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A Contribution to the Study of the Association and Interactions between Psychosocial Dimensions and Biological & Clinical Aspects of Crohn's Disease

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Doctoral Thesis in Biomedical Sciences

Leuven, June 2013

Thesis submitted in partial fulfillment of the requirements for the degree of
“Doctor in Biomedical Sciences”.

GRANT SUPPORT
The candidate, Philippe Persoons, was a research fellow of the Fund for Scientific Research – Flanders, Belgium for four years

Leuven, Belgium. 2013

ISBN 978-90-9027-627-4
NUR 882

Voor An

Voor Liene en Mattijs

Arrange whatever pieces come your way
(Virginia Woolf)

Once you've got a task to do, it's better to do it than to live with the fear of it
(LNF by Joe Abercrombie)

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LIST OF ABBREVIATIONS

5-HT: serotonin (5-hydroxytryptamine)
ADM: Anxiety Disorder Module
ANS: the autonomic nerve system
ATD: Acute TRP depletion
BBB: blood-brain barrier
BC: Bonferroni correction
BD: bowel dimension
BDI: Beck Depression Inventory
BMI: Body Mass Index
CD: Crohn's Disease
CDAI: Crohn's Disease Activity Index
CDF: chronic disabling fatigue
CFS: Chronic Fatigue Syndrome
CIDI: Composite International Diagnostic Interview
CIS20R: Checklist of Individual Strength 20 items
CNS: central nervous system
CRF: corticotropin-releasing factor
ED: emotional dimension
ENS: Enteric Nerve System
FACIT: Functional Assessment of Chronic Illness Therapy
FIS: Fatigue Impact Scale
FQ: Fatigue Questionnaire
GI: gastrointestinal
HADS: Hospital Anxiety and Depression Scale
HPA: hypothalamic-pituitary-adrenergic
HRQL: Health related quality of life
IBD: inflammatory bowel disease
IBDQ: Inflammatory Bowel Disease Questionnaire
IDO: indoleamine 2,3 dioxygenase-1
IFN-γ: interferon-γ
IFX: infliximab
IL: Interleukin
IQR: interquartile range
LNAA: large, neutral amino-acid
MDD: major depressive disorder
MDM: Mood Disorder Module
MFI: Multidimensional Fatigue Inventory
MINI: Mini-International Neuropsychiatric Interview
NK: natural killer
NOD: nucleotide oligomerization domain
NV: nervus vagus
PHQ: Patient Health Questionnaire
POMS: Profile of Mood States
RTD: rapid TRP depletion
SD: Standard Deviation
SDM: Somatoform Disorder Module
SNS: sympathetic nerve system
SocD: social dimension
SSL-I: Social Support List – Interactions
STAI-Y1: State and Trait Anxiety Inventory Form Y
SysD: systemic dimension
TAS-20: Toronto Alexithymia Scale 20 items
TLR: Toll-like receptor
TNF-α: tumor necrosis factor alpha
TRP: tryptophan
UC: ulcerative colitis
VAS: visual analogue scale
TRYCATs: tryptophan catabolites

Chapter 1

1 Introduction

Crohn's Disease (CD) is not a mere disease (*i.e. the structural and functional abnormalities of organs and tissues*), but a chronic "illness" (*i.e. a broader concept, determined by the subjective experience of Crohn's disease, determined by the disease activity and severity, its effect on functioning and its psychosocial effects)*, having a major impact on every aspect of the life of patients confronted with this diagnosis. Therefore, it is important to have a thorough understanding of the biological, psychological and social mechanisms at play, their complex interactions and the impact on patients' well-being and daily functioning.

1.1 CROHN'S DISEASE – AN OVERVIEW

In 1932, doctor Burrill B. Crohn presented a paper on "Terminal ileitis" to the American Medical Association, thus naming the ileocecal inflammatory, tuberculosis-like disease with granulomatous lesions, Crohn's Disease.[1] Crohn's disease (CD) is one of the inflammatory bowel diseases (IBD). IBD are chronic, idiopathic intestinal syndromes and also include ulcerative colitis (UC). IBD has an insidious onset, a relapsing-remitting course and can present with variable symptoms and extraintestinal manifestations. This can complicate recognition and it may take months or years to establish a diagnosis of IBD. The classification of the type of IBD remains difficult, but CD and UC seem to be fundamentally different. Therefore, the focus of this doctoral project was on CD rather than on IBD.

1.1.1 Epidemiology of Crohn's disease

The epidemiology of CD is highly variable around the world, with the highest incidence rates and prevalence in Europe, North America, and also Australia and New Zealand (10.6 to 29.3 per 100,000). The prevalence in Europe is up to 322 per 100,000.[2] The relatively high incidence rates in Australia and New Zealand question the long-standing North-South

gradient hypothesis in CD. Gender does not influence the occurrence of CD. The highest incidence rates for CD occur between the second and the fourth decade of life. Race or ethnicity seem to play a significant role, with the highest incidence in Caucasian and Jewish people.[3] However, races with lower incidence rates showed increases in the past decades and the ethnic groups, especially the first-generation children, migrating from regions (e.g. Asia) with low CD prevalence to higher prevalence countries, are at increased risk for developing CD. Thus, CD might be associated with industrialization of nations which may also – at least in part – explain the recent rising of incidence of CD in developing nations and suggests the influence of environmental risk factors (urbanization and industrialization) in the development of CD.[2] Furthermore, an increase of CD incidence during the last part of the 20th century has been reported globally. This will lead to a continual rise of prevalence of CD since mortality is low and given the young age at diagnosis.

1.1.2 Clinical presentation of Crohn's Disease

Crohn's disease (CD) is characterized by a relapsing and remitting course of transmural inflammation of the gastrointestinal mucosa. Its clinical features can be highly variable. It can affect the entire gastrointestinal tract from the mouth to the perianal area and typically presents with a discontinuous involvement of different sections of the gut. Complications are very common in CD and patients frequently develop fistulas, abscesses and strictures. The clinical features are to a large extend dependent on the location of the disease. At diagnosis, the terminal ileum is the most common initial presentation site (40-47%) and the majority of the patients (up to 80%) have small bowel involvement, of which about 30% exclusively have ileitis.[4] Ileocolitis is present in approximately 50% of the patients and up to 20% of the patients have Crohn colitis. Perianal disease is present in approximately one-third of the patients. Involvement of the upper gastrointestinal tract (mouth, esophagus, gastroduodenal area and proximal small bowel) occurs rarely (3-5%).[3]

The initial manifestations of CD are often vague and symptoms, such as unexplained fever, prolonged diarrhea with abdominal pain, weight loss and fatigue can persist for many years prior to diagnosis. CD is a fibrostenotic disease due to the transmural nature of its inflammatory process and the resulting strictures can lead to episodic small bowel, or less commonly colonic, obstruction. In the case of severe transmural bowel inflammation, (micro)perforation of the bowel wall is possible. This will rarely present as an acute abdomen, but will rather lead to fistula formation or to a walled off inflammatory mass.[4] Systemic symptoms such as fatigue and weight loss are common features in CD. A number of extraintestinal manifestations are also related to the inflammatory activity of CD, including

arthritis, eye involvement (e.g. uveitis, iritis), skin disorders (e.g. erythema nodosum), primary sclerosing cholangitis, secondary amyloidosis, osteoporosis and vitamin B12 deficiency.

1.1.3 Diagnosis of Crohn's disease

Because of the variety in clinical presentation, the chronicity of the illness and the different possible sites of involvement, the differential diagnosis for Crohn's disease is extensive. Especially when CD is limited to or mainly involves the colon, it can be challenging to distinguish it from the other frequent inflammatory bowel disease, Ulcerative Colitis (UC). The distinction is important since the course of both diseases and the medical and surgical management can differ significantly. Table 1 gives an overview of the main differences between CD and UC. If the distinction cannot be made (10-15%), the IBD is referred to as indeterminate colitis. Irritable bowel syndrome, lactose intolerance, infectious colitis, appendicitis and diverticulitis are examples of other possible differential diagnoses for CD.

Table 1. Differential diagnosis between CD and UC.[3]

	CD	**UC**
Anatomopathology		
Transmural mucosal inflammation	Yes	No
Granulomas	Yes	No
Fissures and skip lesions	Common	Rarely
Cryptitis and Crypt abcesses	Yes	Yes
Distorted Crypt architecture	Yes	Uncommon
Clinical Features		
Heamatochezia	Rare	Common
Passage of mucus or pus	Rare	Common
Small-Bowel disease	Yes	No
Upper GI involvement possible	Yes	No
Abdominal mass	Sometimes right lower quadrant	No
Extraintestinal manifestations	Common	Common
Small-bowel obstruction	Common	Rare
Colonic obstruction	Common	Rare
Fistulas & perianal disease	Common	No

CD: Crohn's Disease
UC: Ulcerative Colitis

The diagnosis of CD in clinical practice is usually based on the clinical presentation of the patient supplemented with laboratory and imaging studies. Endoscopic findings and the subsequent histological studies often establish the diagnosis. Whereas in clinical practice the disease activity is described with broad denominators (mild to moderate, moderate to severe,

severe to fulminant), in research disease activity indices are used. They are intended to objectify the clinical global assessment and several indices exist. In the research presented in this thesis, the Crohn's Disease Activity Index (CDAI) was used.

1.1.4 Classification of Crohn's disease

Initially, CD was classified **by site of disease**, i.e. ileal, colonic and ileocolonic disease. However, since this was only an anatomical classification, it was rather limited. The first phenotypic classification – the **Rome classification** – was based on anatomical distribution, history of surgery and clinical behavior (inflammatory, fistulizing or structuring), but proved to be impractical and was replaced by the **Vienna classification.**[5] The latter phenotypic classification comprised of (1) the age of onset (below 40 years or above 40 years), (2) disease location (ileal, colonic, ileocolonic, upper GI tract) and (3) disease behavior (nonstricturing and nonpenetrating, stricturing, penetrating).[6] In the **Montreal classification,** the age of onset definition was modified by adding an early onset of disease category (A1 = patients diagnosed < 17 years, A2 = 17 – 40 years and A3 > 40 years), because genetic studies have found a link between specific genetic markers and the young subgroup.[5] Additionally, perianal fistulizing disease was separated from fistulizing disease in general. In this work, the Vienna classification has been used as the phenotypic classification system in the studies, since all data was collected before the Montreal consensus meeting in 2005.

1.1.5 The course of Crohn's disease

The course of inflammatory Crohn's disease is highly variable between patients and only a minority of patients remain in long term remission (10-13%). The majority of patients (67-73%) are confronted with a chronic remitting and recurring disease course and about 13-20% of patients with CD have a chronic course of continuous disease activity.[3,7] The location of CD is a rather stable factor of the disease, whereas the disease behavior can change over the course of the disease: from inflammatory to stricturing (in 27%) or penetrating (29%) disease. Although surgery does not cure CD, most patients will require surgical intervention. Recurrence after surgery is the rule rather than the exception.[4,8] CD patients have a slightly increased mortality rate compared to the general population (standardized mortality ratio 1.3, 95%CI: 1.03-1.89), especially due to severe CD.[9]

1.1.6 Medical Management

A broad introduction to the full medical management of CD is beyond the scope of this dissertation, but since the treatment is an important variable, a short introduction is necessary. The medical management of active CD is based on the control of the local intestinal inflammation and is aimed at rapidly inducing clinical response or, ideally, remission. For induction therapy in patients with mildly to moderately active CD, 5-aminosalicylic acid (5-ASA) derivatives (sulfasalazine and mesalazine) and locally active corticoids (budesonide) are recommended, whereas induction with systemic corticosteroids (e.g. prednisolone) is the gold standard treatment in moderate to severe disease.[3] Infliximab is a murine-human monoclonal chimeric antibody against tumour necrosis factor-α (TNF-α) and has proven to be effective in severe, refractory luminal and fistulizing CD. If patients do not enter remission with corticosteroids, infliximab at 5mg/kg bodyweight at weeks 0, 2 and 6 is given intravenously. A number of studies in this dissertation have been done with patients receiving infliximab. The maintenance of remission is the other main goal of the medical management of CD. Oral 5-ASA is also widely used for maintenance of medically induced remission. Methotrexate is also used as immunosuppressant, especially in patients who are steroid dependent. For steroid dependent patients, despite immunosuppression by thiopurines or methotrexate and for patients who are unable to maintain remission, infliximab is also an effective option for maintenance of remission.[3]

1.1.7 Mechanisms of Crohn's disease: cause and immunobiology

1.1.7.1 Genetic & Environmental factors

Genetic factors play a prominent role in Crohn's Disease, given the familial aggregation and the disease aggregation in twins. The importance of a positive family history was recognized early in 20th century and it remains the largest independent risk factor for developing CD, with relatives being most at risk of developing the same type of disease as their affected family member. For CD patients, the respective prevalence of CD and of IBD in first-degree relatives ranges between 2.2% to 16.2% and between 5.2 to 22.5%.[10] There is a high concordance for disease type, disease pattern, and presence of extraintestinal disease manifestation. The concordance studies which have been done in twins provide the strongest evidence of genetic factors contributing to susceptibility to IBD. The genetic contribution to the development of IBD seems to be more important in CD with a pooled concordance of 37.3% in monozygotic twins and 7% in dizygotic twins, than in UC.[11] Multiple gene products contribute to the risk of CD. Linkage analysis and genome-wide association studies have

identified susceptibility regions on 12 chromosomes.[12] However, no single locus has been reported, as is to be expected given the genetic heterogeneity of CD.

In addition to genetic factors, epidemiological evidence suggests a prominent role of environmental factors such as geographical, temporal, and seasonal variability, and also other life variables. Factors such as different access to health care and a lower standard of medicine might play an important role, as well as different extents of industrialization, sanitation and hygiene. As such, geographical factors which have been implicated can probably be attributed to those environmental factors. A number of factors have been implicated in the risk of developing IBD or in negatively influencing the course of IBD, such as diet and hygiene.[13] Smoking influences CD negatively: the course of CD in smokers is worse, with a higher number of disease exacerbations, and they are at higher risk of developing fistulas and strictures. Smoking cessation is beneficial in CD. Non-steroidal anti-inflammatory drugs can exacerbate IBD. There is currently no unequivocal evidence that infections are causative factors in Crohn's disease, but the impaired handling of microbial antigens by the intestinal immune system certainly plays a role.[13]

Psychological stress has always been suggested as an important contributor to the etiopathogenesis of Crohn's disease. In the past decade, the research into this field has increased significantly and has established that psychological stress plays an important role in influencing CD symptoms and the course of the disease. This dissertation aims to contribute to this field of research and the possible interactions between CD and psychological factors will be discussed.

1.1.7.2 Immunobiology of inflammation in Crohn's disease

1.1.7.2.1 Normal conditions

In normal conditions, the mucosal immune system is in immunohomeostasis and not in a constant uncontrolled state of inflammation, despite of the high antigen load presented by the largest and most diverse microbiota – both commensal and pathogen – and food-derived antigens. The mucosal surfaces of the gut is intimately associated with a large part of the lymphoid tissue in the human body (approximately 25% of the total lymphoid cells present).[14] A healthy steady-state can be maintained through the interaction between the intestinal microbiome, the intestinal barrier and the intestinal immune system. In IBD, this well controlled balance is disturbed at all levels.

The intestinal microbiome consists of the symbiotic micro-organisms inhabiting the gut which are essential for the development of the intestinal immune system. They are also important for nutrient absorption, mucosal barrier fortification, angiogenesis, etc. In inflammatory bowel disease, intestinal inflammation might be induced by host-microbiome interactions. The positive effect of antibiotics in some patients is an example of the possible role of bacteria in inflammation in IBD. However, no specific pathogen has been confirmed as a causing factor in the development of IBD, whereas microbial antigens that are normally present in the intestinal lumen seem to drive inflammation in the intestine. It is also unknown if the changes of the microbiome observed in IBD patients contribute to the disease or are secondary to the intestinal changes caused by the inflammation.[15]

The epithelium of the intestine is the first line of defense against the external milieu and constitutes a physical barrier against excessive entry of bacteria and other antigens from the intestinal lumen into the circulation. Mucus covers the epithelial cells and defects in mucus production in patients with CD have been reported, thereby increasing the contact between bacteria and the epithelium.[13] Additionally, antimicrobial peptides such as α-defensins, are secreted by Paneth cells, specialized epithelial cells, in the small intestine. The intercellular junctions help to seal the space between adjacent epithelial cells and the tight junctions (comprising occluding and claudin proteins and controlling the paracellular route of fluxes through the intestinal epithelium) are essential to the seal. These elements form an intact mucosal barrier. However, in IBD the permeability of the paracellular space has increased and a defect in the regulation of tight junctions has been demonstrated. It remains unclear if these defects are primary or if they are the consequence of the inflammation.[13,15]

The mucosal immune system of the intestine consists of a complex network of immune cell populations and humoral factors which are responsible for balancing sufficient immune tolerance of the intestinal microbiome with the defense against pathogens and/or the invasion of excessive symbiotic micro-organisms. Luminal antigens interact with antigen-presenting cells which regulate the different immune cells and secreted cytokines in the lamina propria. In the absence of inflammation, the response of both the adaptive immune response and the innate immune response is controlled through the cytokine network. Anti-inflammatory mediators such as interleukin-10 that down-regulate immune responses are present. The regulation of the adaptive immune response through proinflammatory cytokines results in the balanced differentiation of naive T cells into effector T cells (Th1, Th2, Th17) to defend against pathogens and regulatory T cells (Tr). Similarly, the regulation of the innate immune response results in the activation of e.g. natural killer cells. This balance between effector and regulatory immune cells maintained by the streamlined cytokine network is

essential for the immunohomeostasis. Furthermore, the additional release of inflammatory cytokines and chemoattractants is prevented. This tight regulation of the initial immune response to intestinal microbiotica is essential to maintain immune tolerance and to prevent a defensive inflammatory response.

1.1.7.2.2 Crohn's disease

In inflammatory bowel disease and in CD in particular, this immunohomeostasis is upset at all levels. The physical defense of the mucus layer is disrupted and the intestinal permeability is increased because of a leaky barrier. Consequently, the luminal bacteria and antigens penetrate into the mucosa where they come into contact with the cells of the innate and adaptive immune system which express a different profile and number of molecular pattern-recognition receptors, such as toll-like receptors (TLR) and nucleotide oligomerization domain (NOD) proteins. An inadequate inflammatory response ensues as a result of the false recognition of commensals as pathogens by the antigen presenting cells of the innate immune system, which produce increased levels of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12, IL-23 and other chemokines. Furthermore, immune activation promotes the differentiation of T cells in effector T cells (in Crohn's disease Th1 > Th2 and Th17) and natural killer (NK) T cells) and these effector T cells predominate over regulatory T cells (Th3 and Tr). These high numbers of activated effector T cells cause a marked expansion of the lamina propria and secrete increased levels of proinflammatory cytokines and chemokines, which in turn induces recruitment of additional leukocytes from the mucosal vasculature. These inflammatory cells will amplify and perpetuate the cycle of intestinal inflammation.

From a bottom up point of view, some evidence has surfaced for an interaction between the enteric nervous system, which is part of the brain-gut axis (to be discussed later) and the immune system. The reciprocal communication between both systems might be through direct interaction between cells or through cytokine signaling with substance P, serotonine, vanilloids, etc. Through this intricate way of communication, the brain-gut axis might influence or contribute to the course of intestinal inflammation and motility disturbance in Crohn's disease.

