanied by a raised C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), or other laboratory parameters of inflammation. On the other hand, patients may have characteristic symptoms without clear endoscopic disease activity, which can be explained by a high frequency of irritable bowel syndrome-like symptoms in patients in remission.[1;2] Thus, endoscopy is indispensable to determine whether or not symptoms are generated by mucosal inflammation.

Before the era of biologicals and immunosuppressive agents, the main treatment goal in CD was to achieve clinical remission. In the 1990s, azathioprine[3;4] and tumor necrosis factor-α antibodies (TNFα; infliximab)[5;6] were found to induce mucosal healing in CD patients, as a secondary outcome of clinical trials. In the Accent I trial, CD patients who were found to have complete mucosal healing following treatment with infliximab, needed fewer hospitalizations, surgeries, and intensive care unit stays.[7;8] Current guidelines for treatment of CD restrict the use of immunosuppressive agents to patients who fail corticosteroids. However, this strategy is not effective in the long-term prognosis. An alternative is to introduce more potent immunomodulators in an earlier course of disease. Recently presented preliminary data from a controlled study comparing early administration of infliximab and azathioprine ("top-down" therapy) versus conventional "step-up" therapy showed superior mucosal healing, a more rapid remission and higher remission rates in patients in the top-down treatment arm.[9] Whether healing of the mucosa will lead to a more benign course of the disease in the long run remains to be proven in longitudinal studies.

From this, it is evident that a reliable and easy-to-use parameter of disease activity is highly needed in trials and in clinical practice. A number of disease-specific instruments have been created over the last 30 years to measure disease activity in CD.

These various, in particular, clinical and laboratory disease activity indices have been reviewed in the past, apart from their relation with mucosal inflammation.[10-17] In the present article the most frequently used endoscopic disease activity indices, and the correlation between mucosal inflammation and clinical disease activity indices, quality of life questionnaires, and biochemical markers are reviewed.

Endoscopic Disease Activity Indices

Mucosal inflammation of the GI tract in CD is characterized by a number of reproducible mucosal (skip) lesions; erythema, edema, pseudopolyps, aphthoid ulcers, (longitudinal) ulcers, and stenosis.[18] It has been shown that it is feasible for a cooperative multicenter group to collect reproducible endoscopic data in CD in a standardized way.[19]

Crohn's disease Endoscopic Index of Severity (CDEIS)

In 1989, the French "Groupe d' Etudes Thérapeutiques des Affections Inflammatoires Digestives"(GETAID) developed an endoscopic index for assessing the severity of mucosal inflammation in CD, the CDEIS.[19] This index is based on the previous mentioned endoscopic findings in 5 segments of the gut (ileum, right colon, transverse colon, left colon, and rectum), prospectively collected in 75 patients with colonic or ileocolonic CD. It correlates with the endoscopist's global evaluation of lesion severity. The elaboration of the score requires analog scale transformation.

The agreement between paired evaluations of the CDEIS as assessed by 2 endoscopist of data recorded during colonoscopy was excellent (intraclass correlation of 0.96, $P < 0.001$). The CDEIS was validated in 103 colonoscopies. Hereafter, it was used as a marker of mucosal healing in a number of therapeutic trials.[3-7] At present, the CDEIS represents the gold standard for evaluation of mucosal inflammation in CD. The CDEIS score generally ranges from 0-30. A higher score indicates more severe mucosal inflammation. A clinically meaningful reduction in score or a specific cut off value which defines remission has not been determined.

Simple Endoscopic score for Crohn's disease (SES-CD)

Recently the SES-CD has been developed and validated to simplify endoscopic activity assessment.[20] The score does not require analog scale transformation as in the CDEIS. It is based on 4 endoscopic variables (ulcer size, ulcerated and affected surfaces, stenosis) in the same 5 ileocolonic segments considered in the CDEIS. The endoscopic parameters are scored from 0-3. The SES-CD was derived from the results of 70 patients, and validated in 121 CD patients. The interobserver agreement for all selected variables was excellent (kappa coefficient 0.791-1.000). It does not come as a surprise that the SES-D is highly correlated with the CDEIS ($r = 0.920$).

Rutgeerts' score

Rutgeerts developed an endoscopic score system to measure the presence and severity of endoscopic recurrence in the neoterminal ileum after ileolcecal resection. This scorings system was derived from observations in a cross-sectional study of 114 patients after ileal resection.[21; 22] Scores range from 0 (no lesions in the distal ileum) to 4 (diffuse inflammation with already larger ulcers, nodules, and/or narrowing).

Clinical Disease activity scores

The Crohn's Disease Activity Index (CDAI)

To date, the most used and broadly accepted clinical activity score worldwide in trials is the Crohn's Disease Aactivity Index (CDAI).[23] It is recommended as the gold standard for disease activity in CD, by the European Medicines Agency (EMEA) for the development of new medicinal products for the treatment of CD.[24] The score, developed in 1976, is the product of multivariate regression analysis with data prospectively collected from 187 visits from 112 patients. The physician's overall evaluation of "how the patient was doing" ("very well", "fair to good", "poor", and "very poor") was correlated with 8 independent variables (selected out of 18 potential predictor variables), which were the number of liquid stools, the severity of abdominal pain, general well being, the occurrence of extraintestinal manifestations, the need for antidiarrheal drugs, the presence of an abdominal mass, the hematocrit value and body weight. The calculation of the CDAI is based on a 7-day diary. In a following study, the values of the 8 coefficients of the CDAI were rederived using data from 1058 visits of patients.[25] The rederived coefficients were similar to the original one. New and original index values calculated on the same data from patient visits correlated highly; therefore, continued use of the original version was suggested. A CDAI score of 150 or less indicates remission, 150 to 220 mild disease activity, a score of $\geq$220 or $\leq$ 450 moderate disease activity, and >450 severe disease activity. Several reports show that the endoscopic disease activity correlates poorly with the CDAI (r =0.32; P<0.001; r =0.13 n.s.).[26; 27]

The Harvey Bradshaw index

The Harvey Bradshaw index or "simple index"[28] is a simplified version of the CDAI. In all, 112 consecutive patients were assessed with the CDAI and the Harvey Bradshaw index. This last index consists of 5 of the 8 items of the CDAI, based on data of the day before the visit. The Harvey Bradshaw index is independent of (but complementary to) laboratory criteria of inflammatory activity, such as measurement of ESR, plasma viscosity, or CRP.[28] As can be expected, the correlation with the CDAI is excellent (r =0.88; P <0.01).[29] In 1986, Gomes et al.[29] reported a significant

correlation between their endoscopic score and the Harvey Bradshaw index in 22 CD patients with colonic disease ($r = 0.68$, $P < 0.05$).

The van Hees or Dutch index

This index is a combined clinical and laboratory index based on data of 63 CD patients who had been submitted to a total of 85 clinical examinations. The index has been prospectively validated.[30] On the basis of 18 predictor variables 3 physicians gave an overall evaluation of the severity of inflammatory activity in each patient. The index correlates poorly with the CDAI,[30] probably due to its use of laboratory-based items (albumin, ESR), when compared to the CDAI. Simonis et al[31] found a low predictive value of the van Hees index for endocopic disease activity.

Several other clinical disease activity indexes have been developed in the past, including the Organisation Mondiale de Gastroenterologie (OMGE) index,[32] the Cape Town index,[33] the Bristol score,[34] and the St. Mark's index.[34] These validated indices all correlate well with the CDAI, because a large part of the score in all these indices is contributed by the symptoms of well-being, frequency of liquid stools and abdominal pain. The relation between these indices and endoscopic disease activity has not been studied, but a poor correlation can be expected because of the large overlap of these indices with the CDAI.

Quality of life

Quality of life of CD patients is usually assessed by employing the Inflammatory Bowel Disease Questionn-aire (IBDQ). The IBDQ is a disease-specific, health-related, quality-of-life questionnaire, containing 32 items, with a graded response range of 1 (worst) to 7 (best) and a total score of 32 to 224.[35] The 32 items can be divided in 4 dimensional scores, including bowel symptoms (e.g. stool frequency and abdominal pain or cramps; 10 items), systemic symptoms (e.g. fatigue and energy loss; 5 items), emotional well-being (e.g. depressed feelings; 12 items), and social function (e.g. limited sexual activity; 5 items). A substantial portion of the total CDAI score is derived from the "the general well-being" and "the intensity of abdominal pain" items. In this respect there is an overlap between the CDAI and both the bowel symptoms domain and the systemic domain of the IBDQ. In 2004 Casellas et al.[36] found a significant correlation between clinical activity (Harvey Bradshaw index) and quality of life (using an extended version, the IBDQ-36 and a reduced version, the IBDQ-9), in 68 CD patients. Since the Harvey Bradshaw index is a simplified version of the CDAI, it is not surprisingly that quality of life did not correlate with mucosal inflammation.

Laboratory activity markers

The subjective nature of clinical CD indices led investigators to search for laboratory markers that would independently measure mucosal disease activity. Overall, these markers are not specific for CD, but reflect a general degree of systemic inflammation.

In 2000 Nielsen et al [37]published a comprehensive review on classical disease markers (including ESR, acute phase proteins, white cell and platelet counts, albumin, neopterin, and β_2 microglobulin) together with the emerging disease markers such as antibodies of the ANCA/ASCA type, cytokines and various adhesion molecules in CD and their relation with (clinical) disease activity. It was concluded that none of the laboratory markers of disease activity in CD is specific or sensitive enough to replace basic clinical observations such as the number of daily bowel movements, general well-being and other parameters in parallel. In this review, we only focus on parameters of which the correlation with endoscopic inflammation has been studied.

Serum activity markers

One of the first publications in which the relation between mucosal inflammation and serum activity markers was studied dates from 1986.[29] Mucosal inflammation confirmed by colonoscopy in 22 CD patients was defined as no, mild, severe or more severe inflammation. The severity of mucosal inflammation and CRP, ESR, white blood count, platelet count or albumin were not found to be correlated.

Modigliani et al.[27] assessed whether or not the CDEIS, hemoglobin, ESR, and serum albumin were correlated in 42 CD patients. A significant correlation existed between serum albumin levels and the CDEIS (r = -0.31, P <0.001). Hemoglobin and ESR were not related to mucosal inflammation.

Tromm et al.[38] evaluated various laboratory tests in relation to the endoscopic disease activity. Seventy-five CD patients were divided into two groups based on endoscopy; a group with severe inflammation and a group with low-grade or no inflammation. Except for the hematocrit, significant differences were found between both patient groups for the mean values of the ESR, albumin, α_1 protease inhibitor, cholinesterase, and CRP.

In 1994 the GETAID group[26] described in 121 consecutive CD patients rather week, but significant correlations between the CDEIS and serum albumin (r =-0.30), α_2- globulin(r = 0.48), α_1- antitrypsin (r = 0.39), ESR (r = 0.40), platelets (r = 0.28), hemotocrit (r = 0.16), and CRP (r = 0.20), while no relations with orosomucoid and white cell counts were found.

Moran et al.[39] found an impressive high correlation for serum albumin (r =0.8; P <0.001) in 28 patients with mucosal disease activity in either CD colitis or ulcerative colitis employing multiple regression analysis using all endoscopic grades of disease activity.

Simonis et al.[31] investigated the suitability of objective parameters (serum α_1- antitrypsin, acid α_1- glycoprotein (orosomucoid), CRP, sialic acid, prealbumin, albumin) accessible for routine management to act as surrogate indicators for endoscopic alterations. Endoscopic findings were classified on the basis of the pattern of alterations found and were globally labeled as to whether they were remission-related or exacerbation-related. A validated index for endoscopic disease activity, e.g. the CDEIS, was not used. Thirty-six patients were included, 18 with clinically exacerbated disease and 18 after acute phase conservative therapy. Orosomucoid and prealbumin were found to be good predictors for endoscopically active disease. The model was validated in 44 patients; 29 with active disease and 15 controls.
Finally, Solem et al.[40] reported a significant association between disease activity at colonoscopy and the CRP in 104 CD patients in 2005.

Fecal activity markers

Fecal markers comprise a heterogeneous group of substances that either leak from, or are generated by the inflamed intestinal mucosa.

α1-antitrypsin

Fecal α1-antitrypsin clearance reflects protein loss through the bowel wall. It is a protease inhibitor produced by the liver, macrophages, and the intestinal epithelium. A small but significant correlation between this parameter and the CDEIS was reported by the GETAID group(r =.37; P <0.001).[26] Moran et al.[39] found a high correlation coefficient (r =.82; P <0.001) but his study population comprised CD patients as well as colitis ulcerosa patients. Fecal α1-antitrypsin has been generally accepted as a useful marker of IBD; however, the method is not routinely available. Moreover, other fecal markers have been found to be more accurate or cost-effective than α1-antitrypsin in CD patients.[41]

Calprotectin

Calprotectin is a calcium and zinc binding protein, and accounts for 60% of the total soluble proteins in the cytosol fraction of neutrophil granulocytes. It is a marker of neutrophil turnover, since it is released from neutrophils shed from the colonic mucosa by activation of leucocytes. Calprotectin is stable during intestinal transit, resistant to colonic bacterial degeneration, and can easily be assessed in stools by means of ELISA tests. A small raise of calprotectin has been found in patients with colorectal cancer, but patients with mucosal inflammation have highly elevated fecal calprotectin levels, which appeared to be related to the degree of mucosal inflammation in IBD. D'Inca et al[42] reported a significant correlation between endoscopic disease activity and calprotectin levels (r =0.480; P=0.008) in 23 CD patients. A validated index for endoscopic disease activity, e.g. the CDEIS, was not used. Roseth described normalization of fecal calprotectin levels along with mucosal healing in CD.[43]

Overall, studies focused on the correlation between mucosal inflammation and laboratory activity markers are not conclusive. This can be due to the different endoscopic scorings systems used, the different study designs, but also to the small sample size in most of the studies. The levels of CRP and albumin seems to be the most useful in predicting mucosal inflammation,[26; 38; 40] whereas white blood cell count and ESR are less suitable for this purpose. The fecal marker calprotectin seems to be highly useful as well.