1.1.7.2.3 Pathways to inflammation

A defective mucosal immune system in IBD will respond defectively to the microbiome and other luminal antigens, probably through several pathways which results in inflammatory cascades. These pathways are not mutually exclusive. In Crohn's disease, a number of

initiating events have been hypothesized which can upset the immunohomeostasis of the intestine. First, the epithelial barrier is functioning suboptimally due to lowered epithelial resistance and increased permeability of the inflamed and non-inflamed mucosa in CD.[16] Second, CD patients have disturbed innate immune mechanisms of the epithelial layer, with a different pattern expression of toll like receptors.[13] Third, evidence suggests that professional antigen-presenting cells such as dendritic cells, do not adequately recognize or process antigens, leading to a high number of activated dendritic cells in the inflamed mucosa of patients with active CD. Fourth, atypical antigen-presenting cells, such as epithelial cells become potent effect-T-cell activators in people with IBD. Fifth, the clearance of activated T-cell populations is disturbed in CD. Sixth, the naive Th0 cells preferably differentiate into Th1 cells, disturbing the balance of regulatory and effector T cells (Th1 and Th2), which predominate in active CD. Finally, increasing evidence points to a probable role of psychosocial stress which might trigger or augment the inflammatory cascade through neuroimmunological interaction.[13]

1.2 PSYCHOSOCIAL FACTORS IN CROHN'S DISEASE

1.2.1 Psychogastroenterology in Crohn's disease – an evolving model

Gastroenterological symptoms and gastrointestinal disorders on the one hand, and psychosocial factors and stress on the other hand, have always been associated throughout history. More specifically, IBD has long been considered as a **psychosomatic illness** by the medical community. Franz Alexander classified UC among his 7 classical psychosomatic illnesses.[17] At that time, the main focus was on the psychogenic hypothesis or the role of depth-psychological factors (preceding or aggravating) in the pathogenesis of physical diseases while trying to develop a psychoanalytical treatment for UC and CD patients. The psychosomatic hypothesis was largely abandoned, with the development of adequate diagnostic tools and the discovery of effective medical treatment, such as corticosteroids. Psychosocial influences were subsequently discounted as causes of the disease or as modifiers of the disease course by scientific research, in favor of a rigorous **biomedical model**. The latter model implicitly propagated a strict mind-body separation in IBD, most likely as a reaction to the disproven theories on psychogenesis. Regardless, a majority of patients with IBD still believe that psychosocial factors, stress or their personality might have contributed to the development of their disease and more than 90% is convinced these factors influence the course or severity of their disease. According to clinicians, psychosocial factors play an important role in the exacerbation of clinical symptoms.[18,19]

To general practitioners and medical specialists, it often seems logical that chronic conditions such as IBD, make patients prone to "stress" and psychological problems. The severe symptoms and consequences of the disease, combined with the impact on the daily functioning and quality of life of the patient, will invariably cause psychosocial difficulties during flares. Moreover, the unpredictability of IBD often leads to a feeling of loss of control and a marked increase in subjective distress. Thus, the association between chronic gastroenterological conditions and psychosocial difficulties should be easily explained and a causal directionality is routinely presumed in daily clinical practice. However, such a causal relationship has never been shown unequivocally in research.[20] Furthermore, there are often important discrepancies between the "objective disease" activity on the one hand and the severity of the illness experienced by the patient and the patient's behavior on the other hand. Additional factors, such as psychosocial modulators must be considered to explain the patient's health status completely.[21]

George Engel, a psychoanalyst and internist who specialized in IBD, emphasized that "*all three levels, biological, psychological and social must be taken into account in every health care task*".[22] He proposed the **biopsychosocial model,** a more appropriate and accurate model, which encompasses the complex, reciprocal biological and psychosocial interactions between several subsystems and determinants at multiple levels, thus explaining the IBD illness and its effects on the patient. The biopsychosocial model abandons the unidirectional cause and effect view of the psychosomatic and biomedical models, in favor of the bidirectional nature of the interaction, illustrated in Figure 1.

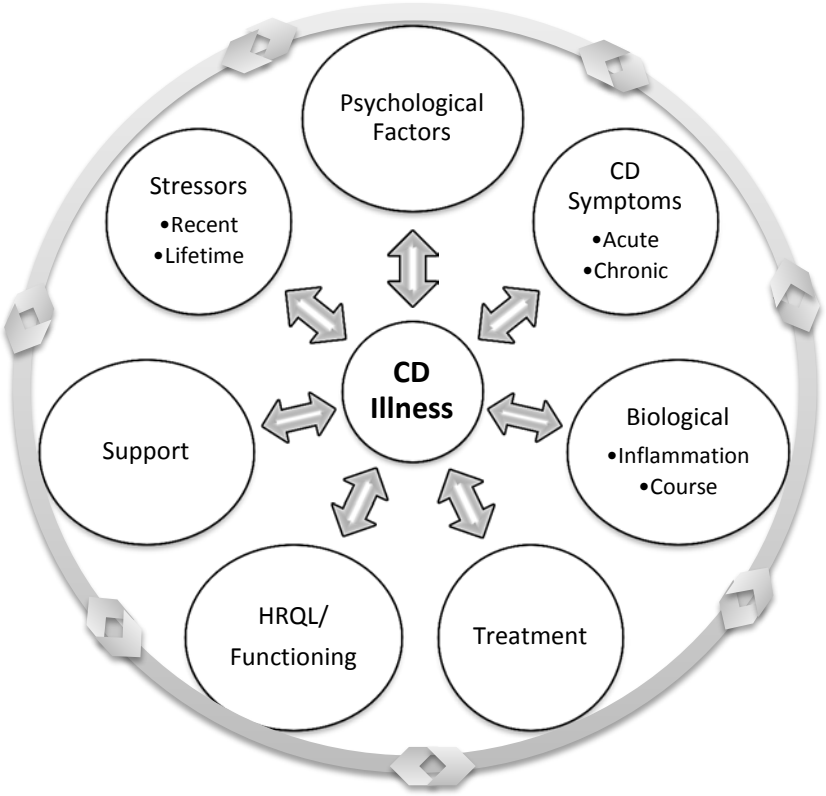

Figure 1. The illness in Crohn's disease is determined by multiple factors which are also interrelated (represented by the outer circle) and this association is bidirectional, indicating that the state of the illness also affects the determining factors (adapted from Drossman & Ringel[69]).

Through the increasing insight provided by the relatively new fields of psychoneuroimmunology, neuroendocrinology and brain-gut interactions, the biopsychosocial model has been provided with a concrete framework of possible pathways through which these bidirectional interactions might be mediated in patients with CD. The "brain-gut-axis", the bidirectional communication system between the intestine and the brain, is of particular interest when discussing the association between psychosocial factors and Crohn's disease. It is one of the core subsystems through which biopsychosocial effects are mediated by autonomic (autonomic nervous system, ANS), neuroendocrine and neuroimmune pathways. Several studies have demonstrated the involvement of the brain-gut-axis in the stress-related modulation of inflammation in CD.[23,24]

1.2.2 Stress in Crohn's Disease

1.2.2.1 The conceptualization of Stress and its importance in CD

First and foremost it is crucial to conceptualize stress as accurately as possible, since conceptual problems are at the basis of the methodological issues in research into the field of stress and psychosocial factors in IBD. Because the definition of stress is often unclear, it can be anything to anyone and by extension, this could lead to the impression that stress can contribute to any disease. When studying the link between the disease course of CD and psychosocial factors, it is necessary to define and to choose what to measure within the stress concept. Differences in concepts, definitions or measures of stress are also one of the important reasons for the heterogenic results between the different studies assessing the association between stress and inflammation in CD and it remains an unanswered question which measure of stress is most appropriate.

If stress is conceptualized as a three-level cascade,[25] stress is initiated by one or more **stressors**, described as an "*event or events that are interpreted as threatening to an individual's or system's homeostasis and which elicit physiological and behavioral responses*".[26] If stressors are measured, it is very difficult to compare the impact among the different levels of stressors, such as daily hassles, which are qualitatively and quantitatively different from major life events.[25] The *"stressor-based" approach* tries to determine which types of events trigger an adaptive response and hypothesizes that an event requiring more effort to cope, is also more stressful.[27] A number of life event studies have been done in IBD.[28–31] However, the subjective appreciation of the event is not taken into account, which is a major disadvantage for this approach and which might explain the conflicting results of these studies.

The way stress is perceived (**stress perception**; distress) is the second level and can be conceptualized as the feelings elicited by the stressors and the coping strategies that are triggered.[25,32] In research, these concepts are often measured as separate constructs, such as psychological symptoms (e.g. depressive or anxiety symptoms), coping strategies or social support.[33,34] For this doctoral project, such a *"perception-based" approach* was mainly used, by using the **construct of depression**.

The **stress response** to stressors is the third level and comprises the biopsychosocial reactions regulated by the stress response system to maintain homeostasis. A *"response-based" approach* as originally described by Hans Selye, proposes that any sufficient stimulus (i.e. "stressor", positive or negative) will elicit a non-specific, physiological response (i.e. the

fight-flight response). The measurement of the stress response, such as heart rate variability or skin conductance, and its correlation with inflammation or disease course in CD would require an experimental stress provocation or continual physiological monitoring and mutual assessment of the stressors. These responses to the stressor, such as physiological changes, emotions, behavior and symptoms than can be measured often confound stress with the subjective feeling of the stress response, thus precluding a clear distinction between cause and effect. This is a major disadvantage of the "response-based" approach.

1.2.2.2 The pathways and mechanisms mediating the effects of stress in Crohn's disease

Recent research has strongly supported the hypothesis that psychosocial stress interacts with the course and the symptoms of CD as a triggering and exacerbating factor.[25,27,32,35] The exact underlying mechanism by which psychosocial stress can influence the course of CD, or possibly even its onset, has not yet been determined and there is most likely not a single, unidirectional pathway responsible, but rather a complex intricately, interconnected network in which the brain-gut axis plays a pivotal role.

1.2.2.2.1 The brain-gut axis: mediating gateway of the effects of stress in CD

A stressor is an environmental signal or factor which will be perceived by the central nervous system (CNS), which will activate the stress response pathway, governed by a network of interconnected limbic regions (e.g. hypothalamus, amygdala, hippocampus) which receive input from visceral and somatic afferents, and from higher cortical structures. The hormonal and neuroendocrine stress response is then regulated via the hypothalamic-pituitary-adrenergic (HPA) axis and the autonomic nerve system (ANS). Through the HPA-axis, stress will initiate the cascade (production of corticotropin-releasing factor or CRF by the hypothalamus, promoting the release of adrenocorticotropic hormone or ACTH from the anterior pituitary gland), which will lead to cortisol secretion from the adrenal cortex. Stress activates the descending neural pathways from the hypothalamus, stimulating the sympathetic nervous system and causing the release of adrenaline and noradrenaline. Both the sympathetic and parasympathetic ANS provide reciprocal communication through afferent and efferent neurons with the Enteric Nerve System (ENS), which contains 100 million neurons and regulates essential functions such as motility and endocrine functions. The brain-gut axis comprises of the richly innervated ANS-ENS plexus and some authors have also implicated elements of the neuroendocrine (HPA-axis) and neuroimmune pathways in this network.[36–38] The brain-gut axis is the communication gateway, responsible

for the bidirectional exchange of information between the CNS and the intestine and it is part of a larger system through which the inflammation might be modulated. Figure 2 illustrates the pathways mediating the effects of stress on the gastrointestinal tract.

The underlying mechanisms by which psychological stress or one of its conceptualizations, such as MDD, can affect the course of IBD, and/or systemic and intestinal inflammation are not fully understood. Moreover, the effects of stress are most likely the result of an intricate, reciprocal and subtle interaction of these multiple factors, which contribute to disrupt the homeostasis. Evidence from recent research proposes a role for several mechanisms through which stress probably plays a role.

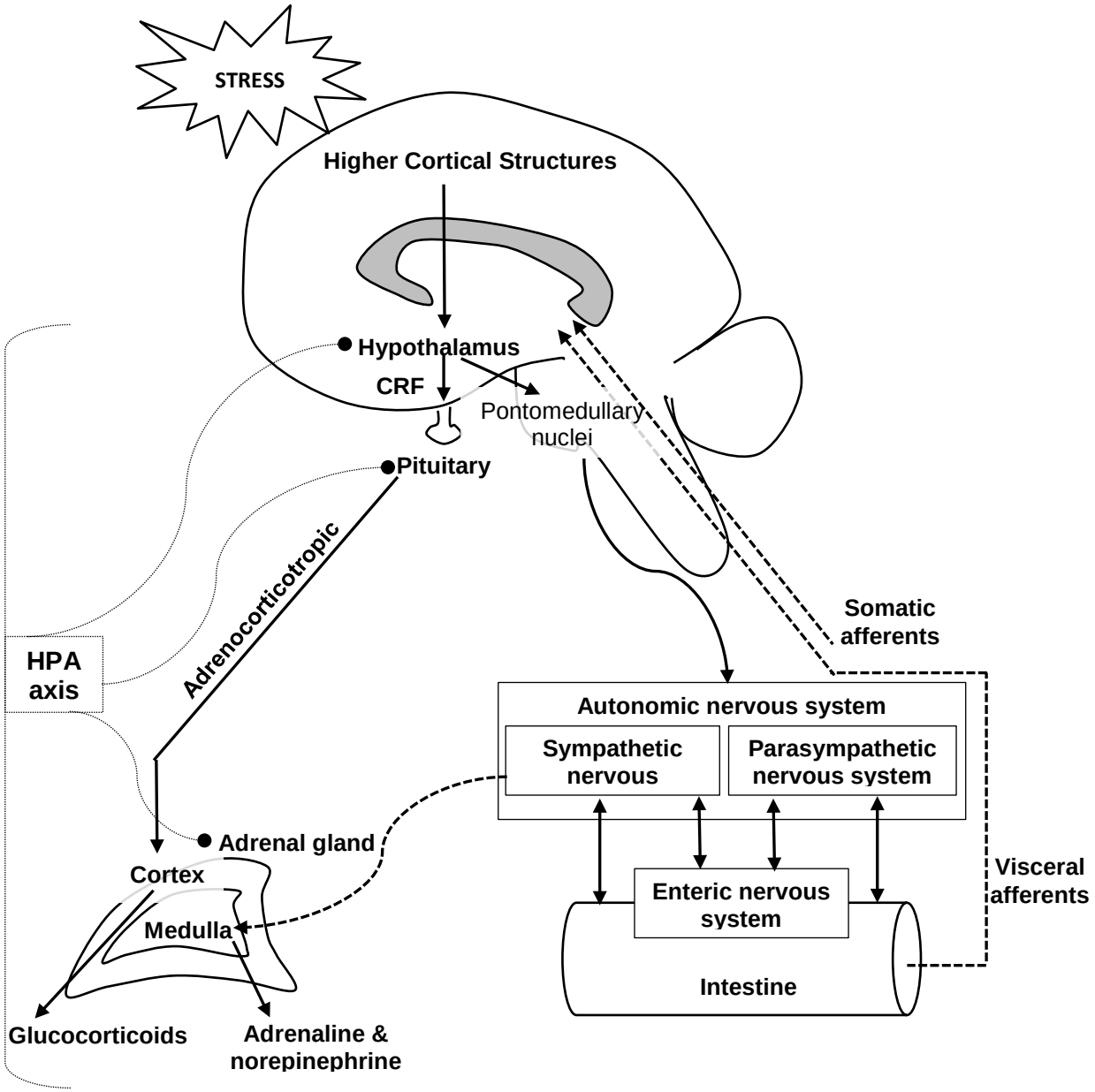

***Figure 2**. The effects of stress on CD: pathways modulating the effects of stress on the intestine. Adapted from Mawdsley & Rampton, 2005*[37]

1.2.2.2.2 Hypothesized mechanisms of stress mediation in CD

Psychological stress and its neuroendocrine effects might modulate gastrointestinal (GI) symptoms in CD patients. Functional gastrointestinal disorders are common in patients with IBD and the GI motor, sensory and secretory function can be influenced through the brain-gut axis.[39] Furthermore, pain processing might also be altered with lowered pain thresholds in situations of chronic psychological stress.

Psychoneuroimmunological mechanisms might play a role. As explained previously, inflammation in IBD results from a malfunctioning intestinal immune system which leads to inappropriate cross-reactivity against host epithelial cells. Evidence from recent research on the stress response system supports the notion that a bidirectional interaction exists between the HPA-axis, the ANS and the ENS on the one hand, and the immune system (systemic and local) on the other hand through direct innervation, neurotransmitters and hormones.[37,38] Psychosocial stress might mediate its effects on the inflammatory cascade through neuroimmunological interaction.[13,24] The ANS forms close connections with immunological cells distributed in the body and in the intestinal mucosa. Additionally, a variety of neurotransmitters and neuropeptides in the neurons of the ANS and ENS can interact and will affect inflammatory cells at the neuro-immune cell junction. The hormones and neuropeptides of the HPA-axis can also influence inflammatory cells through the presence of receptors. Both chronic and acute psychological stress leads to complex effects on the systemic immune and inflammatory system which may be relevant to the pathogenesis of CD. Research has demonstrated stress-induced changes in pro-inflammatory cytokines known to be important in the pathophysiology of CD, such as TNF-α, IL-6, IL-10, or hormones such as cortisol (for full review see[24,37]). Psychological stress will also affect gastrointestinal immune and inflammatory function. Although the topic of animal models of IBD is beyond the scope of this dissertation, they have demonstrated the importance of environmental stress in the initiation and reactivation of gastrointestinal inflammation.[24,36,37]

Stress might **modify the epithelial barrier function and alter intestinal permeability** in CD patients. The suboptimal functioning of the epithelial barrier with lowered epithelial resistance due to increased overall permeability in CD is most likely an important factor in the disease onset and course. Alterations in both the mast cell function and the cholinergic ANS increase the susceptibility to changed intestinal barrier function due to stress.[36,40] Although the influence of stress on intestinal permeability has primarily been researched in animals, which makes it difficult to extrapolate the results to humans, the effect is probably important for the pathophysiology of human CD.

Indirect effects of stress could also influence the course and the symptoms of CD. Psychological stress can also have behavioral effects on CD patients which can influence the course of the disease, such as change in diet, non-compliance to medication and smoking.[41]

1.2.3 Role of stress in the development and clinical course of CD: methodological difficulties with the epidemiological evidence

Psychosocial factors and stress are systematically designated as important "*environmental factors which influence the pathophysiology of CD*" in recent review papers in major biomedical journals,.[13,15] This formulation ignores the inherent bidirectional nature of the hypothesized interaction between stress and CD Furthermore, as discussed previously, plausible evidence for possible mechanisms by which stress could influence the pathophysiology of CD has been provided.[42–45] There is also increasing epidemiological evidence, albeit with the inevitable controversy, that stress plays is a contributing factor to the disease course and the development of CD.[27,32,37,38,41]

Methodological difficulties in this field lead to conflicting results and studies testing the hypothesis of association between stress and CD are hard to design and conduct. It is a challenge to study this association because of the **complexity of the conceptualization of stress and determining which aspect should be measured**. This has been discussed earlier in this introduction.