Discussion

Mucosal healing is presently considered the ultimate goal of treatment in CD, but this has only been within grasp since the introduction of azathioprine,[3; 4] and most notably, infliximab.[5; 6; 44] The clinical relevance of this treatment goal is underscored by the finding that induction of mucosal healing is associated with a reduction of hospitalizations and surgical procedures.[7; 8] Therefore, in our opinion, the CDAI cannot longer remain the primary endpoint in clinical trials, since it relates poorly with the inflammatory status of the intestinal mucosa.[26; 27] The poor association is probably due to several factors. Most important, in the development of the CDAI, mucosal inflammation has not been taken into consideration; instead the physician's over-all evaluation of "how the patient was doing" was used as the gold standard. Furthermore, as pointed out previously, most laboratory markers presently used in the clinical disease activity indices (ESR, albumin, and Ht) have not been found to be reliable predictors for mucosal inflammation. Another factor, potentially explaining the poor performance of the CDAI in predicting mucosal inflammation, is the fact that the location of the disease is not incorporated in the CDAI. While patients with distal inflammatory activity will usually present with diarrhea and macroscopic blood loss, a proximal location of disease will mostly be accompanied by abdominal discomfort, pain or systemic complains such as anorexia, weight loss, fatigue or fever. The heterogeneous behavior of the disease leading to different phenotypes (stenosing, perforating and luminal disease), will further complicate the interpretation of complaints, from which most clinical activity scores are derived. At last, a substantial part of the total CDAI score is derived from subjective items (the extent of abdominal pain, general well being) and reflects the patients' perception and interpretation of the disease and its symptoms. This is unarguable important for the quality of life of patients, and needs to be measured in clinical trials. However, the items of the CDAI show a considerable overlap with both the bowel symptoms domain and the systemic symptoms domain of the Inflammatory Bowel Disease Questionnaire (IBDQ), and it can be argued that the CDAI is redundant and the IBDQ can sufficiently meet these aspects of the patients' perception of

disease. An additional practical impediment when employing the CDAI in daily practice and in clinical trials is the fact that the calculation of the CDAI is based on a 7-day diary, which can be cumbersome for the patient and the investigator. A substantial variability exists when different observers review the same case histories and calculate the CDAI score.[45]

The discrepancy between CDAI and endoscopic disease activity might explain the high placebo rates of clinical response and remission in clinical trials. Su et al.[46] reported in their meta-analysis even remission rates of up to 50% in CD patients treated with placebo. Solem et al.[40] stated that in post-hoc analyses of some clinical trials, the subgroup of CD patients with elevated CRP concentrations had lower rates of placebo response compared with patients with normal CRP concentrations, reflecting probably a lower endoscopic disease activity in the latter group.

Using endoscopic disease indices to assess the mucosal condition poses other problems. Endoscopy is invasive, time consuming, and expensive and, hence, unsuitable for frequent use in daily practice as well as in clinical trials. Furthermore, no clinical useful and validated cut off levels for response or remission following medical therapy have been defined.

The ideal activity index should consist of parameters reflecting the extent of mucosal involvement, a parameter of systemic inflammation to assess transmural disease and/or infiltration outside the gut, a clinical parameter (such as diarrhea) and a correction for phenotypical disease behavior. Furthermore, a disease activity index should be simple and preferably consist of cheap, widely available, and easy-to-collect parameters. Presently, such an index is not available, but we feel that to reach new and ambitious goals in the treatment of CD, clinically as well as in studies, a new disease index predicting mucosal inflammation and reducing placebo response is highly needed.

Conclusions

Currently, endoscopy still is indispensable to determine whether or not symptoms are due to active mucosal inflammation and to monitor the effect of different therapeutic interventions in patients with CD. A new clinical activity index predicting mucosal disease activity reliably without the need for endoscopical procedures would simplify and probably improve the management of CD patients in trials as well as in clinical practice.

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Predicting mucosal inflammatory activity in Crohn's disease: a new reliable non-endoscopic index

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Submitted

Chapter 7

Abstract

Background and aims

Mucosal healing is presently considered the ultimate goal in treatment of Cronn's Disease (CD), but this can only be assessed by endoscopy. The most used clinical disease activity index, the Crohn's Disease Activity Index, correlates poorly with the inflammatory status of the intestinal mucosa. Therefore, we aimed to design a new CD activity index, based on a combination of clinical characteristics, and readily available laboratory parameters, predicting endoscopic disease activity in patients with both quiescent and active disease.

Methods

Thirteen clinical and specific laboratory variables were considered for analysis after a thorough review of literature. Mucosal inflammation was assessed by the Crohn's Disease Endoscopic Index of Severity. A linear regression model was based on 93 ileocolonoscopies performed in 82 CD patients, and internally validated by bootstrap resampling.

Results

The predicted severity of mucosal inflammation was given by the formula: number of liquid stools during one day*0.25 + C-reactive protein (mg/L) *0.1 + platelet counts (10^9/L) *0.01 + fecal calprotectin (mg/L) *0.001 - mean platelet volume (fL) *0.2. This model had good predictive ability (bootstrap adjusted R^2=0.50).

Conclusions

The proposed prediction formula is easy to use with readily available patient characteristics. It may not only be useful for randomized trials but also in daily clinical patient management.

Keywords

Crohn's Disease, mucosal inflammation, endoscopic disease, disease activity index, linear regression model

Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract characterized by a relapsing course. It may affect any part of the intestinal tract with a broad spectrum of clinical presentations. Before the era of biologicals and immunosuppressive agents, the main treatment goal in CD was to achieve clinical remission, mostly defined using the Crohn's Disease Activity Index (CDAI).[1] Following the introduction of azathioprine and tumor necrosis factor-α (TNF-α) antibodies, mucosal healing was found to be within grasp.[2-6] Moreover, mucosal healing was associated with a more favorable course of disease,[7; 8] and is now considered the gold standard for disease activity in the treatment of CD. Assessment of this parameter, however, is cumbersome and requires endoscopy. To quantify endoscopic inflammatory activity, various indices have been developed from which the Crohn's Disease Endoscopic Index of Severity (CDEIS) [9] is most frequently employed. Several reports show that the CDAI correlates poorly with the CDEIS,[10; 11] and therefore this clinical disease activity index cannot replace endoscopy. Endocopy is associated with an unpleasant bowel preparation, discomfort and is time consuming and expensive. Therefore, a non-invasive index, reliably predicting endoscopic disease activity would simplify and probably improve management of CD patients in trials as well as in clinical practice.

The primary aim of the present study was to design a new CD activity index, based on a combination of clinical characteristics and readily available laboratory parameters, predicting endoscopic disease activity in patients with both quiescent and active disease.

Subjects and Methods

Patients

Patients with a diagnosis of CD for at least 6 months and a clinical indication for ileocolonoscopy were invited to participate in the study from December 2003 until December 2005. The diagnosis of CD was based on the usual clinical, radiological, endoscopic and histological features.[12] A (sub)total colectomy was considered an exclusion criterion, as was an ileostomy, colostomy or disease activity in the proximal part of the GI tract.

Study protocol

Participating patients were asked to fill out the CDAI diary and the Inflammatory Bowel Disease Questionnaire (IBDQ) before they underwent ileocolonoscopy. In addition to the usual demographic data, details of duration of disease, localization

and behavior of the disease (Montreal classification),[13] current and previous medication use, and previous surgeries were obtained from the medical records of the patients.
On the day of ileocolonoscopy, blood, and urine samples were collected for multiple tests. Two days before the endoscopy, feces samples were collected. Ileocolonoscopy was performed in all patients by the same endoscopist (B.O.). Endoscopic disease activity was assessed using CDEIS.[9] Representative parts of the ileum, right colon (including cecum), transverse colon, left colon (including sigmoid), and rectum were recorded on videotape. An independent gastroenterologist (T.S.) scored the disease activity by reviewing the videotapes. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, and written informed consent was obtained from all patients.