It would be a methodological challenge to study the **possible association between stress and the onset of Crohn's disease**. Preferentially, a prospective study design, in a predisposed population (e.g. first degree relatives of CD patients), over a long period of time with a large number of participants is necessary to have enough stressful events. A high degree of participant compliance is necessary for keeping diaries with daily hassles, life events and symptoms. Until now only few studies have addressed this topic, with mixed results. No true prospective studies have been published and one large, controlled retrospective study with prospective characteristics based on the Danish national register, found no significant association between the loss of a child and the onset rate of IBD afterwards.[46] Another controlled, retrospective study did find significantly more life events in CD patients, but not in ulcerative colitis patients, 6 months before the onset of the illness.[28] The variables which are related to the onset of the disease may differ considerably from those involved in the relapse or aggravation of inflammation in CD. A study of the association between psychological stress and other psychosocial variables on the one hand, and the onset of CD on the other hand, was beyond the scope of this doctoral project.

Most epidemiological evidence of an association between IBD/CD and stress, is derived from studies investigating **the association between stress and the course of the disease, or alternatively between stress and symptoms accompanying IBD/CD**. This was also the starting point for the approach investigating the possible modulation of the course and symptoms of CD by psychosocial factors in this doctoral project (see aims in chapter 2). There is consistent evidence in favor of a contribution of stress or its conceptualizations, to the course of IBD/CD, albeit with considerable heterogeneity in the results, due to methodological limitations which have been taken into consideration at the start of this doctoral project. It should be noted, however, that part of the research was published after the termination of the experimental phase.

A first methodological issue has been the **use of mixed patient samples**, including both UC and CD patients. The role of stress might differ significantly between UC and CD, due to the heterogeneity of the diagnoses and an underestimation of effect size is plausible.[32] Therefore we decided to narrow the focus of this doctoral project to 1 patient group, i.e. CD. CD should be divided further into phenotypic groups, since this is relevant for the disease course.[5,25]

Only a prospective **study design** is an appropriate methodology for answering questions about the association between stress and the course of CD. Since the inherent bidirectional nature of the interaction, the severity of CD can induce a stress response with a psychosocial reaction. A cross-sectional design would be unable to determine any directionality. In a prospective design, patients should not only be followed up for a time period which is sufficiently long enough, but ideally retrospective information on previous disease activity should also be considered.[25]

An additional methodological problem in this type of studies is the **disease activity**. In CD, the CDAI is used most often as a standardized measure of disease activity, but this scale is based on a symptom diary and subjective well-being is an important item. Conversely, it is difficult to use “objective measures” of inflammation or relapse in CD, such as endoscopy or blood samples. Thus, a bias might be introduced and the stress perception and symptom perception might be confounded.

1.3 "GUT FEELING" IN CROHN'S DISEASE: STRESS CONCEPTUALIZED AS PSYCHOLOGICAL FACTORS & CONTROVERSIES

In IBD research, stress is often conceptualized as "stress perception" and thus defined and measured as psychological disorders, factors or constructs, such as depression. Given the suggested association between CD and depression, this is particularly relevant.[32] There are however a number of controversies surrounding this suggested association. For the introduction of this dissertation, the further focus will be on the psychological symptoms and psychiatric disorders, more specifically on mood/depression, and their pertinence for this doctoral project, as the conceptualization of stress perception, rather than on "stress" per se.

Due to the unpredictable nature of the disease and its chronicity and severity, it is often expected that CD patients are prone to develop so called "reactive" psychological symptoms such as depressive and anxious feelings. However, the prevalence of "*psychological disorders*", such as "*depression*", appears to be higher in patients with IBD in general and in CD patients in particular in comparison to the general population.[47] In the literature, the commonly cited prevalence rates of "*depression*" are approximately 30% in IBD during remission and up to 60% for "*depression*" during relapse.[47–51] However, it remains unclear what these prevalence rates exactly indicate since the conceptualization of "*psychological disorders*" and "*depression*" differ between studies. The term "*psychological disorders"* can either refer to categorical psychiatric disorders or to the intensity or severity of psychological symptoms or dimensions. Thus, investigators may study different constructs, related to the various research questions, and different studies are therefore difficult to compare. It is not known which specific construct of these differently approached "*psychological disorders*" is most relevant to CD and inflammation.[32] The lack of clear conceptualization of this vague concept of "*psychological disorders*" in CD leads again to a heterogeneity in study results and should therefore be avoided. Furthermore, the study of "*the association between psychological symptoms/psychiatric disorders and CD*" has been approached in different ways (e.g. a simple correlation versus the modulation of the disease course). These factors are at the basis of many of the current controversies surrounding psychological and psychiatric factors in CD.

1.3.1 Psychiatric disorders/Psychological symptoms - depression and the association with CD: concepts & controversies

Concepts such as "psychological disorders", "psychiatric disorders", "psychological symptoms", "psychological issues", "mood disorders" and "depression" are often used

interchangeably in the literature, without a clear delineation of their meaning. The exact definition of the psychological construct which is studied, often depends on the aim of the study or is determined by the measurement instrument which is used. A possible way to conceptualize psychological factors and more specifically categorical disorders, such as depression, would be as a **formal psychiatric disorder** according to the Diagnostical and Statistical Manual. Depression is then defined according to specific criteria. Ideally, this requires a structured, psychiatric interview to establish a firm current and/or lifetime diagnosis and this methodology has been used in patients with IBD.[49,52–57] For this doctoral project, a self-report questionnaire, the PRIME-MD Patient Health Questionnaire has been used as an alternative.[58]

In most studies in IBD, psychological factors are conceptualized as **symptom dimensions**, such as "mood" or "depressive symptoms". Here the analysis is aimed at the dimensional assessment of the degree of intensity of various psychological dimensions. Although such continuous measures do not provide categorical diagnoses, prevalence data are almost always reported on the basis of certain cutoff values. Thus, the concept of these categorical data, such as the diagnosis of "depression", can differ significantly between studies and depend on many different determinants (type of questionnaire, cutoff, interpretation by the investigator etc.).[47] Therefore, the results from different studies are often very heterogenic.

The "*association between psychological factors and CD"* has been approached in two different methodological ways. Firstly, the association has been studied as the **co-occurrence of a psychiatric disorder in CD**. A number of studies found an association between CD and a high prevalence of formal psychiatric disorders.[49,53,54] A high rate of co-occurrence was confirmed in studies which used self-report questionnaires and reported prevalence data on the cutoff value of the symptom dimensions (e.g. the Hospital Anxiety and Depression Scale).[47,59] North & Alpers concluded in their review that CD seemed to be associated with a higher lifetime burden of psychiatric disorders than UC.[50] Although these results suggest a specific association of CD with both concepts of psychiatric disorders and a high intensity of psychological symptoms, a firm conclusion cannot be drawn, because of remaining methodological problems, such as the use of inadequate control groups and weak study design.[47] It has been hypothesized that the difference between CD and UC might be due to the worse disease severity in CD.[59] It is also unclear if psychiatric disorders are more prevalent in CD patients than in patients with other chronic medical conditions. Although research has found lower or similar prevalence rates of depression and anxiety of approximately 30%,[49,53,60,61] other studies have reported higher rates.[48,55,59,62] Most likely, psychological symptoms or psychiatric disorders are at least equally prevalent in CD as in

other chronic medical conditions. In general, it is often unclear if the psychological symptoms or psychiatric disorders have adequately been assessed since no disease-specific instruments to measure psychological symptom dimensions or psychiatric disorders exist for IBD/CD. The currently used questionnaires (and their cutoff values) have not been validated and often contain items which might reflect the clinical disease activity of CD (e.g. sleep disturbance, fatigue, appetite). Finally, a high prevalence of psychological/psychiatric comorbidities alone is not enough evidence for their role in the cause and/or in the course of the disease.

The "*association between psychological factors and CD"* has been also studied as the **influence of psychological symptoms or psychiatric disorders on the pathophysiology of CD**, which is related to the research into the association between stress and CD. Here, the psychological factor is often an operationalization of the concept of stress by measuring symptom dimensions, e.g. depressive symptoms with the use of a cutoff to determine a "*psychological disorder*". Nevertheless, these studies often also report prevalence data, although this was not the aim of the study and they often do not include a control group.[47,51] Therefore, it is hard to say anything about the co-occurrence of psychological symptoms in CD from the results of these studies. However, the influence of a formal psychiatric disorder, such as a Major Depressive Disorder (MDD), could also be investigated as a construct in its own right, related to the course or symptoms of CD. MDD may have a direct influence on immune deregulation associated with Crohn's disease. Several studies have shown that MDD is associated with enhanced production of cytokines, such as IL-6 and TNF-α, and successful pharmacological treatment of MDD decreases the elevated cytokine levels.[63] MDD might also have an indirect influence through adverse health behaviors, such as smoking and inappropriate diet, or through noncompliance to self-care regimens. Additionally, IBD patients with a comorbid psychiatric disorder present with significantly more medically unexplained symptoms and have an altered perception of disease severity.[57] The possible influence of psychological symptoms or psychiatric disorders on the severity of symptoms contributes to the ongoing controversy since the activity index of CD, the CDAI, relies heavily on subjective items, such as gastrointestinal symptoms and pain, rather than on "objective" indices, such as intestinal inflammation. It also remains unclear if psychological symptoms or psychiatric disorders precede the onset CD, or if they are a consequence of the illness, due to the severity of the disease, the unpredictability of its course and the impact on functioning and quality of life. Furthermore, there is also no definite answer to the question if psychiatric disorders such as depression and anxiety are only worse during relapse of CD and comparable to the general population during episodes of

remission or if CD patients are equally vulnerable during both relapse and remission of the disease.[19,47,64]

1.3.2 Conceptualization of psychological factors throughout this doctoral project: methodological remarks

In order to make a meaningful contribution to a better understanding of the association between psychological factors and CD, it was important to choose beforehand which type of association would be studied, how the psychological factors would be conceptualized and measured and how the outcome would be quantified.

The focus for the association between psychological factors and CD for this doctoral project was on the modulation of disease-related factors, rather than on prevalence rates and the specificity of co-occurrence.

The formal psychiatric diagnosis, MDD was of particular interest because of the hypothesized specific association between CD and co-occurring psychiatric disorders.[50] A significant association between depressive symptoms, but not MDD, and the course of CD had been demonstrated in several studies.[51,65] Conceptually, MDD is also more easily comparable over the boundaries of studies than perceived stress, daily hassles or life events. MDD has well-defined diagnostic criteria for which both pharmacological and psychotherapeutic interventions exist. As such, it is possible to identify patients at risk who could benefit from such a treatment which could positively influence the course of their disease and possibly their symptoms.[66] MDD should be considered as a separate construct and cannot be reduced to a mere conceptualization of perceived stress, although these concepts are certainly correlated.

For this doctoral project the **PRIME MD Patient Health Questionnaire (PHQ)**, the first self-administered instrument to assess **categorical psychiatric disorders** according to the diagnostic criteria of DSM-IV was translated and validated in 2 populations, and subsequently used. We found that the PHQ was a valid and valuable screening and diagnostic instrument for mood and anxiety disorders in otorhinolaryngology (ORL) patients.[58] We also validated the PHQ in Gastroenterology patients (n = 639; IBD: n = 101) as a part of this doctoral project. The results of this validation were presented during the annual meetings of the American Psychiatric Association in Chicago (2000) and of the American Gastroenterological Association (2001).[67,68] The results demonstrated a good criterion validity of the PHQ subscale for Major Depressive Disorder (MDD) (PHQ-9;

sensitivity = 80.0%, specificity = 98.1%, positive predictive value = 88.9%, negative predictive value = 96.2%, and κ = 0.81). The convergent validity of the PHQ-9 in the gastroenterological patients was demonstrated. Thus, the PHQ-9 can be used as a diagnostic instrument for MDD in gastroenterological patients given the satisfying sensitivity, specificity, positive predictive value and negative predictive value, indicative for a good criterion validity with a high kappa-value.

1.4 A PARADIGM SHIFT: THE ROLE OF INFLAMMATION IN PSYCHOLOGICAL SYMPTOMS IN CROHN'S DISEASE

The association between psychological factors and CD should also be approached form a different point of view: does CD play a role in the development of psychological symptoms or psychiatric disorders, such as MDD?

In part, the higher prevalence of depressive and anxiety symptoms than in the healthy population can be attributed to the severe, aggressive disease course of CD, especially during flares. Furthermore, this psychological distress caused by CD can influence in its turn lower pain thresholds, affect disease activity scores and health seeking behavior.[69] This would imply a very strong correlation between the disease activity and psychological factors, which remains controversial in CD and in other chronic, inflammatory diseases.[47,70] Therefore, other mechanisms might play a role and a possible biological mediating factor should be considered.

Inflammation, more specifically proinflammatory cytokines, on the one hand, and psychological symptoms, psychopathology and particularly MDD on the other hand, are closely linked. This is underpinned by the findings in several research fields. First, immunotherapy with recombinant cytokines (e.g. IFN-α) for cancer or viral infection increased the risk of developing MDD in some patients.[71] Second, MDD is associated with the presence of acute phase proteins and cytokines.[72] Finally, increased rates of MDD have been found in inflammatory medical illnesses such as multiple sclerosis, rheumatoid arthritis and psoriasis,[63,70] which has also been suggested for CD.[50]

Several possible mechanisms by which cytokines may induce psychological symptoms in general and MDD in particular have been proposed, such as modulating the activity of the HPA-axis, through direct action on hippocampal neurogenesis, or via modulation of neurotransmission.[63] The latter mechanism was the focus of the last part of this doctoral project, more specifically, the possible impairment of serotonergic neurotransmission through inflammation-induced tryptophan (TRP) catabolism.

TRP is an essential, large, neutral amino-acid (LNAA) which is absorbed from the intestine. TRP is relatively scarce and there are no tissue reserves. TRP is the precursor of serotonin (5-hydroxytryptamine, 5-HT), which is a neurotransmitter in the central nervous system (CNS) with a central role in the current theories on mood regulation, cognition, behavior, and the pathophysiology of depression and anxiety.[73] About 1% of the total TRP is converted to

5-HT, primarily in the intestine. A small proportion of the TRP passes through the blood-brain barrier (BBB) via a non-specific, active, transport protein, in competition with other LNAA's. In the CNS, TRP is hydroxylized into 5-hydroxyTRP, which is decarboxylized into serotonin (5-HT). The hydroxylation is rate-limiting and a decrease in TRP levels also leads to a decrease in 5-HT synthesis and to a change in central 5-HT neurotransmission,[74] and possibly a change in central serotonergic neurotransmission.[75]

In normal conditions, the majority of the absorbed TRP is metabolized. Together with Tryptophan 2,3-dioxygenase (exclusively expressed in hepatocytes), the enzyme indoleamine 2,3 dioxygenase-1 (IDO) catalyzes the rate-limiting step of the oxidative tryptophan (TRP) catabolism along the kynurenine pathway.[70,76] In active, inflammatory Crohn's disease, toll-like receptor activation and pro-inflammatory cytokines, such as interferon-γ (IFN-γ), IFN-α and TNF-α induce the enzyme indoleamine 2,3 dioxygenase-1 (IDO).[77–79] IDO is expressed in the cells (macrophages, monocytes, dendrites) of the innate immune system and can essentially be found in all tissues, including the intestine.[76] IDO has immunoregulatory properties by suppressing T-cell responses of the adaptive immune system, which is highly important for the intestinal immune tolerance.[76,77] Evidence for the activation of IDO after immune activation and its effect on serum TRP concentrations comes from in vitro experiments, experimentally cytokine-treated animals[80,81] and from measurements in patients undergoing cytokine therapy (e.g. in cancer or hepatitis C).[82,83]. A decrease of serum TRP concentrations has also been shown in other inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis and in Alzheimer's disease.[70,84,85] Previous studies have shown increased IDO expression in active CD[86] and its association with significantly decreased TRP levels[77].

In CD therefore, it is possible that the decreased TRP levels in active CD, might contribute to the pathogenesis of psychological symptoms or even psychopathology such as MDD, via impaired 5-HT synthesis and a dysfunctioning central serotonergic neurotransmission, or "serotonergic vulnerability".[87]

1.5 CONCOMITANTS OF CROHN'S DISEASE: THE EFFECT IS MORE THAN THE SIMPLE SUM OF SYMPTOMS

1.5.1 Crohn's Disease and Health Related Quality of Life

Abdominal symptoms, laboratory findings and diagnostic techniques such as radiology and endoscopy can approximate to assess the degree of inflammation and the severity of Crohn's disease as objectively as possible. Thus, a disease activity measurement is achieved which can be used to stratify the patients for optimal management and to determine the outcome of disease or the response to therapy.[88] However, patients will often experience a marked decline in their level of functioning and general well-being, which correlates poorly with the objective parameters of disease activity. To adequately and comprehensively understand the impact of Crohn's disease as a chronic, biopsychosocial illness, it is essential to take additional patient-dependent dimensions and symptoms into account. This warrants the introduction of the concept of **Quality of Life (QoL)**, for which different studies have used a wide array of different definitions (global, component, focused), for example general QoL can be defined *globally* as the "degree of satisfaction or dissatisfaction felt by people with various aspects of their lives"[89] or can be described using a *component* definition, emphasizing the multidimensional nature of the concept by identifying different dimensions, which can be "objective" (e.g. health and functional status or socio-economic status) or "subjective" (e.g. satisfaction, well-being). The functional effect of a medical condition and/or its consequences upon a patient's QoL is more *focused* and can be described as Health Related QoL.[90]

Health related quality of life (HRQL) is a broad, multidimensional concept which encompasses the patient's subjective perceptions of physical and mental health, functional status and illness experience in relation to the medical condition, i.e. Crohn's Disease.[69] HRQL is influenced by several factors, such as disease-related factors, social support, psychological factors, socioeconomic status and functional status. By definition, the measurement of HRQL is dependent of a subjective evaluation by the patient, which is optimally done with a multidimensional scale, either generic or disease-specific. For IBD, a disease-specific, multi-dimensional, self-report measure has been developed and validated, the Inflammatory Bowel Disease Questionnaire (IBDQ,[91]). This scale has also been used in this thesis and a contribution to the validation process of this scale will be discussed. An additional disease specific conceptualization of HRQL in IBD has been proposed by Drossman et al. with the Rating Form of IBD Patient Concerns (RFIPC,[92]), which focuses on the perceived health status of the patient. The different aspects of HRQL in CD, the psychometric properties of the different questionnaires and an overview of the state of the art

of HRQL in IBD are beyond the scope of this dissertation and have been reviewed extensively elsewhere.[88,93]

HRQL is severely impaired in patients with IBD, but not more than in other chronic diseases despite IBD's complications, severe symptoms, possible side effects of medical treatment or surgery and the unpredictable course.[60,69,94–97] As is to be expected, HRQL is poorer in patients with active CD than in patients with inactive CD.[98,99] HRQL is also more impaired in patients with CD than in patients with UC and it has been suggested that this is due to the more severe disease activity in CD than in UC.[61,100] Of note is that the psychosocial dimensions of HRQL are affected to a larger extend than the dimensions related to physical functioning and the impairment of the psychological and social dimensions is worse in CD patients than in patients with UC.[94,101] These findings in the HRQL research in IBD also suggested a relationship between CD and psychosocial factors and provided a further starting point for the hypotheses of this dissertation.

As expected, further research into the determinants of HRQL showed a rather poor correlation with the disease activity and the intensity of the intestinal symptoms. Factors such as gender (women scored lower on HRQL than men) and other sociodemographic variables determined the variability of HRQL. Additionally, psychopathology, such as depression and anxiety have been established predictors of poor HRQL in Crohn's disease, independent of the severity of disease activity,[53,102] and this was also confirmed during the preparatory work for this dissertation.[103] This association between HRQL and psychopathology in CD warranted further research into the reciprocal relationship between CD itself and psychosocial factors.

1.5.2 Crohn's Disease and Subjective Physical Symptoms: Fatigue as a Paradigm

Subjective symptoms, independent of disease activity, which are experienced and reported by CD patients, are an important group of determinants of HRQL. It has been shown that functional gastrointestinal disorders are common in patients with CD and that they are associated with impaired HRQL, independent of disease activity and psychological symptoms or psychiatric disorders.[104] Fatigue is another important symptom which is highly prevalent among CD patients.[105,106] The impact of fatigue on the quality of life in CD patients has also been established.[107,108] Fatigue has consistently emerged as one of the major concerns for IBD patients, even exceeding the concerns about gastrointestinal symptoms and a recent study also showed that chronic fatigue in IBD patients was associated with

increased levels of disease-related worries and concerns.[106,109] Therefore, fatigue was further explored as a symptom for this dissertation. Fatigue is a complex concept which is inherently subjective in nature and therefore difficult to measure to define. Van Langenberg and Gibson described fatigue in chronic diseases as "*a persistent, overwhelming sense of tiredness, weakness or exhaustion resulting in a decreased capacity for physical and/or mental work, which is typically unrelieved by adequate sleep or rest. Furthermore, it can exist as a unique entity and not merely as a component of psychological comorbidity or 'illness behavior'*".[106] Rather than formulating clear criteria for severe fatigue in CD, this description of fatigue encompasses more the exclusion of "normal fatigue", psychopathology and the consequences of inflammation (cytokines) on the general behavior of the patient. Other descriptions of fatigue in chronic diseases will stress the importance of including multiple dimensions in the conceptualization. Typically, these dimensions reflect the domains where fatigue will impact the level of functioning, such as physical dimensions, cognitive dimensions and affective dimensions. These dimensions can be measured with either objective or subjective measurement tools. No fatigue scales have been specifically validated for CD, until now.[110]

Clinically, fatigue is highly relevant for patients and for many physicians it remains a frustrating and enigmatic symptom to treat. The prevalence of fatigue is difficult to assess due to the variable definitions and cutoffs which are used by different research groups. Most IBD patients experience fatigue at some point during their illness and it is evident from clinical experience and research that rarely a clear cause, such as iron deficiency, can be determined.[106] Especially chronic fatigue in IBD can be a difficult to manage problem which is highly prevalent.[111] In order to improve the understanding and the management of this complex problem, it is important to explore the role of the possible underlying mechanisms and determinants in the pathogenesis of fatigue. There is evidence for multiple determinants of fatigue in IBD in general and CD in particular. Fatigue in IBD has been associated with disease-related factors, such as inflammation, disease activity and anemia,[110,112–115] demographic factors, such as gender,[110,114] and psychological factors.[110,112,113] However, the number of studies is limited and most studies are cross-sectional in design, which leaves much work undone and further research is needed to improve the insight in the intricate interaction between fatigue and the different factors. As a physical complaint, fatigue is truly located at the cross-roads of the biological, psychological and social aspects of Crohn's disease, which is reflected in its multidimensional nature. Therefore, the biological and psychosocial determinants of fatigue and the possible inflammatory influence of CD through the modulation of serotonergic neurotransmission in the central nervous system will be explored in this dissertation.

Chapter 2

2. Hypothesis & Objectives

2.1 NARROWING THE FOCUS: FROM IBD TO CROHN'S DISEASE

Originally, the intended study population of this doctoral project were patients with Inflammatory Bowel Disease (IBD). However, ulcerative colitis (UC) and Crohn's disease (CD) are two very different diseases, with a multitude of differences: onset, clinical course, treatment, impact, etc. Furthermore, about 10% of the patients are diagnosed with indeterminate colitis. The heterogeneity of the results from the existing studies on psychosocial factors in IBD exposed the use of mixed patient samples as an important methodological confounder. Due to the heterogeneity of the diagnoses, an underestimation of effect size is plausible.[32] Therefore, Crohn's disease was chosen as the focus of research for this doctoral project. Furthermore, even within CD it is important to take subtle sources of heterogeneity into account, such as different clinical phenotypes, gender differences and genetic differences, as described in the first chapter.

2.2 BASIC HYPOTHESIS

For a thorough understanding of the biopsychosocial nature of Crohn's disease (CD), the association between psychosocial factors and CD illness cannot be reduced to a simple, directional pathway of cause and effect. We therefore hypothesized that **the clinical outcome and disease exacerbation of CD should be approached as influencing and strongly influenced by both psychosocial and biological factors**. Such a bidirectional influence can be achieved through various interfacing pathways or frameworks. The reciprocal nature of the association between psychosocial factors and CD was central as a starting point for the research questions for this doctoral project and we approached the putative interaction from 2 different points of view.

In the **first point of view**, the hypothesis can be approached by assessing if psychosocial factors (independent) modulate (the course of) CD (dependent). As discussed in chapter 1, a notion of the different intestinal factors (e.g. the barrier function, the microbiome) and the peripheral mediators of inflammation is essential in the understanding of the pathogenesis and the course of CD. However, clinical practice and previous research have demonstrated that the phenotypic expression of CD illness is not only driven by these local factors, but is also modulated by a broad array of biopsychosocial variables. Research findings in the field of psychoneuroimmunology have provided an operating framework for the reciprocal interactions between psychosocial factors and intestinal inflammation in this point of view: the brain-gut-axis. The brain and the intestine communicate through this complex, bidirectional system with mutually interacting systems at multiple levels, comprised of the HPA-axis, the autonomic nerve system, the enteric nerve system and elements of the immune system. Emerging animal studies suggested stress-induced modifications through the brain-gut axis of intestinal function and sensitivity, and of intestinal permeability and local inflammatory responses.[115] The findings showed hyperpermeability and activation of mucosal immune function under stress conditions, through the activation of mast cells, the end effectors of the brain-gut axis. Other studies demonstrated the relapse of experimental colitis, further underpinning the putative adverse impact of psychosocial factors on the course of IBD.[116] Additionally, human studies suggested an association between depression and CD and demonstrated an effect of mood on IBD.[50,51,57]

In the **second point of view**, the hypothesis can be approached by assessing if (the course of) CD (independent) modulates psychosocial factors (dependent). Previous research showed that disease activity negatively influences HRQL and psychosocial outcomes in IBD.[117–119] This could be interpreted straightforward: the severity of CD, its chronicity and the disease activity (independent variables) are more than likely an important part of the explanation for the psychosocial consequences (dependent variables): high prevalence of psychological symptoms, such as depressive or anxious feelings and even the high prevalence of psychiatric disorders in CD.

However, the approach we proposed for this doctoral project assumes a paradigm shift and thus an alternative operating framework as the basis for the modulation of psychosocial factors by CD, i.e. inflammation-induced tryptophan catabolism. The overall severity of the illness seems insufficient to explain the higher lifetime burden of formal psychiatric disorders in patients with CD.[50] This suggests that a CD-related factor might play a role. Based on the findings in other inflammatory diseases and on the consequences of the administration of therapeutic cytokines for mood,[71,120] we hypothesized that the decreased availability of tryptophan (TRP, the precursor of serotonin) might be associated with psychosocial

problems in CD, due to inflammation-induced increased oxidative TRP catabolism. There is some evidence of impaired TRP availability in CD[121] and of an increased oxidative TRP catabolism to kynurenine during an exacerbation of CD.[86,122] Furthermore, the majority of patients with CD in clinical remission, still have local intestinal inflammation.[123] CD patients might be prone to psychological symptoms and even psychopathology due to a "serotonergic vulnerability" caused by this increased TRP catabolism.

2.3 OBJECTIVES OF THE DOCTORAL PROJECT

The aim of this doctoral project was to contribute to the understanding of the biopsychosocial model of CD through the investigation of the complex, bidirectional interaction between psychosocial factors on the one hand, and the pathophysiological aspects of CD on the other hand. More specifically, the project can be subdivided into 3 main sections, each with specific objectives, as illustrated in table 1. The starting points and aims of each section and the different studies are detailed in the following subheadings.

Table 1. Overview of the objectives in the 3 sections of the doctoral project

Section	Section Titles	Chapter	Chapter Running Titles
Section 1	Methodological studies	Chapter3	IBDQ Responsiveness & cutoff
		Chapter 4	Validation of the CIS20R
Section 2	Impact of biopsychosocial aspects on CD disease factors	Chapter 5	Effects of depression on the outcome of treatment in CD
Section 3	Impact of CD inflammation on psychosocial factors in CD	Chapter 6	Evolution of TRP after treatment and its effects on HRQL
		Chapter 7	Rapid TRP depletion in CD

2.3.1 Section 1 – Methodological Studies

As discussed in chapter 1, the majority of the heterogeneity in the results and the controversies surrounding the studies on the association between psychosocial factors and CD, are due to methodological problems and conceptual inconsistencies. The methodological studies of the first section reflect the preparatory work which was done in the context of this doctoral project to address some of these issues before starting the planned experiments of sections 2 and 3.

The aim of *the first study of this section, presented in* ***chapter 3****,* was to assess the ability of the Inflammatory Bowel Disease Questionnaire (IBDQ) to reflect rapid changes in the clinical

condition of patients with CD and to propose the remission and relevant clinical response cutoff values for the IBDQ. The IBDQ is a disease specific measure designed to determine the HRQL in patients with IBD and was described in detail in chapter 1. The sensitivity of the IBDQ and its 4 dimensions (bowel function, emotional status, systemic symptoms, and social functioning) to promptly reflect the changes in a patient's condition is a crucial parameter for the assessment of short-term response to therapeutic interventions in CD. Furthermore, if the IBDQ is to be used as an outcome parameter of a certain therapy in clinical trials, the cutoff values for remission and for partial clinical response would be crucial for assessing the effect of the therapeutic intervention. The IBDQ will be used to assess the convergent validity of the Checklist of Individual Strength 20 items (CIS20R) in chapter 4 and as an outcome parameter and dependent variable in the study presented in chapter 6.

Fatigue, although poorly understood, is highly prevalent in CD and should be approached as a multidimensional concept. There was no disease specific instrument which could measure fatigue in CD. Therefore*, the aim of the third study of the first section, presented in* ***chapter 4****,* was to validate the Checklist of Individual Strength (CIS20R) extensively for the measurement of fatigue in CD patients with a multidimensional questionnaire. The CIS20R was initially designed and validated for patients with the Chronic Fatigue Syndrome. The CIS20R has already been used extensively in other patients samples with inflammatory diseases and other medical diagnoses. The aim was to check the underlying dimensions of the questionnaire, its internal consistency, its reliability, its reproducibility and its responsiveness, together with the concurrent and convergent validity. In addition, we aimed to assess the prevalence of fatigue in this sample of CD patients. The CIS20R or a derivative of the scale was subsequently used in the context of this doctoral project (chapter 7).

2.3.2 Section 2 – Impact of psychosocial aspects on disease factors of CD

Despite the controversy of "classic psychosomatic medicine" and the errors of its ways, the majority of the patients with CD and the clinicians remain convinced that psychosocial factors play an important role in the course of the disease and its symptoms.[69] In this second section, the association between psychosocial factors and CD is approached from the "*psyche-somatic*" point of view (i.e. biopsychosocial variables as independent variables and CD-related variables, such as disease course, as dependent variables; this concept is thus not related to the classic definition of psychosomatic medicine, which implied a cause and effect relationship). A study is presented in which biopsychosocial factors are investigated as possible determinants of the course of CD after treatment with infliximab.

The aim of the study of the 2nd section, presented in ***chapter 5****, was* to assess if the presence of MDD in CD patients at the time of treatment affects the short-term effectiveness and the long-term duration of response to infliximab. Moreover we aimed to study whether the usefulness of the instruments we use in drug trials in Crohn's disease, i.e. the CDAI is influenced by the presence of MDD. As a secondary objective we assessed the impact of other biopsychosocial factors, such as anxiety, perceived social support, sleep and alexithymia. Our hypothesis was that biopsychosocial factors might influence the short- and long-term outcome of treatment of active CD. Since therapy needs to be very effective to evaluate the importance of predictive factors, treatment with Infliximab (Remicade®) was used as the experimental condition.

2.3.3 Section 3 – Impact of CD pathophysiology/inflammation on psychosocial factors in CD

In the 3rd section the associations between psychosocial factors and CD is approached from the "*somatic-psyche*" point of view.

The aim of the first study of the 3rd section, presented in ***chapter 6***, was to assess the total and free serum TRP concentrations in patients with a severe exacerbation of CD compared to CD patients in remission and the changes in TRP concentrations induced by treatment with anti-TNF-α (infliximab, Remicade®). Free TRP was assessed separately because this fraction is relevant for central 5-HT neurotransmission, since only free TRP, unbound to protein, will pass the blood brain barrier. Finally, we also studied the relationship between total and free serum TRP concentrations and the quality of life (HRQL) at different time points in patients with Crohn's disease.

The second study of the 3rd section, which is presented in ***chapter 7***, aimed to investigate if the technique of rapid TRP depletion (RTD) can induce a temporary, acute, clinically significant mood reduction in patients with CD. RTD was developed to investigate the response of behavior, mood and cognitive functions in humans to a pharmacological challenge of the central serotonergic neurotransmission. It is a non-invasive way to investigate the vulnerability of the central 5-HT neurotransmission and to assess if a serotonergic vulnerability is present in CD patients. The secondary aim of this study was to assess the effect of RTD on the level of anxiety and fatigue of patients with CD and to investigate if an acute, clinically significant increase of these levels can be induced.

References Chapter 1 & Chapter 2

1. Baron JH. Inflammatory bowel disease up to 1932. *Mt Sinai J Med*. 2000;67:174–189.
2. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. 2012;142:46–54.
3. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet*. 2007;369:1641–1657.
4. Lashner BA. Clinical features, laboratory findings, and course of Crohn's disease. In: Kirsner JB, ed. *Inflammatory Bowel Disease*.Vol 5. Philadelphia, Pennsylvania: W.B. Saunders Company; 2000:305–314.
5. Vermeire S, Assche G Van, Rutgeerts P. Classification of inflammatory bowel disease: the old and the new. *Curr Opin Gastroenterol*. 2012.
6. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis*. 2000;6:8–15.
7. Loftus Jr. E V, Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. *Aliment Pharmacol Ther*. 2002;16:51–60.
8. Cosnes J, Cattan S, Blain A, et al. Long-term evolution of disease behavior of Crohn's disease. *Inflamm Bowel Dis*. 2002;8:244–250.
9. Jess T, Winther K V, Munkholm P, et al. Mortality and causes of death in Crohn's disease: follow-up of a population-based cohort in Copenhagen County, Denmark. *Gastroenterology*. 2002;122:1808–1814.
10. Russell RK, Satsangi J. Does IBD run in families? *Inflamm Bowel Dis*. 2008;14 Suppl 2:S20–1.
11. Gaya DR, Russell RK, Nimmo ER, et al. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet*. 2006;367:1271–1284.
12. Limbergen J Van, Russell RK, Nimmo ER, et al. The genetics of inflammatory bowel disease. *Am J Gastroenterol*. 2007;102:2820–2831.
13. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet*. 2007;369:1627–1640.
14. Elson CO. The Immunology of Inflammatory Bowel Disease. In: Kirnser JB, ed. *Inflamm Bowel Dis*.Vol 5. W.B. Saunders; 2000:208–239.
15. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med*. 2009;361:2066–2078.
16. Soderholm JD, Olaison G, Peterson KH, et al. Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. *Gut*. 2002;50:307–313.
17. Alexander F. *Psychosomatic Medicine: Its Principles and Applications.* Scranton, Pennsylvania: W.W.Norton & Co.Inc. 1950.
18. Lewis MC. Attributions and inflammatory bowel disease: patients' perceptions of illness causes and the effects of these perceptions on relationships. *AARN News Lett*. 1988;44:16–17.
19. Robertson DA, Ray J, Diamond I, et al. Personality profile and affective state of patients with inflammatory bowel disease. *Gut*. 1989;30:623–626.
20. Ringel Y, Drossman DA. Psychosocial aspects of Crohn's disease. *Surg Clin North Am*. 2001;81:231–52, x.
21. Garrett JW, Drossman DA. Health status in inflammatory bowel disease. Biological and behavioral considerations. *Gastroenterology*. 1990;99:90–96.
22. Engel GL. The biopsychosocial model and the education of health professionals. *Ann NY Acad Sci*. 1978;310:169–87.
23. Kiank C, Tache Y, Larauche M, et al. Stress-related modulation of inflammation in experimental models of bowel disease and post-infectious irritable bowel syndrome: role of corticotropin-releasing factor receptors. *Brain Behav Immun*. 2010;24:41–48.
24. Mawdsley JE, Rampton DS. The role of psychological stress in inflammatory bowel disease. *Neuroimmunomodulation*. 2006;13:327–36.
25. Camara RJ, Ziegler R, Begre S, et al. The role of psychological stress in inflammatory bowel disease: quality assessment of methods of 18 prospective studies and suggestions for future research. *Digestion*. 2009;80:129–139.
26. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res*. 2000;886:172–189.
27. Keefer L, Keshavarzian A, Mutlu E. Reconsidering the methodology of "stress" research in inflammatory bowel disease. *J Crohns Colitis*. 2008;2:193–201.
28. Lerebours E, Gower-Rousseau C, Merle V, et al. Stressful life events as a risk factor for inflammatory bowel disease onset: A population-based case-control study. *Am J Gastroenterol*. 2007;102:122–131.
29. Wietersheim J von, Kohler T, Feiereis H. Relapse-precipitating life events and feelings in patients with inflammatory bowel disease. *Psychother Psychosom*. 1992;58:103–112.
30. Vidal A, Gómez-Gil E, Sans M, et al. Life events and inflammatory bowel disease relapse: a prospective study of patients enrolled in remission. *Am J Gastroenterol*. 2006;101:775–81.
31. Wietersheim J von, Overbeck A, Kiel K, et al. [The significance of recurrence-inducing events for patients with chronic inflammatory bowel diseases. Results of a prospective longitudinal study over three years]. *Psychother Psychosom Med Psychol*. 1994;44:58–64.