Endoscopic disease activity

Crohn's disease Endoscopic Index of Severity (CDEIS) - Endoscopic disease activity was assessed by the CDEIS.[9] This index is based on a number of reproducible mucosal (skip) lesions; erythema, edema, pseudopolyps, aphthoid ulcers, (longitudinal) ulcers, and stenosis.[14] It correlates with the endoscopist's global evaluation of lesion severity. The elaboration of the score requires analogue scale transformation. The CDEIS score generally ranges from 0-30. A higher score indicates more severe mucosal inflammation.

Variables analyzed

After a thorough review of literature, we selected the following 13 variables for analysis: platelets count, mean platelet volume (MPV), C-reactive protein (CRP), serum albumin, fecal lactoferrin, fecal calprotectin, fecal α-1 antitrypsin, and microalbuminuria. From the wide spectrum of symptoms in CD patients, the most objective parameter, the number of liquid stools during one day was selected. Furthermore, two out of 4 dimensional scores of the IBDQ, i.e. the bowel and systemic symptoms, were included in the analysis. These dimensions largely overlap with parameters from which the CDAI is composed, and may add important complementary value to an index. The dimension" bowel symptoms" overlaps with abdominal complaints and the dimension "systemic symptoms" overlaps with general wellbeing. Location and behavior of disease were also included in the analysis.

Questionnaires

Crohn's disease activity index (CDAI) – Clinical disease activity was assessed using the CDAI.[1] Eight independent variables (the number of liquid stools, the extent of abdominal pain, general well being, the occurrence of extraintestinal manifesta-

tions, the need for antidiarrheal drugs, the presence of an abdominal mass, hematocrit value and body weight) determine the CDAI score. A CDAI score of 150 or less indicates remission, 150 to 220 mild disease activity, a score of ≥220 or ≤ 450 moderate disease activity, and >450 severe disease activity.

Quality of life

The Inflammatory Bowel Disease Questionnaire (IBDQ) – Quality of life was measured by the IBDQ. The IBDQ is a disease-specific, health-related quality of life questionnaire, containing 32 items, with a graded response range of 1 (worst) to 7 (best) and a total score of 32 to 224.[15] The 32 items can be divided in 4 dimensions, including bowel symptoms (e.g. stool frequency and abdominal pain or cramps; 10 items), systemic symptoms (e.g. fatigue and energy loss; 5 items), emotional well-being (e.g. depressed feelings; 12 items), and social function (e.g. limited sexual activity; 5 items).The IBDQ has been translated and validated in the Dutch language and has been proven to be valid, discriminative and reliable.[16]

Laboratory tests

Blood tests

Biochemical and hematological tests were performed at the day of ileocolonoscopy (CRP, hemoglobin, hematocrit, platelet count, MPV, white cell count, erythrocyte sedimentation rate (ESR), and serum albumin)

Urine test

Microalbuminuria was assessed in all patients by an immunochemistry method (Beckman Coulter, type analyzer DxC 800, Fullerton, USA).

Feces tests

α-1 antitrypsin -Total fecal α-1 antitrypsin was determined with a commercially available antiserum against α-1 antitrypsin (Dade Behring, Marburg, Germany) using a Behring Nephelometer II, BN II (Dade Behring).

Calprotectin -After extraction of calprotectin from the feces using the extraction buffer of commercially available kit, (Calprotectin-Elisa kit, Orange Medical, The Netherlands) standards and samples were incubated in micro wells coated with a polyclonal rabbit anti-calprotectin. The amount of calprotectin bound was determined by an ELISA. Samples were tested in duplicates.

Lactoferrin - Lactoferrin was detected with an ELISA kit (TechLab, Blacksburg, Va., USA (IBD-CHEK). After dilution of 50 milligram of feces in buffer, standards and samples were analyzed. To quantify the results, a standard curve was prepared by serial dilution from normal serum. Samples were tested in duplicate.

Statistical Analysis

The distributions of the continuous predictors and scores were evaluated by means of descriptive statistics (mean or median; Standard Error of the Mean (SEM) or range). Dichotomous values (gender, localization and behavior of the disease, current medication, surgery in the past) were expressed as proportions (%).The reproducibility level of endoscopic scores (CDEIS) was evaluated through the intraclass correlation coefficient. We assumed that missing data were missing at random, and we imputed them by estimated means. The proportion of imputed data was 3.4%, which included all of the 13 selected variables. We used the entire data set, after missing value imputation, for analyses. Univariate analysis was done for all 13 selected variables. A further selection of predictors was made based on the mutual Pearson's or Spearman correlation coefficients. Predictors mutually associated with a correlation coefficient of 0.5 or higher were first put in a linear regression model. Of these, the strongest predictors were selected. The final model was based on multivariable linear regression. Backward elimination was assessed to obtain a parsimonious model, removing variables with $p > 0.20$. A P value of < 0.05 was considered to indicate statistical significance.

Internal validation

The internal validity of the regression model was assessed by bootstrapping techniques, which included the stepwise selection of variable for the model. This technique gives an impression of how over-optimistic the model is, i.e. how much the performance of the model deteriorates when applied to a new group of similar patients.[17]

Statistical analyses were performed with SPSS version 12.0 for Windows (SPSS Inc, Chicago, Ill) and R software version 2.3.1 (http://www.R-project.org).

Results

All patients approached for this prospective study were willing to participate. In 83 CD patients, 94 colonoscopies were performed. One ileocolonoscopy was discontinued because of pain and was therefore excluded from the analysis. In nine colonoscopies the ileum could not be reached. This was in 4 patients due to a stenosis, and in 5 due to technical problems. Representative parts of the ileum, right colon, transverse colon, left colon, and rectum were recorded on videotape in 72 colonoscopies and were scored by the second gastroenterologist, blinded for the results of his colleague and disease-specific data. The reproducibility of the endoscopic scores (CDEIS) was very good (intraclass correlation 0.86; $P < 0.001$).

Table 1
Montreal Classification, demographic, and clinical characteristics of the participating patients and analyzed ileocolonoscopies.