32. Maunder RG, Levenstein S. The role of stress in the development and clinical course of inflammatory bowel disease: epidemiological evidence. *Curr Mol Med*. 2008;8:247–52.
33. Bitton A, Dobkin PL, Edwardes MD, et al. Predicting relapse in Crohn's disease: a biopsychosocial model. *Gut*. 2008;57:1386–1392.
34. Camara RJ, Lukas PS, Begre S, et al. Effects of social support on the clinical course of Crohn's disease. *Inflamm Bowel Dis*. 2011;17:1277–1286.
35. Hisamatsu T, Inoue N, Yajima T, et al. Psychological aspects of inflammatory bowel disease. *J Gastroenterol*. 2007;42 Suppl 1:34–40.
36. Hollander D. Inflammatory bowel diseases and brain-gut axis. *J Physiol Pharmacol*. 2003;54 Suppl 4:183–190.
37. Mawdsley JE, Rampton DS. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. *Gut*. 2005;54:1481–1491.
38. Rampton DS. The influence of stress on the development and severity of immune-mediated diseases. *J Rheumatol Suppl*. 2011;88:43–47.
39. Mikocka-Walus AA, Turnbull DA, Andrews JM, et al. The effect of functional gastrointestinal disorders on psychological comorbidity and quality of life in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2008;28:475–83.
40. Soderholm JD, Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G7–G13.
41. Sajadinejad MS, Asgari K, Molavi H, et al. Psychological issues in inflammatory bowel disease: an overview. *Gastroenterol Res Pract*. 2012;2012:106502.
42. Mawdsley JE, Macey MG, Feakins RM, et al. The effect of acute psychologic stress on systemic and rectal mucosal measures of inflammation in ulcerative colitis. *Gastroenterology*. 2006;131:410–419.
43. Collins SM. Stress and the Gastrointestinal Tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G315–G318.
44. Farhadi A, Keshavarzian A, Kar LD Van de, et al. Heightened responses to stressors in patients with inflammatory bowel disease. *Am J Gastroenterol*. 2005;100:1796–1804.
45. Kuroki T, Ohta A, Aoki Y, et al. Stress maladjustment in the pathoetiology of ulcerative colitis. *J Gastroenterol*. 2007;42:522–527.
46. Li J, Norgard B, Precht DH, et al. Psychological stress and inflammatory bowel disease: a follow-up study in parents who lost a child in Denmark. *Am J Gastroenterol*. 2004;99:1129–1133.
47. Mikocka-Walus AA, Turnbull DA, Moulding NT, et al. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflamm Bowel Dis*. 2007;13:225–34.
48. Addolorato G, Capristo E, Stefanini GF, et al. Inflammatory bowel disease: a study of the association between anxiety and depression, physical morbidity, and nutritional status. *Scand J Gastroenterol*. 1997;32:1013–1021.
49. Andrews H, Barczak P, Allan RN. Psychiatric illness in patients with inflammatory bowel disease. *Gut*. 1987;28:1600–1604.
50. North CS, Alpers DH. A review of studies of psychiatric factors in Crohn's disease: etiologic implications. *Ann.Clin.Psychiatry*. 1994;6:117–124.
51. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. *Psychosom Med*. 2004;66:79–84.
52. Helzer JE, Stillings WA, Chammas S, et al. A controlled study of the association between ulcerative colitis and psychiatric diagnoses. *Dig Dis Sci*. 1982;27:513–518.
53. Helzer JE, Chammas S, Norland CC, et al. A study of the association between Crohn's disease and psychiatric illness. *Gastroenterology*. 1984;86:324–330.
54. Tarter RE, Switala J, Carra J, et al. Inflammatory bowel disease: psychiatric status of patients before and after disease onset. *Int J Psychiatry Med*. 1987;17:173–181.
55. Magni G, Bernasconi G, Mauro P, et al. Psychiatric diagnoses in ulcerative colitis. A controlled study. *Br J Psychiatry*. 1991;158:413–415.
56. Walker EA, Gelfand AN, Gelfand MD, et al. Psychiatric diagnoses, sexual and physical victimization, and disability in patients with irritable bowel syndrome or inflammatory bowel disease. *Psychol Med*. 1995;25:1259–67.
57. Walker EA, Gelfand MD, Gelfand AN, et al. The relationship of current psychiatric disorder to functional disability and distress in patients with inflammatory bowel disease. *Gen Hosp Psychiatry*. 1996;18:220–229.
58. Persoons P, Luyckx K, Desloovere C, et al. Anxiety and mood disorders in otorhinolaryngology outpatients presenting with dizziness: validation of the self-administered PRIME-MD Patient Health Questionnaire and epidemiology. *Gen Hosp Psychiatry*. 2003;25:316–323.
59. Nordin K, Pahlman L, Larsson K, et al. Health-related quality of life and psychological distress in a population-based sample of Swedish patients with inflammatory bowel disease. *Scand J Gastroenterol*. 2002;37:450–457.
60. Drossman DA, Leserman J, Mitchell CM, et al. Health status and health care use in persons with inflammatory bowel disease. A national sample. *Dig Dis Sci*. 1991;36:1746–1755.

61. Mikocka-Walus AA, Turnbull DA, Andrews JM, et al. Psychological problems in gastroenterology outpatients: A South Australian experience. Psychological co-morbidity in IBD, IBS and hepatitis C. *Clin Pract Epidemiol Ment Health*. 2008;4:15.
62. Kurina LM, Goldacre MJ, Yeates D, et al. Depression and anxiety in people with inflammatory bowel disease. *J Epidemiol Community Health*. 2001;55:716–720.
63. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry*. 2012;83:495–502.
64. Simren M, Axelsson J, Gillberg R, et al. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. *Am J Gastroenterol*. 2002;97:389–396.
65. Mardini HE, Kip KE, Wilson JW. Crohn's disease: a two-year prospective study of the association between psychological distress and disease activity. *Dig Dis Sci*. 2004;49:492–497.
66. Mikocka-Walus AA, Gordon AL, Stewart BJ, et al. The role of antidepressants in the management of inflammatory bowel disease (IBD): a short report on a clinical case-note audit. *J Psychosom Res*. 2012;72:165–7.
67. Fischler Persoons, P., Luyckx, K. B. PRIME-MD PHQ in the specialist medical setting: validation for anxiety and mood disorders. In: *J Psychosom Res*.Vol 48.; 2000:A1018.
68. Persoons P, Luyckx K, Fischler B. Psychiatric diagnoses in gastroenterolgy: Validation of a self-report instrument (PRIME-MD Patient Health Questionnaire), epidemiology and recognition. *Gastroenterology*. 2001;120:A114.
69. Drossman DA, Ringel Y. Psychosocial factors in ulcerative colitis and Crohn's disease. In: Sartor RB, Sandborn W, eds. *Kirsner's Inflammatory Bowel Disease*.Vol 6th. Philadelphia: W.B. Saunders Co; 2004:340–356.
70. Russo S, Kema IP, Fokkema MR, et al. Tryptophan as a Link between Psychopathology and Somatic States. *Psychosom Med*. 2003;65:665–671.
71. Capuron L, Dantzer R. Cytokines and depression: The need for a new paradigm. *Brain Behav Immun*. 2003;17:119–124.
72. Maes M. Major depression and activation of the inflammatory response system. *Adv Exp Med Biol*. 1999;461:25–46.
73. Stahl S. Chemical neurotransmission as the mediator of disease actions. In: *Essential psychopharmacology: neuroscientific basis and practical applications*. 2nd ed. Cambridge: Cambridge University Press; 2000:99–135.
74. Williams WA, Shoaf SE, Hommer D, et al. Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5-Hydroxyindoleacetic Acid in Normal Volunteers. *J Neurochem*. 2001;72:1641–1647.
75. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry*. 2001;178:399–405.
76. Cherayil BJ. Indoleamine 2,3-dioxygenase in intestinal immunity and inflammation. *Inflamm Bowel Dis*. 2009;15:1391–6.
77. Gupta NK, Thaker AI, Kanuri N, et al. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis*. 2012;18:1214–20.
78. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol*. 2012.
79. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004;4:762–74.
80. Carlin JM, Borden EC, Byrne GI. Interferon-induced indoleamine 2,3-dioxygenase activity inhibits Chlamydia psittaci replication in human macrophages. *J Interferon Res*. 1989;9:329–37.
81. Lestage J, Verrier D, Palin K, et al. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun*. 2002;16:596–601.
82. Capuron L, Ravaud A, Neveu PJ, et al. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry*. 2002;7:468–73.
83. Capuron L, Neurauter G, Musselman DL, et al. Interferon-alpha–induced changes in tryptophan metabolism. *Biol Psychiatry*. 2003;54:906–914.
84. Widner B, Leblhuber F, Walli J, et al. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm*. 2000;107:343–53.
85. Schroecksnadel K, Winkler C, Duftner C, et al. Tryptophan degradation increases with stage in patients with rheumatoid arthritis. *Clin Rheumatol*. 2006;25:334–7.
86. Wolf AM, Wolf D, Rumpold H, et al. Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. *Clin Immunol*. 2004;113:47–55.
87. Wichers M, Maes M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. *Int J Neuropsychopharmacol*. 2002;5:375–88.
88. Irvine EJAN. Measuring quality of life in inflammatory bowel disease. In: Targan SR, Shanahan F, Karp LC, eds. *Inflammatory Bowel Disease: From Bench to Bedside*. 2nd Ed. Springer US; 2005:481–494.
89. Abrams MA. Subjective Social Indications. *Social Trends*. 1973;4:35–56.

90. Guyatt GH, Feeny DH, Patrick DL. Measuring health-related quality of life. *Ann Intern Med*. 1993;118:622–9.
91. Irvine EJ. Development and subsequent refinement of the inflammatory bowel disease questionnaire: a quality-of-life instrument for adult patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 1999;28:S23–S27.
92. Drossman DA, Leserman J, Li ZM, et al. The rating form of IBD patient concerns: a new measure of health status. *Psychosom Med*. 1991;53:701–12.
93. Russell AS, Gulliver WP, Irvine EJ, et al. Quality of life in patients with immune-mediated inflammatory diseases. *The Journal of rheumatology. Supplement*. 2011;88:7–19.
94. Drossman DA, Patrick DL, Mitchell CM, et al. Health-related quality of life in inflammatory bowel disease. Functional status and patient worries and concerns. *Dig Dis Sci*. 1989;34:1379–1386.
95. Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology*. 1989;96:804–810.
96. Lesage AC, Hagege H, Tucat G, et al. Results of a national survey on quality of life in inflammatory bowel diseases. *Clin Res Hepatol Gastroentero*. 2011;35:117–124.
97. Patrick DL, Deyo RA. Generic and disease-specific measures in assessing health status and quality of life. *Med Care*. 1989;27:S217–S232.
98. Graff LA, Walker JR, Lix L, et al. The relationship of inflammatory bowel disease type and activity to psychological functioning and quality of life. *Clin Gastroenterol Hepatol*. 2006;4:1491–1501.
99. Sainsbury A, Heatley R V. Review article: psychosocial factors in the quality of life of patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2005;21:499–508.
100. Drossman DA. Functional versus organic: an inappropriate dichotomy for clinical care. *Am J Gastroenterol*. 2006;101:1172–1175.
101. Farmer RG, Easley KA, Farmer JM. Quality of life assessment by patients with inflammatory bowel disease. *Cleve Clin J Med*. 1992;59:35–42.
102. Vidal A, Gomez-Gil E, Sans M, et al. Health-related quality of life in inflammatory bowel disease patients: the role of psychopathology and personality. *Inflamm Bowel Dis*. 2008;14:977–983.
103. Persoons P, Fischler B, Vandenberghe J, et al. Determinants of quality of life in Crohn's disease. In: *Gastroenterology*.Vol 122 (Suppl.; 2002:S1375: A216.
104. Farrokhyar F, Marshall JK, Easterbrook B, et al. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. *Inflamm Bowel Dis*. 2006;12:38–46.
105. Singh S, Blanchard A, Walker JR, et al. Common symptoms and stressors among individuals with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2011;9:769–75.
106. Langenberg DR Van, Gibson PR. Systematic review: fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2010;32:131–143.
107. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is associated with impaired health-related quality of life in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2011;33:106–14.
108. Romberg-Camps MJL, Bol Y, Dagnelie PC, et al. Fatigue and health-related quality of life in inflammatory bowel disease: results from a population-based study in the Netherlands: the IBD-South Limburg cohort. *Inflamm Bowel Dis*. 2010;16:2137–47.
109. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is associated with increased disease-related worries and concerns in inflammatory bowel disease. *World J Gastroenterol*. 2012;18:445–52.
110. Czuber-Dochan W, Ream E, Norton C. Review article: Description and management of fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2013;37:505–16.
111. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is more prevalent in patients with inflammatory bowel disease than in healthy controls. *Inflamm Bowel Dis*. 2011;17:1564–72.
112. Graff L a, Vincent N, Walker JR, et al. A population-based study of fatigue and sleep difficulties in inflammatory bowel disease. *Inflamm Bowel Dis*. 2011;17:1882–9.
113. Minderhoud IM, Samsom M, Oldenburg B. Crohn's disease, fatigue, and infliximab: is there a role for cytokines in the pathogenesis of fatigue? *World J Gastroenterol*. 2007;13:2089–93.
114. Bager P, Befrits R, Wikman O, et al. Fatigue in out-patients with inflammatory bowel disease is common and multifactorial. *Aliment Pharmacol Ther*. 2012;35:133–41.
115. Banovic I, Gilibert D, Cosnes J. Crohn's disease and fatigue: constancy and co-variations of activity of the disease, depression, anxiety and subjective quality of life. *Psychol.Health Med.* 2010;15:394–405.
116. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory bowel disease. *Gastroenterology*. 2013;144:36–49.
117. Qiu BS, Vallance BA, Blennerhassett PA, et al. The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. *Nat med*. 1999;5:1178–82.
118. Petrak F, Hardt J, Clement T, et al. Impaired health-related quality of life in inflammatory bowel diseases: psychosocial impact and coping styles in a national German sample. *Scand J Gastroenterol*. 2001;36:375–382.
119. Porcelli P, Zaka S, Centonze S, et al. Psychological distress and levels of disease activity in inflammatory bowel disease. *Ital J Gastroenterol*. 1994;26:111–5.

120. Porcelli P, Leoci C, Guerra V. A prospective study of the relationship between disease activity and psychologic distress in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 1996;31:792–796.
121. Russo S, Kema IP, Haagsma EB, et al. Irritability rather than depression during interferon treatment is linked to increased tryptophan catabolism. *Psychosom Med*. 2005;67:773–7.
122. Beeken WL. Serum tryptophan in Crohn's disease. *Scand J Gastroenterol*. 1976;11:735–40.
123. Forrest CM, Youd P, Kennedy A, et al. Purine, kynurenine, neopterin and lipid peroxidation levels in inflammatory bowel disease. *J Biomed Sci*. 2002;9:436–442.
124. Baars JE, Nuij VJAA, Oldenburg B, et al. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis*. 2012;18:1634–40.

SECTION I

METHODOLOGICAL STUDIES

Chapter 3

3 Evaluation of Short-Term Responsiveness and Cutoff Values of Inflammatory Bowel Disease Questionnaire in Crohn's Disease

Published in Inflammatory Bowel Diseases:

Hlavaty T., Persoons P., Vermeire S., Ferrante M., Pierik M., Van Assche G., & Rutgeerts P. Evaluation of short-term responsiveness and cutoff values of inflammatory bowel disease questionnaire in Crohn's disease. Inflamm.Bowel.Dis. 2006; 12:199–204.

3.1 ABSTRACT

***Background**: The inflammatory bowel disease questionnaire (IBDQ) is a frequently used outcome parameter in clinical trials. Whereas the validity and reproducibility of the IBDQ have been extensively studied, there are limited data on its short-term responsiveness and cutoff values for remission and partial clinical response.*

***Methods**: The IBDQ score and its bowel (BD), systemic (SysD), emotional (ED), and social (SocD) dimensions were tested for responsiveness in a cohort of 224 patients with Crohn's disease (CD) treated with infliximab for refractory luminal disease. Changes in the IBDQ score and its dimensions 4 weeks after therapy were analyzed and correlated with changes in the Crohn's Disease Activity Index (CDAI). The responsiveness ratios of the IBDQ and its dimensions were analyzed. Using regression line with ΔCDAI, the cutoff values for the IBDQ remission and response were calculated.*

***Results**: Overall, there was a good correlation between the CDAI and IBDQ at week 0 (correlation coefficient, 0.69; P < 0.001) and week 4 (-0.76; P < 0.001) and change after 4 weeks (0.74; P <0.001). The correlation coefficients for ΔCDAI and changes in BD, SysD, ED, and SocD were 0.753, 0.552, 0.620, and 0.631, respectively; all P <0.001. The responsiveness ratios for ΔIBDQ, BD, SysD, ED, and SocD were 2.6, 2.1, 1.9, 1.7, and 1.9, respectively. Regression line for the IBDQ (r = -0.76, P < 0.001) resulted in a cutoff value for remission of 168 points and for ΔIBDQ resulted in a cutoff value of 22 and 27 points for clinical improvement based on ΔCDAI ≥-70 and ≥-100 points.*

***Conclusions**: The IBDQ is a responsive instrument for reflecting quick change in the quality of life of patients with CD. Cutoff values for the IBDQ remission and partial response were 168 and ≥27 points.*

3.2 INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) with negative impact on the quality of life of affected patients.[1,2] Several instruments assessing the disease-specific quality of life have been developed and validated including the Inflammatory Bowel Disease Questionnaire (IBDQ) and the Rating Form of IBD Patient Concerns.[3,4] Whereas there is limited experience with the Rating Form of IBD Patient Concerns, the IBDQ has evolved into a standard for measuring disease-specific quality of life in patients with CD. The IBDQ is a self-administered, 32item questionnaire concerning 4 dimensions of quality of life: bowel function (BD), emotional status (ED), systemic symptoms (SysD), and social functioning (SocD).[5] With 1 to 7 points for each item, the total IBDQ score ranges from 32 to 224, with higher scores indicating a better quality of life. Since its development, the IBDQ has been used in many clinical trials, usually as a secondary endpoint.[6-12] Several shortened versions have been proposed as well.[13,14] The IBDQ is recommended to be used routinely as a secondary outcome in all prospective, randomized, controlled trials to ensure that the quality of life is improved in medically treated patients with IBD.[15] The sensitivity of the IBDQ to promptly reflect the changes in a patient's condition is a crucial parameter for the assessment of short-term response to therapeutic interventions. Although the validity and reproducibility of the IBDQ have been extensively studied, there are limited data on its short-term responsiveness and the evolution of its 4 dimensions.[16-19] Furthermore, when the IBDQ is to be used as an outcome parameter of a certain therapy in clinical trials, the cutoff values for remission and eventually for partial clinical response are crucial for assessing the effect of the studied medication or other therapeutic intervention. The aim of our study was to evaluate the ability of the IBDQ to reflect the rapid changes in the clinical condition of patients with CD. We also intended to study and propose the remission and relevant clinical response cutoff values for the IBDQ.

3.3 METHODS

3.3.1 Patient Cohort

The IBDQ score and its BD, SysD, ED, and SocD were tested for responsiveness in a cohort of 224 patients with CD treated with infliximab for refractory luminal disease. Clinical characteristics of the patients are summarized in Table 1. All patients were infliximab naive and received a single 5-mg/kg infliximab infusion at our center (University Hospital Gasthuisberg, Leuven, Belgium) between the years 1998 and 2003. The clinical condition

and quality of life of the patient was assessed on the subjects' written consent at baseline and 4 weeks after the therapeutic intervention as a part of prospective follow-up of all patients receiving infliximab in this center. Clinical activity of the disease was assessed using the Crohn's Disease Activity Index (CDAI), and the quality of life was evaluated by means of the self-administered validated Dutch version of IBDQ.[18]

TABLE 1. Clinical Characteristics of the Patient Cohort

Clinical Characteristics	Cohort of Patients With CD ($N = 224$)
Male/female, no. (%)	78/126 (38.2/61.8)
Age at diagnosis >40 yr, no. (%)	16 (7.8)
Mean ± SD age at diagnosis of CD, yr (range)	24.9 ± 11.1 (6–71)
Mean ± SD age at first IFX infusion, yr (range)	34.6 ± 12.1 (14–76)
Mean ± SD disease duration until first IFX infusion, yr (range)	9.6 ± 7.3 (0–39)
Location of disease	
Ileitis, no. (%)	21 (10.3)
Colitis, no. (%)	53 (26.0)
Ileocolitis (%)	107 (52.5)
Upper GI tract, no. (%)	23 (11.3)
Smokers/nonsmokers,* no. (%)	52/99 (34.4/65.6)
Concomitant medication	
Aminosalicylates, no. (%)	88 (43.1)
Steroids, no. (%)	79 (38.7)
6MP/AZA, no. (%)	91 (44.6)
MTX, no. (%)	21 (10.3)
Previous abdominal surgery	84 (41.2)
Mean ± SD baseline CDAI (range)	272 ± 114 (11–590)
Mean ± SD baseline CRP, mg/Lz (range)	30.4 ± 36.9 (0–223.9)

*Data on smoking were not available for all patients.
CRP, C-reactive protein; IFX, infliximab; MP/AZA, mercaptopurine/azathioprine; MTX, methotrexate.

3.3.2 Evaluating Short-term Responsiveness of IBDQ

Changes on the IBDQ and its dimensions between 4 weeks after the therapy and baseline (ΔIBDQ = IBDQweek4 - IBDQweek0) were analyzed and correlated with changes on the CDAI (ΔCDAI = CDAIweek4 - CDAIweek0). To make our data comparable with those of previous studies on the IBDQ, the responsiveness ratio (RR) was used to quantify the responsiveness of the IBDQ.[17] The RR is calculated by dividing the mean change in particular score in those who improved by the standard deviation of the score in stable

patients.[20] Clinical improvement was defined as a decrease in the CDAI of ≥70 points, stable clinical condition as ΔCDAI of 70 points in any direction, and clinical deterioration as an increase in CDAI of ≥70 points.

3.3.3 Calculation of the IBDQ Cutoff Values

A regression line was drawn between the IBDQ and CDAI at week 4 after the therapy and between ΔIBDQ and ΔCDAI. Using regression lines, the cutoff values for the IBDQ remission corresponding to clinical remission based on a CDAI of 150 points and for the IBDQ partial response corresponding to clinical response based on ΔCDAI of -70 and -100 were calculated. Receiver operating characteristic curves were then calculated, and sensitivity and specificity of the IBDQ partial response to predict clinical response were evaluated.

3.3.4 Statistical Analyses

All statistical tests were performed using the SPSS 12.0 statistical software package (SPSS, Chicago, Ill). After testing for normality of the IBDQ and CDAI with the Kolmogorov-Smirnov test (at $P > .05$), the relationship between the IBDQ and CDAI was evaluated using a bivariate correlation and correlation coefficient. Linear regression and regression line were used to calculate corresponding IBDQ and CDAI values. Receiver operating characteristic curves were used to explore the cutoff values to predict the clinical remission and response based on the CDAI. $P = .05$ was considered statistically significant.

3.3.5 Ethical Considerations

The racial, gender, and ethnic characteristics of the subject population reflect the demographics of the Belgian population. No exclusion criteria for infliximab administration have been based on race, ethnicity, gender, or human immunodeficiency virus status. All of the subjects gave written permission to use the collected data for research purposes.

3.4 RESULTS

3.4.1 Correlation Between the IBDQ and CDAI

Follow-up CDAI 4 weeks after the therapy and consequently ΔCDAI were available for 201 patients and the follow-up IBDQ with ΔIBDQ for 180 patients. There was overall a good

correlation between the CDAI and IBDQ at week 0 (correlation coefficient, 0.69; P < .001), week 4 (correlation coefficient, 0.76; P < .001), and change after 4 weeks (correlation coefficient, 0.74; P < .001). The correlation coefficients for ΔCDAI and ΔIBDQ BD, SysD, ED, and SocD were 0.753, 0.552, 0.620, and 0.631, respectively; all P < .001 (Table 2).

TABLE 2. Correlation Between ΔCDAI and ΔIBDQ and Its 4 Dimensions

	ΔCDAI	ΔIBDQ	ΔIBDQ BD	ΔIBDQ SocD	ΔIBDQ ED	ΔIBDQ SocD
ΔCDAI						
Pearson correlation	1	-0.747	-0.753	-0.552	-0.620	-0.631
P (2-tailed)		<.001	<.001	<.001	<.001	<.001
No.	201	180	180	180	180	180
ΔIBDQ						
Pearson correlation	-0.747	1	0.849	0.829	0.903	0.841
P (2-tailed)	<.001		<.001	<.001	<.001	<.001
No.	180	180	180	180	180	180
ΔIBDQ BD						
Pearson correlation	-0.753	0.849	1	0.621	0.641	0.652
P (2-tailed)	<.001	<.001		<.001	<.001	<.001
No.	180	180	180	180	180	180
ΔIBDQ SocD						
Pearson correlation	-0.552	0.829	0.621	1	0.711	0.659
P (2-tailed)	<.001	<.001	<.001		<.001	<.001
No.	180	180	180	180	180	180
ΔIBDQ ED						
Pearson correlation	-0.620	0.903	0.641	0.711	1	0.684
P (2-tailed)	<.001	<.001	<.001	<.001		<.001
No.	180	180	180	180	180	180
ΔIBDQ SocD						
Pearson correlation	-0.631	0.841	0.652	0.659	0.684	1
P (2-tailed)	<.001	<.001	<.001	<.001	<.001	
No.	180	180	180	180	180	180

TABLE 3. Changes in the IBDQ and Its Dimension in Patients Who Improved, Remained Stable, and Deteriorated on Therapy

Parameter	Improved	Stable	Deteriorated	RR
No. of patients	109	63	8	
ΔCDAI	−197 ± 97	−22 ± 35	121 ± 36	
ΔIBDQ	48 ± 30	10 ± 19	−13 ± 24	2.6
ΔIBDQ BD	15 ± 10	3 ± 7	−3 ± 6	2.1
ΔIBDQ SysD	8 ± 6	2 ± 4	0 ± 6	1.9
ΔIBDQ ED	15 ± 12	3 ± 9	−6 ± 12	1.7
ΔIBDQ SocD	10 ± 8	2 ± 5	−4 ± 7	1.9

3.4.2 Responsiveness Ratio

The RR of the IBDQ was calculated for 109 patients showing a response to infliximab therapy and 63 showing no change in clinical status. The number of those worsening while on therapy (8 patients) was too small to allow for a separate analysis. The RRs for the IBDQ total and BD, SysD, ED, and SocD scores were 2.6, 2.1, 1.9, 1.7, and 1.9, respectively (Table 3). The lowest RR was observed on the ED (1.7), implying that after successful therapy, the emotional aspect of the quality of life also recovers but the most slowly.

3.4.3 Cutoff Points for Remission and Partial Response on the IBDQ

The regression lines between the follow-up IBDQ and CDAI were IBDQ = 201 - 0.22 x CDAI (r = -0.76, P < .001; Fig. 1). Based on the arbitrarily chosen CDAI value for clinical remission of 150 points, the corresponding IBDQ value for the IBDQ remission based on this equation is 168 points. There was a strong regression between ΔIBDQ and ΔCDAI as seen in Figure 2. The regression line between the change in the IBDQ and CDAI was ΔIBDQ = 8.34 - 0.19 x ΔCDAI (r = -0.75, P < .001). Using this regression line, the cutoff values for ΔIBDQ partial response corresponding to ΔCDAI of 70 and 100 points were 22 and 27 points, respectively.

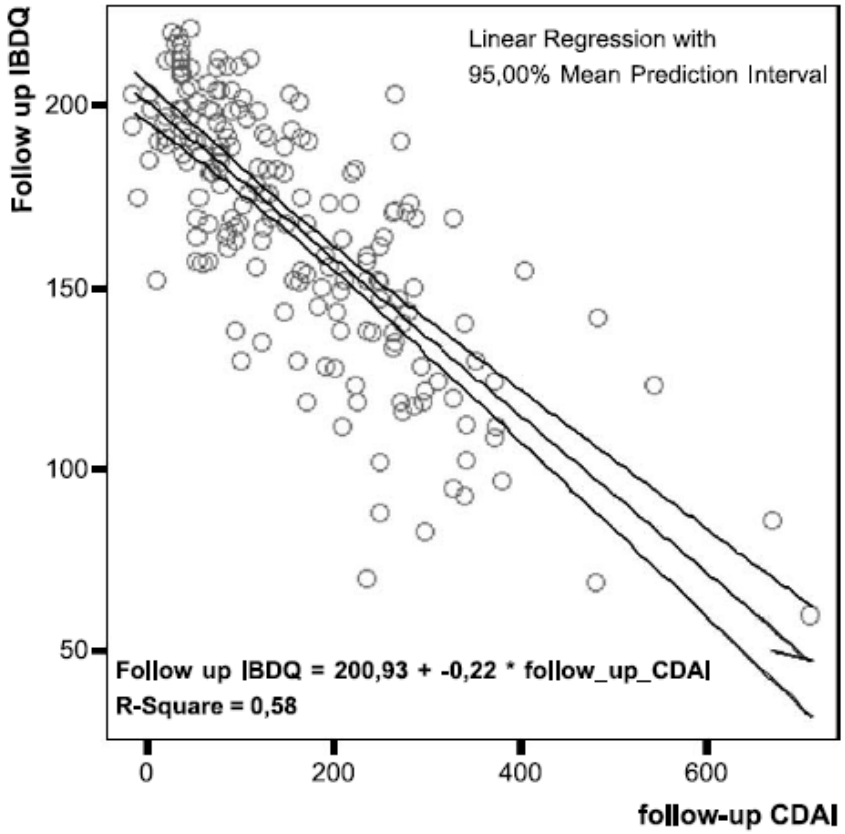

FIGURE 1. Regression line between the follow-up IBDQ and CDAI.

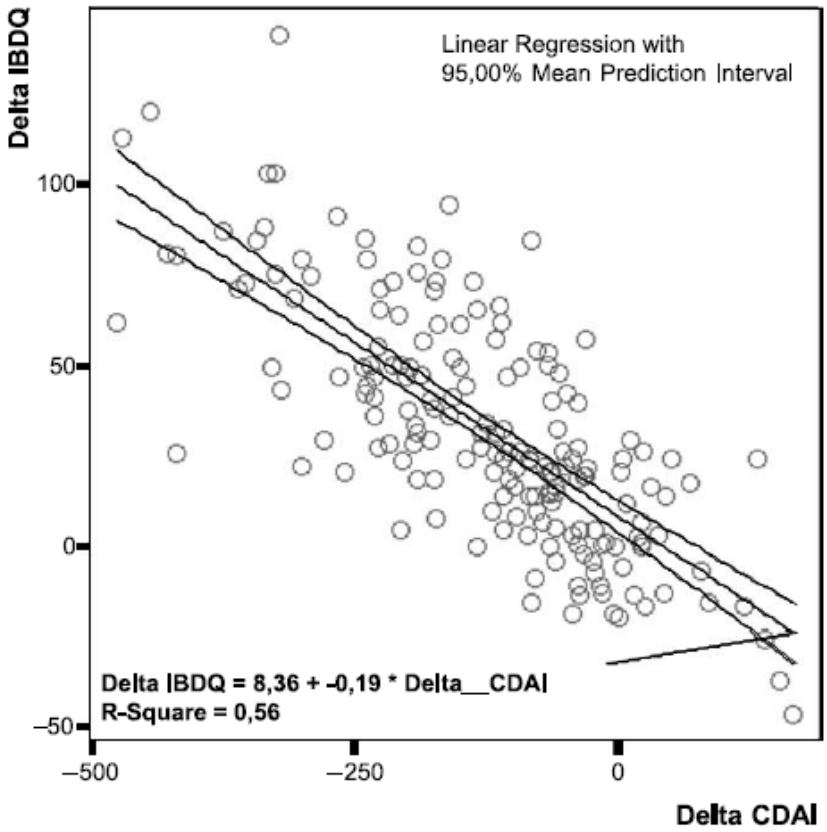

FIGURE 2. Regression line between ΔIBDQ and ΔCDAI.

Based on the receiver operating characteristic curves, the sensitivity and specificity of the IBDQ cutoff value after therapy of 168 to predict the clinical remission were 81.6% and 80.7%, respectively. The sensitivity and specificity of ΔIBDQ of ≥22 points to predict a clinical response based on ΔCDAI of ≥70 points were 80.4% and 73.9%, respectively, and the sensitivity and specificity of ΔIBDQ of ≥28 points to predict a clinical response based on ΔCDAI of ≥100 points were 74.2% and 82.9%, respectively.

3.5 DISCUSSION

The IBDQ proposed by Guyatt et al.[4] is the most widely used health-related quality-of-life instrument in patients with IBD. Although the IBDQ has been developed as a new measure of health status for clinical trials in IBD and is now widely recommended as a secondary outcome measure in all prospective randomized controlled trials, there is still a limited amount of data on the IBDQ responsiveness to rapid changes in a patient's condition (e.g., after induction of remission) and its proper discriminative cutoff values for remission and/or significant clinical improvement.

In the first part of our study, we have shown that all 4 dimensions of this questionnaire (DB, SysD, ED, and SocD) respond quickly to a change in a patient's clinical condition 4 weeks after the therapy. The best response was, as expected, on the BD and SysD of the IBDQ; however, the ED and SocD showed a significant response as well.

Whereas the validity and reproducibility of the IBDQ in patients with CD have been thoroughly studied, the data on the responsiveness of the IBDQ in CD are scarce.[16] The responsiveness was mostly evaluated by the RR parameter. To be able to compare our results with the published data, we opted also to use this parameter in our relatively large cohort. In the original study by Guyatt et al,[4] the responsiveness was studied in a subgroup of 19 patients with CD with selfreported global improvement in their condition after a 4-week period. No therapeutic intervention was reported. The RRs for the BD, SysD, ED, and SocD were 3.0, 1.8, 1.4, and 1.7, respectively. In accordance with our results, the responsiveness was also higher in the BD and SysD and lower in the SocD and especially the ED. In the first larger validation study, performed by the same investigators, the responsiveness of the IBDQ was studied in 171 patients with CD who experienced a clinical relapse in a 1-year multicenter clinical trial setting. The relapse was defined as an increase in the CDAI score of at least 100 points at any time point during the maintenance cyclosporine therapy.[17] The RR for the IBDQ was 0.81, and for its BD, SysD, ED, and SocD, the RRs were 0.85, 0.76, 0.63, and 0.54, respectively. Compared with our study, Irvine et al evaluated the responsiveness of the IBDQ over a longer period and the data for short-term responsiveness (25 patients relapsing at week 8) were not presented separately. Nevertheless, the responsiveness of the IBDQ dimensions in that study was in line with our results. The same trend of greater responsiveness of the IBDQ for the BD and SysD was also observed in the ACCENT I infliximab trial as presented in a substudy on the quality of life.[21] Two national validation studies also tackled the issue of responsiveness of the IBDQ. In a Dutch validation study, the sensitivity to change of the IBDQ corresponding to the responsiveness testing was evaluated in 22 patients with CD reporting "*change in their general condition*" using a paired t-test. In this study, the authors demonstrated that the IBDQ values and all of the dimensions significantly changed in the subgroup of patients with CD, but in 23 patients with ulcerative colitis, only the total IBDQ showed a significant change.[18] In a British study, the issue of responsiveness was addressed in a subgroup of 15 patients with CD reporting a change in the general rating of their bowel condition; the follow-up period was not clearly defined.[19] The RRs were 1.2 for the IBDQ and 0.88 and 1.02, respectively, for Bowel I and Bowel II subscales of the BD and 0.19, 0.44, and 0.91, respectively, for the SysD, ED, and SocD. Given that the earlier validation studies concentrated on the issue of validity and reproducibility, we think that our study of 180 patients with CD provides a significant amount of data on the issue of responsiveness of the IBDQ. This parameter seems to be crucial for

using the IBDQ in a clinical trial focused on the induction of remission in patients with CD.

The second issue that we addressed was the cutoff values for remission and for a meaningful clinical improvement, which, for about a decade, has been often denoted as a clinical response. Similar to previously published reports, we observed a tight correlation between the IBDQ and CDAI ($r = -0.76$, $P < .001$). This is in complete agreement with the findings of Irvine et al[17] and Feagan et al.[21] It is of interest that both regression equations in our article and in that of Irvine et al give almost the same value of the IBDQ (168 and 169 points, respectively), corresponding to a clinical remission based on an arbitrarily defined CDAI score of 150 points. A similar correlation was observed also between the short version of the IBDQ and the CDAI.[14] The correlation is not absolute, implying that the IBDQ is probably measuring the disease burden in a different and presumably more complex way, and it is therefore a useful instrument. The value of the IBDQ that corresponds to a clinical remission has been set between 170 and 190 points. Based on the above-mentioned observations, we propose that the value of ≥170 points should be used.

Based on similar regression lines between ΔCDAI and ΔIBDQ, the corresponding values for the IBDQ partial response based on a decrease in CDAI of 70 and 100 points were 22 and 27 points, respectively. One of these 2 ΔCDAI cutoff values is employed in most of the clinical trials in CD, although many believe that the value of ≥100 points is more appropriate to define a meaningful clinical improvement. In our study, we studied the IBDQ correlates of both CDAI values. In a study by Irvine et al,13 the relapse was defined as an increase in the CDAI value by 100 points. The mean change in the IBDQ in a subgroup of patients who relapsed compared with those who remained stable was 32.6 points. Based on our results and those of Irvine et al, we think that the partial response should be defined as an increase of ≥32 points or improvement by at least 1 point per question answered (in case the patient does not answer all of the questions on the IBDQ). By a less strict definition of meaningful clinical improvement (corresponding to a ΔCDAI of 70 points), the value of ≥22 points (improvement by 1 point in at least two thirds of questions) could be used.

3.6 CONCLUSIONS

To conclude, the IBDQ is a sensitive instrument for reflecting the change in the quality of life of patients with CD. The IBDQ can be reliably used as an outcome parameter in clinical trials studying therapies for the induction of remission in patients with CD. Based on our results, we propose a cutoff value for remission of ≥170 points and a cutoff value for clinical response of ≥32 points.