Age at diagnosis (n=82 patients)	≤16 y (%)	9 (11)
	17 - 40 y (%)	64 (78)
	> 40 y (%)	9 (11)
Location (n=82 patients)	ileal (%)	2 (2)
	colonic (%)	15 (18)
	ileocolonic (%)	63 (78)
	ileocolonic with upper GI disease (%)	2 (2)
Behavior (n=82 patients)	non-stricturing, non-penetrating (%)	38 (46)
	stricturing (%)	13 (16)
	penetrating (%)	4 (5)
	stricturing and penetrating (%)	10 (12)
	non-stricturing, non-penetrating † (%)	8 (10)
	stricturing † (%)	5 (6)
	penetrating † (%)	2 (2)
	stricturing and penetrating † (%)	2 (2)
Age (years ± SEM) (n=93 ileocolonoscopies)		41.0 (1.4)
Disease duration (years ± SEM) (n=93 ileocolonoscopies)		14.0 (1.1)
Gender male (%) (n=82 patients)		30 (37)
Operations (n=82 patients)		
	none (%)	28 (34)
	appendectomy (%)	9 (11)
	ileocecal resection (%)	20 (24)
	right hemicolectomy (%)	10 (12)
	partial small bowel resection (%)	7 (9)
	sigmoid resection (%)	3 (4)
	cholecystectomy (%)	11 (13)
	other[1] (%)	7 (9)
Medication use (n=93 ileocolonoscopies)		
	5 –ASA medication (%)	57 (61)
	Corticosteroids (%)	35 (38)
	Azathioprine (%)	47 (51)
	Methotrexate (%)	4 (4)

† = p, is added when concomitant perianal disease is present, [1]other operations: caesarean section; hysterectomy

Table 2
Descriptive data and univariable coefficients of the CDEIS, the CDAI, the IBDQ, and the selected variables

			Univariable coefficients (95% CI)
CDEIS[1]		(mean ± SEM)	5.0 (0.5)	.
CDAI[2]		(mean ± SEM)	167.1 (10.8)	.
No. of liquid stools during one day		(mean ± SEM)	2.3 (0.3)	1.3 (1.0-1.6)*
IBDQ[3]-total		(32-224; mean ± SEM)	155.6 (3.6)	.
IBDQ[3]- bowel symptoms		(10-70; mean ± SEM)	49.6 (1.1)	0.089 (0.066-0.112)*
IBDQ[3]- systemic symptoms		(5-35; mean ± SEM)	21.3 (0.7)	0.20 (0.15-0.25)*
Serum	Platelet count (10^9/L; mean ± SEM)		308.7 (10.4)	0.017(0.014-0.020)*
	MPV[4] (fL mean; ± SEM)		8.1 (0.1)	0.58 (0.44-072)*
	CRP[5] (mg/L; median; range)		7 (7-143)	0.24 (0.20-0.28)*
	Albumin (g/L; mean ± SEM)		40.7 (0.3)	0.12 (0.09-0.15)*
Feces	Lactoferrin (μ/mL; median; range)		8.8 (0.0-1751.1)	0.014 (0.010-0.017)*
	calprotectin (mg/L; median; range)		231 (19.5-6940)	0.004 (0.003-0.005)*
	α-1 antitrypsin (mg/g; median; range)		0.7 (0.2-10.1)	2.22 (1.77-2.66) *
Urine	microalbumin (mg/L; median; range)		11 (8-86)	0.18 (0.13-0.24)*

[1]Crohn's Disease Endoscopic Index of Severity; [2]Crohn's Disease Activity Index; [3]Inflammatory Bowel Disease Questionnaire; [4]Mean platelet volume; [5] C-reactive protein
* $P < 0.001$

In Table 1, demographic, and clinical characteristics of the participating patients and analyzed colonoscopies are presented. Table 2 shows the descriptive data of the CDEIS, the CDAI, the IBDQ, and the selected laboratory and clinical parameters. A low but statistically significant correlation was found between the CDAI and the CDEIS ($r = 0.38$; $P < 0.001$).
CRP, fecal lactoferrin, fecal calprotectin, and fecal α-1 antitrypsin were highly mutually correlated ($r > 0.50$), as were the systemic dimension of the IBDQ score,

the bowel dimension of the IBDQ score, and the number of liquid stools during one day. When combined in a linear regression model, CRP, fecal calprotectin, the bowel dimension of the IBDQ score, and number of liquid stools during one day were the strongest predictors.

In multivariable analysis, 5 main predictors of the CDEIS were identified: the number of liquid stools during one day, CRP, platelet count, fecal calprotectin and microalbuminuria (adjusted $R^2 = 0.74$). Replacing the latter predictor by MPV, only marginally affected the R^2 (adjusted $R^2=0.73$). Since this resulted in a less laborious index, this was considered justified. The new disease activity index was based on the regression coefficients presented in Table 3. The final formula for this index is as follows: mucosal inflammation (CDEIS) = number of liquid stools during one day*0.25 + C-reactive protein (mg/L) *0.1 + platelet counts (10^9/L) *0.01 + fecal calprotectin (mg/L) *0.001 - mean platelet volume (fL) *0.2. This means a 1 point rise in the CDEIS per 4 stools, 1 point rise per 10 units CRP, 1 point rise per 100 units of platelet counts;1 point rise per 1000 units of fecal calprotectin, and 1 point decrease per 5 units of MPV.

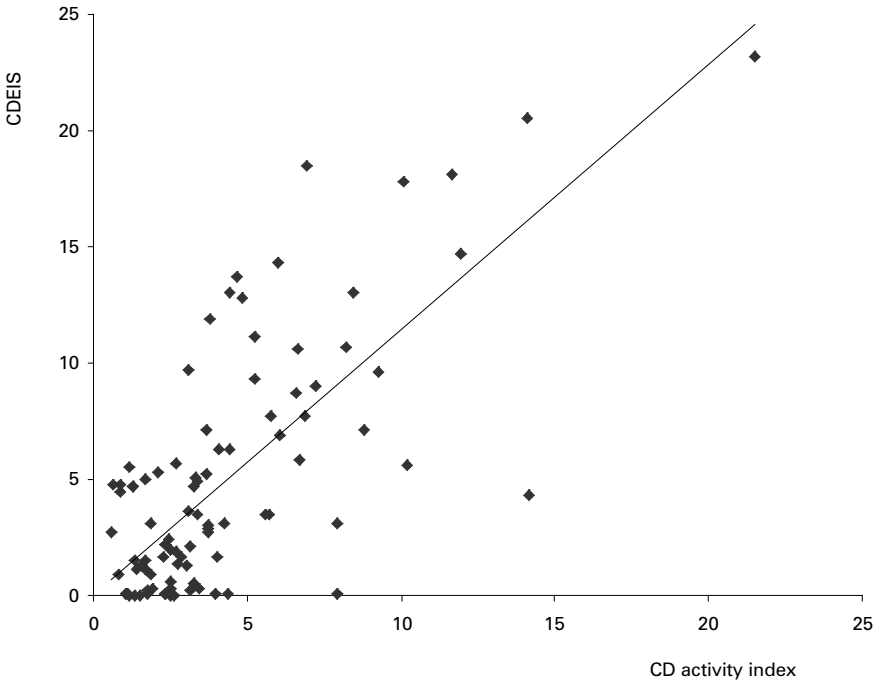

Figure 1
Correlation between the new CD activity index and the Crohn's Disease Endoscopic Index of Severity (CDEIS).

Table 3
Multivariable regression coefficients of the final predictors

Predictor	regression coefficients
liquid stools during one day	0.248
CRP[1]	0.099
platelet count	0.012
fecal calprotectin	0.001
MPV[2]	-0.200

[1]C-reactive protein;[2]mean platelet volume

Figure 1 shows the good correlation between the new activity index and the CDEIS ($r = 0.72$; $P < 0.001$). Applying bootstrapping techniques for internal validation of this model showed a small reduction in R^2 adjusted of 0.50.

Discussion

At present, endoscopy is considered the most adequate tool for assessing disease activity in CD. Endoscopy, however, causes discomfort for the patient, is time-consuming, and is expensive. We have developed a new disease activity index for CD, based on readily available clinical characteristics and laboratory parameters. The index is expected to reliably predict endoscopic inflammation. The obvious advantage of such an index is its potential role in clinical decision making and its use as a reliable endpoint in clinical trials.