3.7 REFERENCES

1. Gazzard BG. The quality of life in Crohn's disease. Gut. 1987;28: 378-381.
2. Sorensen VZ, Olsen BG, Binder V. Life prospects and quality of life in patients with Crohn's disease. Gut. 1987;28:382-385.
3. Drossman DA, Leserman J, Li ZM, et al. The rating form of IBD patient concerns: a new measure of health status. Psychosom Med. 1991;53:701-712.
4. Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. Gastroenterology. 1989;96:804-810.
5. Irvine EJ, Feagan BG, Wong CJ. Does self-administration of a quality of life index for inflammatory bowel disease change the results? J Clin Epidemiol. 1996;49:1177-1185.
6. Greenberg GR, Feagan BG, Martin F, et al. Oral budesonide for active Crohn's disease. Canadian Inflammatory Bowel Disease Study Group. N Engl J Med. 1994;331:836-841.
7. Feagan BG, Rochon J, Fedorak RN, et al. Methotrexate for the treatment of Crohn's disease. The North American Crohn's Study Group Investigators. N Engl J Med. 1995;332:292-297.
8. Feagan BG, McDonald JW, Rochon J, et al. Low-dose cyclosporine for the treatment of Crohn's disease. The Canadian Crohn's Relapse Prevention Trial Investigators. N Engl J Med. 1994;330:1846-1851.
9. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med. 1997;337:1029-1035.
10. Summers RW, Elliott DE, Qadir K, et al. Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol. 2003;98:2034-2041.
11. Rutgeerts P, Feagan BG, Lichtenstein GR, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. Gastroenterology. 2004;126:402-413.
12. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut. 2004;53:108-114.
13. Irvine EJ, Zhou Q, Thompson AK. The Short Inflammatory Bowel Disease Questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. Am J Gastroenterol. 1996;91:1571-1578.
14. Alcala MJ, Casellas F, Fontanet G, et al. Shortened questionnaire on quality of life for inflammatory bowel disease. Inflamm Bowel Dis. 2004;10:383-391.
15. Sandborn WJ, Feagan BG, Hanauer SB, et al. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. Gastroenterology. 2002;122:512-530.
16. Pallis AG, Mouzas IA, Vlachonikolis IG. The inflammatory bowel disease questionnaire: a review of its national validation studies. Inflamm Bowel Dis. 2004;10:261-269.
17. Irvine EJ, Feagan B, Rochon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Canadian Crohn's Relapse Prevention Trial Study Group. Gastroenterology. 1994;106:287.
18. Russel MG, Pastoor CJ, Brandon S, et al. Validation of the Dutch translation of the Inflammatory Bowel Disease Questionnaire (IBDQ): a health-related quality of life questionnaire in inflammatory bowel disease. Digestion. 1997;58:282-288.
19. Cheung WY, Garratt AM, Russell IT, et al. The UK IBDQ-a British version of the inflammatory bowel disease questionnaire. Development and validation. J Clin Epidemiol. 2000;53:297-306.
20. Deyo RA, Diehr P, Patrick DL, et al. Reproducibility and responsiveness of health status measures. Statistics and strategies for evaluation. Control Clin Trials. 1991;12:142S-158S.
21. Feagan BG, Yan S, Bala M, et al. The effects of infliximab maintenance therapy on health-related quality of life. Am J Gastroenterol. 2003;98:2232-2238.

Chapter 4

4 Fatigue in Crohn's Disease: Validity of the Checklist of Individual Strength (CIS20R) and Prevalence

Paper submitted for publication in 2013

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4.1 ABSTRACT

Background: *Fatigue, although poorly understood, is highly prevalent in Crohn's disease (CD) and should be approached as a multidimensional concept. The aim was to validate the multidimensional Checklist of Individual Strength (CIS20R) for measuring fatigue in CD patients and to assess the prevalence of fatigue.*
Methods: *CD patients completed the CIS20R and other questionnaires. Demographic and disease characteristics were gathered. The underlying dimensions, internal consistency and reliability of the CIS20R in CD patients were tested. Reproducibility and responsiveness were assessed, as were concurrent and convergent validity. The prevalence of "significant fatigue" in CD patients was determined, using a previously validated CIS20R cutoff.*
Results: *308 CD patients were included. Factor analysis confirmed the 4 CIS20R dimensions in CD patients, explaining 73.6% of the variance. The internal consistency was good and a Crohnbach's α of 0.95 demonstrated reliability. Reproducibility and responsiveness of the CIS20R were demonstrated in 120 patients, who completed the CIS20R before and after treatment. The correlation ($r=0.80$, $p<0.001$) between the CIS20R and a visual analogue fatigue scale confirmed concurrent validity. Moderate correlations between the CIS20R and measures for quality of life and disease activity demonstrated convergent validity. The CIS20R score was significantly higher in patients with active CD and 73.4% reported "significant fatigue" in contrast to 33.7% of patients with inactive CD ($p<0.001$). Significant, chronic (>6 months) fatigue was present in 39.3% of the CD patients.*
Conclusions: *The CIS20R is a valid responsive multidimensional questionnaire for the assessment of the severity and prevalence of fatigue in CD patients.*

4.2 INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) with an unpredictable, relapsing course, which is potentially associated with severe gastrointestinal symptoms. Often a disruption of social and professional life occurs and CD has an important impact on the physical functioning and the global wellbeing of the patients. Because of the observed discrepancy between "objective" disease parameters (i.e. laboratory or endoscopic variables) and the patients' subjective perception of illness and disease impact on well-being and daily functioning, the measurement of Health Related Quality of Life (HRQL) is an indispensable addition to the global assessment of the severity of CD. Several disease-specific questionnaires measuring HRQL in CD exist.[1–3] These questionnaires have contributed to the recognition of concerns and of the importance of HRQL for clinical care in CD patients, and subjective illness experience has become an important outcome measure of treatment. Patients with chronic illness rate fatigue as one of the key factors leading to a decrease in their HRQL and previous research in chronic, inflammatory diseases such as multiple sclerosis and rheumatoid arthritis identified fatigue as one of the most problematic and challenging aspects for patients.[4] The same pattern has been observed in IBD, as fatigue was identified as one of the most important concerns and this was the case in clinical IBD populations[5,65;6] as well as in a large community sample of IBD patients.[1]

In a recent review on fatigue in IBD, the authors defined fatigue as "*a persistent, overwhelming sense of tiredness, weakness, or exhaustion resulting in a decreased capacity for physical and/or mental work, which is typically unrelieved by adequate sleep or rest and which can exist as a unique entity and not merely a component of psychological comorbidity or illness behaviour*".[7] Nevertheless, fatigue remains a complex concept which is inherently subjective in nature and therefore difficult to measure and define. Czuber-Dochan recently reviewed the literature and found a certain degree of overlap between the definitions which were provided for the concept of "fatigue" which was used in the study. There was, however, also a marked heterogeneity and some studies even used alternative concepts or terms, which were not further defined or conceptualized.[8] Due to this confusion between terms and the absence of a clear cut conceptualization of fatigue in IBD patients, studies are difficult to compare and it is unclear if they assess the same symptom.

An important point of consensus is that the concept of fatigue should be approached as a multidimensional concept, encompassing physical, cognitive and affective aspects.[7,8]. This should also be reflected in the selection of appropriate questionnaire or other assessment tools, since this will ultimately determine the diagnosis and quantification of fatigue. A disease specific, multidimensional questionnaire measuring fatigue in IBD would be ideal. Such a scale does not exist. Until now, most studies have used the Multidimensional Fatigue

Inventory (MFI), but other questionnaires have been used.[8] Most of these questionnaires have been developed for the general population or for other medical conditions and have not been validated for IBD-fatigue. For the Fatigue Questionnaire (FQ), validation data was provided for an IBD population, but not as primary aim of the study.[9] The FACIT-F has also been validated for the IBD population.[10] Other studies which used the same questionnaires (e.g. the MFI), have provided the prevalence of fatigue on the basis of different cutoff points. Furthermore, the concepts on which the prevalence of "fatigue" (e.g. chronic fatigue, severe fatigue, overall fatigue) as a categorical diagnosis were based, or the reasons why a certain cutoff was chosen, were often not explained in detail.[8]

Thus, controversies are unavoidable, e.g. concerning the cause, the course, the determinants, and – not in the least – concerning the exact prevalence of fatigue. Fatigue is undeniably highly prevalent in IBD patients, and more common than in healthy controls.[7,9] The prevalence rates, however are difficult to compare between studies and show a relatively broad range. The prevalence in patients with ulcerative colitis in remission ranges between 22% and 36%.[9,11] In CD patients in remission the prevalence rates might be somewhat higher, ranging between 27% and 41%.[8,12]. A study in CD patients in remission with irritable bowel syndrome (IBS)-like symptoms found a fatigue prevalence rate of 60%.[13] The prevalence in CD patients with active disease or in a population with a mixed disease activity was much higher, ranging from 44% to 86% (for review see Czuber-Dochan et al.[8]).

Fatigue is a cross-cultural phenomenon, which is comparably present both in men and women with IBD.[14] It has also been shown that fatigue is associated with a significant reduction in HRQL in IBD.[15] and it contributes to a large extend to the systemic aspects of QOL (i.e. concerning the impact of IBD on general health) in IBD.[16] Physical, psychological and situational factors may determinate the development, severity or course of fatigue in IBD and several studies have investigated their contribution or the association. Nevertheless, these findings are often also controversial and fatigue remains poorly understood in IBD.

It remains unclear how to measure fatigue in IBD, which contributes to the conceptual confusion and the heterogeneity between studies. Providing a validated measurement tool in the IBD population might help improve this situation. The aim of this paper was therefore, to assess the validity of the Checklist of Individual Strength (CIS20R), a multidimensional questionnaire, initially validated for patients with the Chronic Fatigue Syndrome (CFS), for the measurement of fatigue in CD patients and to assess the prevalence of fatigue in this sample of CD patients, using a previously established cutoff of the CIS20R for significant fatigue .

4.3 METHODS

4.3.1 Data collection

Between December 2003 and July 2004, CD outpatients, who consulted the IBD clinic at a university hospital were asked individually to complete a set of questionnaires including the Checklist of Individual Strength (CIS20R), a visual analogue scale (VAS) measuring fatigue, the Inflammatory Bowel Disease Questionnaire (IBDQ). Only patients with previously diagnosed CD on the basis of clinical, standard radiological or endoscopic criteria, supplemented with the typical histological appearance in mucosal biopsy or resection specimen were asked to participate in the study. Patients younger than 18 years were excluded, as were patients who suffered from a short bowel syndrome, patients with stomas or if they participated in a clinical trial. Written informed consent was obtained from all participants at inclusion and the institutional Ethical Committee approved the study.

4.3.2 Measurements

4.3.2.1 Demographic and disease-related characteristics

Relevant demographic variables, such as gender, age, civil status and work status were gathered. Clinical disease activity was assessed with the CD Activity Index (CDAI[17]) and C-reactive protein was used as a marker for inflammation. Hematocrit levels, disease duration, concomitant drug treatment, smoking and sports were recorded. The phenotype of the CD patients was described using the Vienna classification,[18] since the newer Montreal classification was not available at the time of the study.

4.3.2.2 Checklist of Individual Strength 20 Items (CIS20R)

The Checklist of Individual Strength 20 items (CIS20R[19]) is a multi-dimensional questionnaire developed to assess the severity of subjective fatigue in CFS patients during the previous 2 weeks. The four measured dimensions are Subjective Feeling of Fatigue (8 items), reduction in Concentration (5 items), reduction in Motivation (4 items) and reduction in Activity (3 items). The answers are checked on a 7-point Likert scale with a theoretical total score range of 20 to 140. A higher score corresponds with worse fatigue. The instrument is a relatively short, with good psychometric qualities and it can distinguish between groups of patients with severe fatigue and healthy controls.[20] Previously, a total cutoff point for the CIS20R of > 76 has been determined for "*significant fatigue*" with a specificity of 90% and a sensitivity of 73%.[21] This cutoff point indicates *a fatigue level that puts the individual "at risk" for*

subsequent sick leave or work disability and it is close to the 95% percentile of the CIS-20 score in working population samples.[21] This cutoff point was also used in the current study to determine significant fatigue in CD patients. Chronic fatigue was defined as "*significant fatigue*" with a duration of more than 6 months.[4] The English version of this questionnaire has previously been published.[20] An English version is added to this study as an addendum (addendum 1).

4.3.2.3 Visual Analogue Scale measuring Fatigue (VAS Fatigue)

In addition to the CIS20R, a generic, continuous VAS assessed the subjective feeling of fatigue over the previous 2 weeks. Patients were asked to indicate their level of fatigue on a graph representation ranging from 0 (no fatigue) to 100 (worst imaginable fatigue).

4.3.2.4 Inflammatory Bowel Disease Questionnaire (IBDQ)

The IBDQ is a disease-specific, self-administered quality of life measure for CD patients consisting of 32 items assessing 4 dimensions: bowel function (BD), emotional status (ED), systemic symptoms (SysD) and social functioning (SocD).[16] Answers are indicated on a 7-point Likert scale and the total IBDQ score rages from 32 to 224, with higher scores indicating a better quality of life. A validated Dutch translation was used in this study.[22,23]

4.3.3 Validation procedure

4.3.3.1 CIS20R in CD patients: underlying dimensions, internal consistency and reliability

A principal component analysis with Varimax rotation[24] was used to assess whether the dimensions of the CIS20R completed by CD patients, corresponded with the 4 subscales of the CIS20R which were described in CFS patients.[19] All factors with an "eigenvalue" (a statistical measure of its power to explain variation between patients) exceeding 1.1 would be considered important.[25,26] All questions with a factor loading of at least 0.40 on that factor, would be considered as contributing to a dimension.

The internal consistency and reliability of the CIS20R completed by CD patients was assessed by item-total correlations and Cronbach's alpha.[27] If a question would yield an item-total correlation below 0.2, it would be considered for rejection. Alternatively, items with item-total correlations above 0.9 would provide little additional information and would therefore

also be considered for rejection.[25,27] The Cronbach's alpha for the CIS20R and its subscales should exceed 0.7.[25,27]

4.3.3.2 Assessing reproducibility and responsiveness

Reproducibility and responsiveness were tested in patients who received treatment with anti-TNF-α (infliximab) following their clinic visit. A retest CIS20R questionnaire was presented to these patients at their control visit after 4 weeks and the CDAI was recalculated. Patients without a clinically significant change of CDAI (defined as a change of less than 70 points on the CDAI) were included in the reproducibility analysis and both sets of responses on the CIS20R should be consistent. Intraclass correlation coefficients were used to assess reproducibility.[28]

In contrast, the retest CIS20R scores of patients with a CDAI change of equal or more than 70 points, should also change if the questionnaire is adequately responsive, which can be quantified by using the responsiveness ratio (mean difference of a questionnaire score change in patients with a clinically significant change of CDAI divided by the standard deviation of the mean difference of questionnaire score change in patients without a clinically significant change of CDAI). A higher ratio indicates a more responsive questionnaire.[28]

Assessing concurrent and convergent validity

The concurrent validity was assessed by comparing the CIS20R with a generic VAS Fatigue. If the CIS20R were a valid measure of fatigue in CD patients, we would expect a moderate to high correlation. The convergent validity of the CIS20R was assessed by comparing it with the CDAI and the IBDQ. CD with higher fatigue scores on the CIS20R were expected to score higher on the CDAI and lower on the IBDQ.

4.3.4 Additional statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS 16.0, SPSS Inc., Chicago). Descriptive statistics were calculated for all variables. The Levene's Test was used to examine the equality of variances, Students's t test and One-Way ANOVA were used to compare means. The χ^2 test was used to assess associations between categorical data. Correlations were calculated using the Pearson's r for linear data and the Spearman's ρ for non-parametric data. The level of statistical significance was set at $P < 0.05$ and Bonferroni correction for Type-I errors was used when appropriate. Mean values

are given with one Standard Deviation (SD) and median values with the interquartile range (IQR).

4.4 RESULTS

4.4.1 Study population

Of the 350 patients who were eligible for this study, 26 patients (7.4%) preferred not to participate. Additionally, 16 patients (4.6%) returned a blank or incomplete questionnaire, thus leaving 308 patients (88%) who were included in the statistical analyses. Table 1 shows the descriptive characteristics of the CD patients.

Table 1. Descriptive statistics of the CD patients participating in the study (n = 308)

Mean age (SD)	37.5 (12.0) years
Male Gender n (%)	124 (40.3%)
Civil Status	
Married/cohabitant n (%)	217 (70.5%)
Single n (%)	37 (12.0%)
With parents/in group n (%)	54 (17.5%)
Work/Study	
Full-time n (%)	117 (38.0%)
Part-time n (%)	25 (8.1%)
Sick leave < 6 months n (%)	73 (23.7%)
Sick leave > 6 months n (%)	84 (27.3%)
No job/not studying n (%)	9 (2.9%)
Mean duration of disease (SD)	11.6 (8.7) years
Mean CDAI (SD)	231 (127.1)
CD extend	
L1 terminal ileum n (%)	86 (27.9%)
L2 colon n (%)	71 (23.1%)
L3 ileocolon n (%)	137 (44.5%)
L4 Upper GI n (%)	14 (4.5%)
CD disease type	
Inflammatory n (%)	140 (45.5%)
Stricturing n (%)	29 (9.4%)
Penetrating n (%)	139 (45.1%)
Mean hematocrit (SD)	40.2 (4.4)
Median C-reactive Protein (IQR)	6.7 (17.6) mg/L
Type of medication	
5 ASA n (%)	87 (28.2%)
Systemic steroids n (%)	38 (12.3%)
Metothrexate n (%)	48 (15.6%)
TNF-α inhibitors n (%)	128 (41.6%)
Azathioprine n (%)	111 (36%)
Antidepressants n (%)	35 (11.4%)
Sleep medication n (%)	24 (7.8%)
Sedatives n (%)	26 (8.4%)
Pain medication n (%)	65 (21.1%)
Narcotic medication n (%)	29 (9.4%)
Smoker n (%)	111 (36%)
Regular sports n (%)	98 (31.8%)

SD: Standard deviation - IQR: Interquartile range

4.4.2 Validity of the CIS20R

4.4.2.1 Factor analysis, internal consistency and reliability of the CIS20R in a CD population

The principal component analysis with varimax rotation yielded 4 factors in CD patients corresponding with those originally found in a population of CFS patients (table 2). These 4 factors concur with the 4 subscales of the CIS20R (subjective feeling of fatigue, concentration, motivation and activity), explaining 73.6% of the variance, which is comparable to the explained variance of 67.7% in the original validation study.[19] The respective items with the highest factor loadings on the different factors/subscales were the same as in the original CFS population. Item 2 was an exception and had a high factor loading both on factor 1 (subjective feeling of fatigue) and on factor 3 (motivation) in this CD population.

Table 2. Factor analysis of the CIS20R by Principal Component analysis with Varimax rotation (Kaiser normalisation) yields the same 4-factor structure in a CD population as the original questionnaire designed for a population of CFS patients

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Item 1	0.77			
Item 2	*0.61*		*0.41*	
Item 3		0.73		
Item 4	0.81			
Item 5			0.71	
Item 6	0.86			
Item 7				0.86
Item 8		0.74		
Item 9	0.80			
Item 10				0.82
Item 11		0.82		
Item 12	0.80			
Item 13		0.81		
Item 14	0.72			
Item 15			0.87	
Item 16	0.72			
Item 17				0.67
Item 18			0.62	
Item 19		0.79		
Item 20	0.75			

Factor 1: Subjective feeling of fatigue (30.1% of variance)
Factor 2: Reduction of concentration (18.8% of variance)
Factor 3: Reduction of motivation (11.4% of variance)
Factor 4: Reduction of activity (13.3% of variance)
Cumulative % of variance = 73.6%

The internal consistency of the CIS20R in a CD population was demonstrated by the item-total correlations which ranged between 0.46 and 0.76. There were no questions with item-total correlations below 0.20 or above 0.80. The Crohnbach's α which is an indicator of the reliability of a scale, was 0.95 for the complete CIS20R scale. In this respect, the summary

score of the CIS20R can be used to measure overall fatigue in CD patients. The respective Crohnbach's α for the subscales, were 0.94 for the Subjective Feeling of Fatigue subscale, 0.89 for the Concentration subscale, 0.83 for the Motivation subscale and 0.90 for the Activity subscale in this population of CD patients. These findings are comparable to the Crohnbach's α values in the original validation study (overall Crohnbach's α = 0.92, Crohnbach's α of the subscales subjective fatigue = 0.88, concentration = 0.92, motivation = 0.83 and activity = 0.87).[19]

4.4.2.2 Reproducibility and responsiveness of the CIS20R in a CD population

A total of 120 patients completed the CIS20R for a second time, at 4 weeks after treatment with anti-TNF-α and the CDAI in these patients was calculated at baseline and at re-evaluation. In 37 patients (31%) there was no clinically significant change of CDAI (defined as a change < 70 points on the CDAI). In these patients, paired t-tests of the CIS20R and its subscales showed no significant difference in their baseline test scores and their retest scores after 4 weeks (table 3). The intraclass correlations between their test and retest scores were very good, ranging from 0.80 to 0.89 (table 3).