As opposed to the development of other disease activity indices, we based this new activity index on mucosal disease activity measured by the CDEIS. Previously, the assessment of the CDEIS has been found to be reliable and reproducible, with a good interobserver agreement,[9]which is underscored by the high reproducibility between the paired endoscopic scores in the present study (intraclass correlation 0.86).

Thirteen clinical and specific laboratory variables were selected for analysis. Parameters had to be easy to collect, cheap, readily available, and/or the relation between the parameter and mucosal or histological inflammation had to be demonstrated in previous studies. Microalbuminuria emerged as one of the

predictors in our first model. Including miroalbuminuria in the model would have resulted in a more laborious index, since a portion of urine would be acquired besides feces and blood. Because one of the aims was to develop a simple and easy-to-use index, we computed a model without microalbuminuria. The R^2 adjusted did decrease by only 1% which justifies our choice to leave this variable out of the model.

A disease activity index for CD should ideally consist of a parameter reflecting the extent of mucosal involvement, a parameter of systemic inflammation, and a clinical/functional parameter (such as diarrhea). The incorporation of the number of liquid stool in the model did not come as a surprise, because this parameter represents probably the most objective clinical activity parameter available. The number of liquid stools during one day is also a variable in the Harvey Bradshaw index or "simple index",[18] which is a simplified version of the CDAI. Previously, no attempts have been made to correlate this parameter with mucosal inflammatory activity which explains the paucity of published data.

Systemic inflammation is represented in the index by CRP, platelet count and MPV. CRP is one of the most important acute phase proteins in humans. It is produced almost exclusively by hepatocytes, under normal circumstances in low quantities (<1 mg/L). However, following an acute phase stimulus such as inflammation, hepatocytes rapidly increase production of CRP.[19] It has a short half-life compared with other acute phase proteins and will therefore rise early after the onset of inflammation and rapidly decrease after resolution of inflammation.[19] For this reason it is very well suitable to monitor disease activity in CD patients. Platelets and MPV are less extensively studied compared to the CRP in CD patients. It is known that platelets are capable of amplifying the inflammatory response in CD by releasing inflammatory mediators,[20] and increased platelet count has been associated with clinical disease activity.[20; 21] The platelet size, MPV, was reported to be reduced in CD patients with clinically active disease,[20; 22] which is in line with our finding that MPV is negatively correlated to the CDEIS ($r = -0.290$, $P < 0.001$). The origin of decreased platelet volume observed in CD could be due to a defect in the regulation of trombopoiesis, secondary to the inflammatory process.[20] Because the correlation between MPV and platelet count was -0.477 in the present study, both parameters were considered independent variables in the analysis.

The extent of mucosal involvement in this index is represented by fecal calprotectin levels. Calprotectin is a marker of neutrophil turnover, since it is released from neutrophils shedded from the colonic mucosa by activation of leucocytes. The marker is stable during intestinal transit, resistant to colonic bacterial degeneration, and can easily be assessed in stools by means of ELISA tests.

Overall, studies focused on the correlation between mucosal inflammation and the above mentioned laboratory activity markers are not conclusive.[10; 23-28] This can be

due to the different endoscopic scorings systems used, to the different study designs, but probably mainly to the small sample size in most of the studies.
At this moment the most used and accepted activity score worldwide in clinical trials is the CDAI[1]. We found a poor, but significant correlation between the CDEIS and the CDAI, which is in line with the literature.[10; 11] In the development of the CDAI, mucosal inflammation has not been taken into consideration; instead the physician's over-all evaluation of "how the patient was doing" was used as the gold standard. We feel that the CDAI cannot longer remain the primary endpoint in clinical trials and should be replaced by an improved index or parameter, reflecting mucosal healing better.
Using the CDAI in clinical trials as primary endpoint in the past resulted in remission rates of up to 50% in CD patients treated with placebo.[29] Solem et al.[25] reported that the subgroup of CD patients with elevated CRP concentrations had lower rates of placebo response compared with patients with normal CRP concentrations, reflecting probably a lower endoscopic disease activity in the latter group. Furthermore, a substantial part of the total CDAI score is derived from subjective items (the extent of abdominal pain, general well being) and reflects the patients' perception and interpretation of the disease and its symptoms. This is important for the quality of life of patients, and needs to be measured in clinical trials. However, the items of the CDAI show a considerable overlap with both the bowel symptoms domain and the systemic symptoms domain of the Inflammatory Bowel Disease Questionnaire (IBDQ). Thus, the CDAI may be redundant since the IBDQ can sufficiently measure these aspects of the patient's perception of disease.
Although a large percentage of the enrolled patients in the present study appeared to be endoscopically in remission or have mild to moderate disease, we feel that the new index will perform well in daily practice and in trials. Generally, patients with severe disease activity are easily identified by their high CRP and calprotectin levels and their characteristic symptoms. Patients with mild to moderate disease activity or patients in remission with IBS-like symptoms,[30; 31] however, are often difficult to recognize without the help of endoscopy. The strength of this newly designed index will lay particularly in prediction of the mucosal disease activity in this difficult subset of patients.
In summary, we have created a novel activity index based on readily available parameters, accurately predicting the severity of mucosal inflammation in CD patients. This tool may not only prove to be useful for trials but also in daily clinical patient management.

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General Summary

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Chapter 8

General Summary

Inflammatory bowel disease (IBD) comprises ulcerative colitis (UC) and Crohn's disease (CD). IBD patients frequently complain of fatigue, and a substantial proportion of the patients have gastrointestinal symptoms, resembling irritable bowel syndrome (IBS). These symptoms are not always related to disease activity, since a significant proportion of patients experience them even when in remission. The pathogenesis of these systemic symptoms in IBD is incompletely understood and studies, aimed at the incidence and presentation of the symptoms, are hard to find.

In **Chapter 1**, we have shown that the degree of fatigue experienced by a cohort of 80 IBD patients in clinical remission is significantly higher compared with age- and sex-matched controls. Fatigue was assessed by the multidimensional fatigue inventory, a validated instrument dedicated to measure fatigue in an objective manner. If fatigue is defined as the 95 percentile of the score of a healthy control group, 41% of the IBD patients in remission suffered from fatigue. Comparing the results of the IBD patients with other patient groups, we found mean fatigue scores comparable to the scores reported in cancer patients. Furthermore, the study showed a strong negative correlation between fatigue and quality of life in IBD patients in remission, which supports the thought that fatigue influences the quality of life to a great extend. We postulated that a disturbance in the hypothalamic-pituitary-adrenal axis would contribute to the generation of fatigue, but we could not substantiate this hypothesis as the basal cortisol levels and the low dose ACTH test were not abnormal in the studied patients.

As fatigue seems not to be the result of hypocortisolism in this group of IBD patients, questions can be raised regarding other possible causes of this symptom. Cytokines (e.g. tumor necrosis factor (TNF)-α, interleukin (IL)- 6, IL-10, IL-18) play an important role in IBD, especially in active disease. Studies in patients with chronic fatigue syndrome suggest an immunological basis for fatigue, mediated by cytokines. **Chapter 2** describes the first placebo-controlled study that was conducted to examine the effect of a TNF-α antibody, infliximab, on fatigue in CD patients, and the role of cytokines in this respect. Fourteen CD patients were included in this study. Blood samples were drawn at a regular basis, and questionnaires on fatigue, depression, quality of life, and clinical disease activity were completed at regular intervals. We found a clear reduction of fatigue following administration of infliximab, although a placebo response was observed as well. The effect of infliximab on fatigue, however, persisted while the placebo response disappeared after a short period of time. Again, the impact of fatigue on the quality of life was confirmed, as was the impact on psychological well-being. A clear role of cytokines in the pathogenesis of fatigue could not be substantiated.

In **Chapter 3** we aimed to assess the prevalence of IBS-like symptoms in 107 IBD patients in clinical remission and in 66 controls, according to the Rome II criteria for IBS. We found that about 8% of the controls, one-third of UC patients and 40% of CD patients in remission suffered from IBS-like symptoms. The presence of these symptoms was found to affect the quality of life in these patients significantly.

In **Chapter 3, Chapter 4,** and **Chapter 5**, mechanisms potentially underlying the generation of IBS-like symptoms in IBD patients in remission were studied.

We hypothesized in **Chapter 3** that IBD patients in remission suffering from IBS-like symptoms have coping strategies that are different from those without symptoms. It is known that psychological factors are determinants in the development of persistent bowel symptoms, secondary to inflammatory processes. In this study, we could not confirm a causal relationship between the presence of symptoms and coping strategies.

In **Chapter 4** the question was addressed whether IBD patients inremission with prior disease activity restricted to the colon, develop visceral hypersensitivity throughout the whole gastrointestinal tract, as is seen in IBS patients. Antro-duodenal manometry was performed in 10 IBD patients who were endoscopically in remission and in 13 controls. Small volumes of nutrients and acid were administered intraduodenally. Subtle differences in chemospecific responses to lipids and acid were observed in IBD patients in remission. In our opinion these changes are indicative for changes at the level of chemoreceptors in the duodenal wall in this group of patients. However, the clinical relevance of these findings can be questioned, since none of the infusions induced altered perception of nausea, abdominal pain, bloating, urgency or fullness.

In **Chapter 5** the effect of inflammatory activity on the enteric nerve system (ENS) was explored. Serotonin (5-HT) is a pivotal neurotransmitter and paracrine signaling molecule in the gut, whose availability is altered in IBS patients. We aimed to study the role of serotonin in the generation of IBS-like symptoms in CD patients in re-mission. Ileal- and colonic biopsy specimens were obtained from 20 CD patients in remission (10 with, and 10 without IBS-like symptoms) and 11 healthy controls. Enterochromaffin (EC) cells were counted, and messenger RNA (mRNA) expres-sion levels of tryptophan hydroxylase (TpH)-1 and serotonin reuptake transporter (SERT) were determined.
We demonstrated large regional differences of SERT mRNA expression in the gut. The mRNA levels of SERT found in the colon were lower than the levels measured in the ileum (in controls and CD patients), while expression of TpH-1 in ileum and

colon was comparable. The different uptake capacity might be related to the distinctive functions of the terminal ileum and the colon. Furthermore, we found higher levels of TpH-1 expression in colonic mucosa of CD patients in remission with IBS-like symptoms when compared to subjects without these symptoms (patients and healthy controls), with similar numbers of 5-HT producing cells in all groups. Upregulated 5-HT synthesis in the colon might lead to increased 5-HT availability. Increased 5-HT availability has been associated with symptom generation in IBS. Therefore, elevated TpH-1 levels may contribute to IBS-like symptom generation in CD patients in remission. As it is known that inflammation affects the serotonergic pathway, our data implicate that some of these changes persist in the subset of CD patients in remission with IBS-like symptoms.

Overall, the exact pathogenesis of IBS-like symptoms in IBD patients in remission remains unclear. Although the cause of symptom generation is probably multifactorial, our data suggest that the effect of inflammation on the ENS seems to play an important role.

Assessing disease activity in CD is cumbersome and the tools used to measure inflammatory activity are not always adequate. Mucosal healing is presently considered the ultimate goal of treatment in CD, but this can only be assessed by endoscopy.
The most used endoscopic disease activity indices, and the predictive value for mucosal inflammation of clinical disease activity indices, quality of life questionnaires, and biochemical markers are reviewed in **Chapter 6**. We conclude that the clinical Crohn's Disease Activity Index (CDAI) cannot longer remain the primary endpoint in clinical trials, since it correlates poorly with the inflammatory status of the intestinal mucosa. We propose to develop a new clinical activity index predicting mucosal disease activity reliably without the need for endoscopic procedures. This would simplify and probably improve the management of CD patients in trials as well as in clinical practice.

In **Chapter 7** the development of this new CD activity index is described. Based on the previously mentioned review 13 clinical and specific laboratory variables were selected for analysis. Using multiple linear regression, we developed a new activity index, including cheap, widely available, and easy-to-collect parameters, based on 93 ileocolonoscopies performed in 82 CD patients. We constructed an index that proved to accurately predict the severity of mucosal inflammation in CD patients: number of liquid stools during one day*0.25 + C-reactive protein (mg/L) *0.1 + platelet counts (10^9/L) *0.01 + fecal calprotectin (mg/L) *0.001 - mean platelet volume (fL) *0.2. We feel that this index, after validation, will provide a better endpoint in clinical trials, and contribute importantly to clinical decision making in daily practice.

Nederlandse samenvatting

Symptomen in Inflammatory Bowel Disease; pathofysiologische aspecten en de relatie met ziekte activiteit

Colitis ulcerosa en de ziekte van Crohn zijn chronische darmontstekingen. De ontsteking van de darm is niet continue aanwezig en kan ook perioden afwezig zijn. De ziekten worden in de literatuur aangeduid met de term "Inflammatory Bowel Disease"ofwel IBD. De exacte oorzaak van deze aandoeningen is (nog) niet bekend, maar duidelijk is wel dat meerdere factoren, zoals aanleg en omgevingsfactoren, een rol spelen. De klachten die IBD patiënten ervaren zijn niet altijd gerelateerd aan de mate van ontsteking van de darm, ook wel ziekteactiviteit genoemd. Patiënten die geen aanwijzingen hebben voor ziekteactiviteit kunnen evidente klachten ervaren. In dit proefschrift worden een aantal veel voorkomende symptomen, vermoeidheid en klachten die passen bij het prikkelbaar darm syndroom, belicht. Enkele hypotheses aangaande de oorzaak van deze klachten worden onderzocht.

In het eerste hoofdstuk van dit proefschrift inventariseren we hoe vaak vermoeidheid voorkomt bij IBD patiënten zonder ziekteactiviteit. Ruim 40 % van de patiëntengroep blijkt vermoeid. De mate van vermoeidheid is vergelijkbaar met de mate van vermoeidheid gemeten bij patiënten met kanker, patiënten met reumatoïde artritis en patiënten bekend met lever cirrosis. De vermoeidheid heeft een negatieve invloed op de kwaliteit van leven van de IBD patiënten.
Corticosteroïden zijn medicijnen die vaak worden voorgeschreven aan IBD patiënten met ziekteactiviteit. Het idee dat het gebruik van corticosteroïden in het verleden een rol speelt bij de ontwikkeling van de vermoeidheidsklachten konden we niet bevestigen.
Het is opvallend dat patiënten met de ziekte van Crohn met ziekteactiviteit na de behandeling met infliximab, een krachtige ontstekingsremmer, minder vermoeid zijn dan vòòr de behandeling. Omdat infliximab specifiek TNF-α (een ontstekingsmediator) remt, onderzochten wij de betrokkenheid van deze en andere ontstekingseiwitten bij het ontstaan van vermoeidheid. In hoofdstuk 2 beschrijven we de resultaten van een onderzoek waarin 14 patiënten met de ziekte van Crohn werden behandeld met zowel placebo als infliximab. Uit de studie kwam naar voren dat vermoeidheid kortdurend afnam na behandeling met placebo, terwijl het positieve effect van infliximab op vermoeidheid persisteerde. De rol van meerdere ontstekingseiwitten werd onderzocht in relatie tot vermoeidheid, maar geen van de eiwitten bleek geassocieerd te zijn met vermoeidheid.
Concluderend, vermoeidheid is een veel voorkomende klacht bij IBD patiënten. Het heeft grote invloed op de kwaliteit van leven van de IBD patiënten, maar een evidente rol voor ontstekingseiwitten dan wel corticosteroïden bij het ontstaan van deze klacht hebben we niet kunnen aantonen.

Naast vermoeidheid ervaren veel patiënten met IBD in remissie (zonder ziekte-activiteit) klachten die te vergelijken zijn met de symptomen van het prikkelbaar darm syndroom (PDS). PDS is een frequent voorkomende aandoening waarbij patiënten een veranderd ontlastingpatroon en buikpijn hebben zonder dat er sprake is van aantoonbare afwijkingen bij onderzoek. In hoofdstuk 3 werd de incidentie van op PDS- gelijkende klachten onderzocht in een groep van 107 IBD patiënten zonder ziekte activiteit. Eenderde van de patiënten met colitis ulcerosa en 40% van de patiënten met de ziekte van Crohn bleek deze klachten te hebben, in vergelijking met slechts 8% van de gezonde vrijwilligers. In de hierop volgende hoofdstukken hebben we geprobeerd een oorzaak te vinden voor deze klachten.

Het is bekend dat stress de klachten van het PDS kan uitlokken. In hoofdstuk 3 is onderzocht of *coping* (omgaan met stressvolle of negatieve situaties) invloed heeft op het wel of niet ontwikkelen van de klachten wanneer een patiënt geen ontsteking heeft. Verschil in coping lijkt niet aan de basis te liggen voor het ontwikkelen van deze symptomen bij IBD patiënten.

Uit eerder onderzoek is naar voren gekomen dat de darmen van patiënten met het PDS gevoeliger zijn dan de darmen van gezonde vrijwilligers. De darmen hebben een eigen zenuwstelsel. De gevoeligheid van de darmen van PDS patiënten kan o.a. berusten op veranderingen in dit "darmzenuwstelsel". Wij hebben onderzocht of bij IBD patiënten ontsteking van de darmwand of het darmslijmvlies in het verleden aanleiding geeft tot beschadiging van het zenuwstelsel, resulterend in overgevoeligheid van de darmen, mogelijk ook op plaatsen waar eerder geen ontsteking was.

In hoofdstuk 4 beschrijven we de resultaten van een studie bij 10 IBD patiënten en 13 gezonde vrijwilligers, waarin de sensorische en motorische reacties op infusies van voedingsstoffen in de twaalfvingerige darm werden bepaald met behulp van drukmetingen ter plaatse. We constateerden dat IBD patiënten een veranderde motorische reactie hebben op bepaalde voedingsstoffen in vergelijking met gezonde vrijwilligers. Dit resulteert echter niet in een veranderde perceptie. Anders gezegd, patiënten ervaren geen klachten op het moment van toedienen van deze voedingsstoffen.

In hoofdstuk 5 onderzoeken we één specifieke neurotransmitter van het "darm-zenuwstelsel", namelijk serotonine. In PDS patiënten is er een verandering van de serotonine beschikbaarheid in het darmslijmvlies. Wij vonden bij Crohn patiënten zonder ziekteactiviteit met PDS klachten een verhoging van het eiwit dat essentieel is voor de aanmaak van serotonine, terwijl in het dikke darmslijmvlies van Crohn patiënten zonder deze klachten en gezonde vrijwilligers lagere spiegels van dit eiwit werden gemeten. We denken dat de verhoging van dit eiwit uiteindelijk resulteert in een verhoogde beschikbaarheid van serotonine. Dit kan bijdragen tot de ontwikkeling van PDS klachten.

In het laatste deel van het proefschrift wordt het meten van de ziekteactiviteit bij de ziekte van Crohn onder de loep genomen.
De gouden standaard voor ziekteactiviteit in Crohn patiënten is de mate van ontsteking van het darmslijmvlies. Deze kan alleen worden geobjectiveerd middels endoscopie, wat een onplezierig en tijdrovend onderzoek is. In het verleden zijn meerdere non-invasieve instrumenten ontwikkeld om de mate van ziekteactiviteit te meten bij de ziekte van Crohn, maar deze instrumenten waren nooit gebaseerd op de werkelijke ontstekingsactiviteit in de darm. De op dit moment meest gebruikte klinische activiteit index blijkt slecht te correleren met de ernst van ontsteking van het darmslijmvlies. In het laatste hoofdstuk van dit proefschrift staat een onderzoek beschreven waarin we een model hebben ontwikkeld dat de mate van ontsteking van het darmslijmvlies voorspelt aan de hand van gemakkelijk te verkrijgen variabelen. Met behulp van lineaire regressie werd het nieuwe model gebaseerd op de resultaten van 93 ileocolonoscopiën in 82 patiënten. Het nieuwe model voorspelt aan de hand van de frequentie van ontlasting, enkele bloedwaarden en een bepaling uit de ontlasting accuraat de ernst van de ontsteking van het darmslijmvlies. Wij hopen dat deze nieuwe index, na validatie, een belangrijke rol gaat spelen bij klinisch onderzoek en richtinggevend kan zijn voor het beleid bij individuele Crohn patiënten in de dagelijkse praktijk.

Curriculum Vitae

Itta Minderhoud werd geboren op 9 februari 1975 te Amsterdam. In 1993 behaalde zij het V.W.O.-diploma aan de F.A. Minkema Scholengemeenschap in Woerden. Na aanvankelijk te zijn uitgeloot, werd in 1994 begonnen met de studie Geneeskunde aan de Universiteit Utrecht. Tijdens haar studie werkte zij mee aan het opzetten van de Inflammatory Bowel Disease database op de afdeling Maag-, Darm- en Leverziekten van het Universitair Medisch Centrum Utrecht, onder leiding van Dr. B. Oldenburg. In juni 2001 werd het artsenexamen gehaald. De daaropvolgende jaren was zij werkzaam als artsonderzoeker binnen de afdeling Maag-, Darm- en Leverziekten van het Universitair Medisch Centrum Utrecht. In deze periode werd de basis gelegd voor dit proefschrift. In januari 2005 werd begonnen met de voor-opleiding Interne Geneeskunde in het Meander Medisch Centrum te Amersfoort (opleider: Dr. A. van de Wiel, Dr. C.A.J.M. Gaillard). In mei 2007 startte zij met de opleiding Maag-, Darm- en Leverziekten in het Meander Medisch Centrum (opleider: Drs. J.R. Vermeijden), welke voltooid zal worden in het Universitair Medisch Centrum Utrecht (opleider: Dr. P.D. Siersema).

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