Table 3. Reproducibility of the CIS20R and its subscales in CD patients (n = 37) without a clinically significant change of the CDAI (change < 50 points)

	Mean difference	SD of the difference	95% CI for the difference	Intraclass correlation
CIS Total	2.81	20.45	-4.01, 9.63	0.87
CIS subjective fatigue	2.22	11.07	-1.47, 5.91	0.80
CIS Concentration	0.43	5.87	-1.52, 2.39	0.88
CIS Motivation	0.41	5.22	-1.34, 2.15	0.82
CIS Physical Activity	-0.24	4.00	-1.58, 1.09	0.89

The responsiveness of the CIS20R was assessed for the 83 patients (69%) with a CDAI change of ≥ 70 points. Four weeks after treatment, only 4 patients (5%) had deteriorated. This sample was too small to allow separate analysis. Therefore, these patients were included in a single analysis with the 79 patients who significantly improved by reversing the sign of the difference in scores, as has been done in IBD patients in previous studies validating the IBDQ, by the McMaster and other research teams.[25,29] A separate analysis without these 4 patients yielded no significantly different results (data not shown). Table 4 shows that for the total CIS20R score and all of the subscales, significant differences were found with the paired t-tests. All of the responsiveness ratios exceeded half a standard deviation of the difference of the CIS20R scores after 4 weeks in patients without clinically significant change on the CDAI, indicating that the CIS20R can detect clinically significant change in fatigue after treatment (table 4).

Table 4. Responsiveness of the CIS20R and its subscales in CD patients (n = 83) with a clinically significant change of the CDAI (change >50 points)

	Mean difference	p-value	95% CI for the difference	Responsiveness ratio*
CIS20R Total	21.16	< 0.001	15.39, 26.93	21.16/20.45 = 1.05
CIS subjective fatigue	10.54	< 0.001	7.44,13.64	10.54/11.07 = 0.95
CIS Concentration	3.76	< 0.001	2.13, 5.38	3.76/5.87 = 0.64
CIS Motivation	4.48	< 0.001	3.22, 5.75	4.48/5.22 = 0.86
CIS Physical Activity	3.00	< 0.001	1.82, 4.18	3.00/4.00 = 0.75

* Responsiveness ratio = mean difference of questionnaire score change in patients with a clinically significant change of CDAI (n = 83) divided by the standard deviation of the mean difference of questionnaire score change in patients without a clinically significant change of CDAI (n = 37, see table 3 for SD of mean difference)

4.4.2.3 Concurrent and convergent validity of the CIS20R in a CD population

Concurrent validity of the CIS20R in a CD population was demonstrated by a highly significant correlation between the generic VAS Fatigue and the total CIS20R score (Pearson's r = 0.80, p < 0.001). This VAS also correlated significantly with the 4 CIS20R subscales and all correlations were higher than 0.50: subjective feeling of fatigue (Pearson's r = 0.84, p < 0.001), concentration (Pearson's r = 0.51, p < 0.001), motivation (Pearson's r = 0.61, p < 0.001) and activity (Pearson's r = 0.58, p < 0.001).

Table 5 illustrates the negative correlations of the fatigue questionnaire, CIS20R, with the HRQL questionnaire, IBDQ. All correlations were highly significant, thus confirming the convergent validity between the CIS20R and its subscales and the IBDQ and its subscales. The lowest correlation was found between the CIS20R and the bowel symptoms subscale of the IBDQ. The same pattern was observed for all CIS20R subscales. The CIS20R concentration subscale achieved the lowest correlations with the IBDQ and its subscales. The highest range of correlations was found between the IBDQ systemic symptoms subscale and the CIS20R and its subscales, with the exception of the CIS20R motivation subscale, which was more correlated with the IBDQ emotional functioning subscale. For the CIS20R activity subscale, the correlation with the IBDQ systemic symptoms subscale equalled the correlation with the IBDQ social functioning subscale.

Additionally, the CIS20R and its subscales correlated significantly with the CDAI, especially the CIS20R subjective feeling of fatigue subscale (table 5). Since the CDAI also incorporates quality of life (through the item "how did you feel during the previous week"), correlations with the CIS20R were also calculated with an adjusted CDAI score (= CDAI total score minus the quality of life item). The correlations between, on the one hand this adjusted CDAI, and on the other hand the CIS20R score (Pearson's r = 0.36, p < 0.001), the CIS20R subjective feeling of fatigue subscale (Pearson's r = 0.39, p < 0.001), the concentration subscale

(Pearson's r = 0.21, p < 0.001), the motivation subscale (Pearson's r = 0.26, p < 0.001) and the activity subscale (Pearson's r = 0.30, p < 0.001), were statistically significant but lower. Except for the CIS subjective feeling of fatigue subscale, all correlations of the CIS20R and its subscales with the CRP were lower than Spearman's ρ = 0.20 (table 5). This was also the case for the correlations of the CIS20R and its subscales with the age and gender-adjusted haematocrit, except for the correlations with the CIS20R activity subscale.

Table 5. Correlations between the CIS20R and its subscales with the IBDQ and its subscales and with disease parameters indicative of disease severity in CD patients

Convergent validity with *quality of life measure and disease parameters*	**CIS20R total score**	**CIS20R Subjective feeling of fatigue**	**CIS20R Concentration**	**CIS20R Motivation**	**CIS20R Activity**
IBDQ total score (Pearson's r)	r = -0.67*	r = -0.63*	r = -0.43*	r = -0.58*	r = -0.49*
Bowel symptoms subscale (Pearson's r)	r = -0.48*	r = -0.50*	r = -0.39*	r = -0.31*	r = -0.26*
Systemic symptoms subscale (Pearson's r)	r = -0.67*	r = -0.67*	r = -0.42*	r = -0.56*	r = -0.50*
Emotional functioning subscale (Pearson's r)	r = -0.59*	r = -0.52*	r = -0.37*	r = -0.58*	r = -0.44*
Social functioning subscale (Pearson's r)	r = -0.55*	r = -0.51*	r = -0.30*	r = -0.49*	r = -0.50*
Disease parameters					
CDAI (Pearson's r)	r = 0.51*	r = 0.54*	r = 0.30*	r = 0.38*	r = 0.41*
CRP (Spearman's Rho)	ρ = 0.11§	ρ = 0.20*	ρ = -0.05	ρ = 0.04	ρ = 0.11§
Hematocrit (Pearson's r, adjusted for age/gender)	r = -0.16†	r = -0.13†	r = -0.08	r = -0.14†	r = -0.22*

* < 0.001, † < 0.01, § < 0.05

4.4.2.4 CIS20R ability to discriminate between groups

The CIS20R with its subscales were able to discriminate between patients with clinically inactive (CDAI < 150, n = 86) and clinically active disease (CDAI ≥ 150, n = 222). Patients with clinically inactive CD scored significantly lower than patients with clinically active CD on the CIS20R summary score (63.1 ± 27.0 vs. 91.5 ± 26.1, p < 0.001), the subjective feeling of fatigue subscale score (29.7 ± 13.9 vs. 43.4 ± 11.4, p < 0.001), the concentration subscale score (12.8 ± 7.5 vs 18.1 ± 8.4, p < 0.001), the motivation subscale score (11.4 ± 6 vs. 16.9 ± 6, p < 0.001) and the activity subscale score (9.3 ± 5.4 vs. 13.2 ± 6.0, p < 0.001). Additionally, when the group of CD patients with active disease was further subdivided into 2 groups of mild to moderate disease (CDAI 150 – 400, n = 194) and severe disease activity (CDAI > 400, n = 28), the CIS20R total scores, the physical fatigue subscale and the activity subscale, significantly differed between the 2 groups (figure 1). The differences between the patient group with mild to moderate disease activity and the patient group with severe disease activity, on the CIS20R concentration subscale and the motivation subscale were in the expected direction, but did not reach statistical significance.

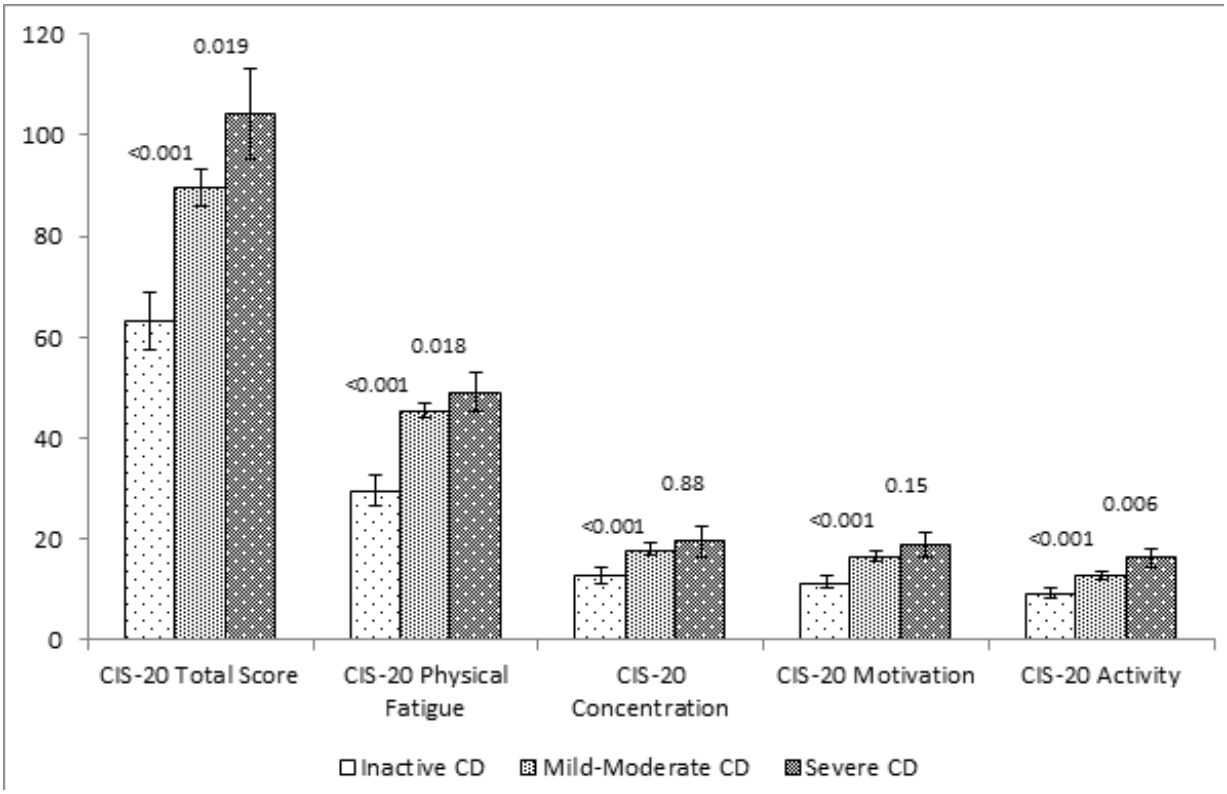

Figure 1. Ability of the CIS20R to discriminate between groups of disease activity

4.4.3 Prevalence of fatigue in CD

The prevalence of significant fatigue (CIS20R score > 76) for this population of CD patients is presented in table 6. More female CD patients reported significant fatigue (73.4%) than male CD patients (46.8%, $p < 0.001$). A significantly higher proportion of patients with active CD (CDAI > 150) also had significant fatigue, than of patients with inactive CD ($p < 0.001$). The proportion of patients with significant fatigue did not differ significantly between mild-moderately active CD and severely active CD. When the former group was arbitrarily subdivided into a subgroup[30] with mildly active CD (CDAI between 150 and 219, n = 56) and moderately active CD (CDAI between 220 and 400, n = 138), the respective prevalence rates of significant fatigue within these groups were 66.1% for mildly active CD (n = 37) and 76.8% for moderately active CD (n = 106) and did not differ significantly ($p = 0.12$). The prevalence rate of significant fatigue in mildly active CD was however significantly higher than in patients with inactive CD ($p < 0.001$).

Table 6 also presents the prevalence rates of patients who indicated that they suffered from chronic (> 6 months), disabling fatigue (CDF). The proportion of female CD patients reporting CDF (48.9%) was also significantly larger than the proportion of male CD patients with CDF (25.0%, $p < 0.001$). Significantly fewer patients without disease activity reported CDF than

patients with disease activity. Within the latter group, a significantly higher proportion of patients with CDF had severe disease activity than mild to moderate disease activity.

Table 6. Prevalence rates of CD patients with and without significant fatigue and chronic, significant fatigue

Patients	Significant Fatigue		p	Chronic Fatigue		p
	Present	Absent		Present	Absent	
CD population (n = 308)	62.7% (193)	37.3% (115)		39.3% (121)	60.7% (187)	
Inactive CD (n = 86)	33.7% (29)	66.3% (57)	< 0.001	19.8% (17)	80.2% (69)	< 0.001
Active CD (n = 222)	73.9% (164)	26.1% (58)		46.8% (104)	53.2% (118)	
Mild – Moderate (n = 194)	73.7% (143)	26.3% (51)	0.24	43.8% (85)	56.2% (109)	0.017
Severely Active (n = 28)	85.7% (24)	14.3% (4)		67.9% (19)	22.1% (9)	

4.5 DISCUSSION

No accepted definition of fatigue exists and no general diagnostic criteria for fatigue have been established for inflammatory bowel diseases in general or CD specifically. Czuber-Dochan et al. advocated the further conceptualization of the symptom of fatigue and suggested the use of standardized, patient-focused definitions.[8] A prerequisite for a better understanding the symptom of fatigue is an adequate measurement thereof. Fatigue, however, is inherently a subjective experience and cannot be assessed with objective measures or assessment tools. Currently no “gold standard” exists to measure the symptom of fatigue, its severity, the impact, its prevalence and the effect of interventions.[31] In a recent review, Van Langenberg and Gibson recommended the use of validated self-report scales which can assess the multidimensionality of the fatigue concept in IBD patients.[7] The CIS20R is such a multidimensional instrument which has been used in several other patient populations and also in patients with chronic, inflammatory disease.[32,33] It has not yet been validated however in patients with IBD in general or specifically in CD patients.

With this validation of the CIS20R in a large population of over 300 CD patients, we wanted to provide an additional, multidimensional measurement scale and we have demonstrated that it is a valid and reliable fatigue scale with good operating characteristics for this population. The original 4 dimensions of the CIS20R (subjective feeling of fatigue, reduction in concentration, motivation and activity) were reproduced in CD patients through factor analysis a high percentage of the variance explained. The second item of the CIS20R had a

significant factor loading on both the first (Subjective Feeling of Fatigue) and the third factor (Motivation). This was probably due to a slight difference in interpretation of this item by a Flemish population of patients, in contrast to the Dutch population, since the questionnaire originated in the Netherlands. Although, a slight linguistic adaptation of this item might be warranted for future use in the Flemish population, we chose not to reject this item, because of the overall excellent psychometric properties of the questionnaire.

The internal reliability of the CIS-20 and its subscales in CD patients was confirmed by the good to excellent internal consistency. The reproducibility of the CIS20R and its subscales in stable CD patients was demonstrated by the high intraclass correlations between the test and retest scores which supports its possible use for longitudinal studies. The overall intraclass correlation coefficient (ICC = 0.87) was somewhat higher for the CIS20R than for the one-dimensional FACIT-F in stable IBD patients (0.81), but the ICC for the subscale Subjective Feeling of Fatigue of the CIS20R, which specifically assesses physical fatigue, was comparable (ICC = 0.80).[10] The instrument was found to be responsive to clinically significant change of fatigue in CD patients who reported changes in their disease activity. There was a clinically significant change in fatigue when a clinical significant change in disease activity was reported after treatment with infliximab. A significant improvement of fatigue after treatment with infliximab has previously been reported.[34] Thus, this instrument could be suitable for the longitudinal measurement of fatigue in CD patients.

The concurrent and convergent validity of the CIS20R and its subscales in CD patients was demonstrated with the respective significant correlations with a VAS measuring fatigue, with HRQL and with clinical disease activity. The strong association between fatigue, i.e. the CIS20R and its subscales, and HRQL, i.e. the IBDQ and its subscales, illustrates the fact that fatigue can have a debilitating effect on daily functioning and quality of life. The correlations between the CIS20R and the IBDQ were comparable to those in a previous study, although a different fatigue questionnaire was used.[35] Previous studies found that fatigue contributes independently to impaired HRQL in CD[11,12,36] and that chronic fatigue has a negative impact on HRQL scores, especially on physical domains.[15] Overall, the correlations between the IBDQ and its subscales and the CIS20R and its subscales were moderate, suggesting that the CIS20R is not merely an alternative for measuring HRQL, but that it truly provides additional information specifically linked to fatigue. This supports the notion that the CIS20R can be used as a designated, validated questionnaire to assess the severity of fatigue.[15]

The mean CIS20R total scores in CD patients with inactive disease score was 63.1 (± 27.0). This was below the cutoff for "significant fatigue" which was defined as a score of > 76 (fatigue level that puts the individual at risk for subsequent sick leave or work disability)[21] and which was higher than a healthy population (47.9). The elevated levels of fatigue in CD patients is a known clinical phenomenon and a major concern of patients and clinicians.[37,38] Possibly, it is due to the presence of on-going mucosal inflammation in the majority CD patients, even when in remission.[39] Patients with active CD scored significantly higher on the CIS20R 91.5 (± 26.1). The correlation between clinical disease activity and the severity of fatigue on the one hand, and the higher fatigue severity in groups with higher severity of disease activity, was in line with previous findings that fatigue severity correlates with symptom severity as a measure of disease activity, rather than with objective measures of gastrointestinal inflammation, such as CRP.[7,9,35,40] Tinsley et al. also reported significantly lower fatigue in patients with inactive CD, parallel to our findings.[10] The correlation between the CIS20R and its subscales and disease activity, measured with an adjusted CDAI without the highly weighed "*subjective well-being*" item which is a recognized limitation in the CDAI,[41] remained significant but was nonetheless weaker. This suggests that the strong association between reported disease activity, measured with the CDAI, and fatigue could at least partly be due to this single item in the CDAI.

The correlations between the subjective fatigue subscale with CRP levels was not clinically significant, except for the weak correlation between CRP and the CIS20R subscale of subjective feeling of fatigue, which is parallel with the findings of other authors.[9,35,40] Nevertheless, the theoretical intrinsic role of inflammation in fatigue pathogenesis remains interesting. Further evidence of a role of systemic inflammation in CD is the significant improvement of fatigue after anti-inflammatory treatment, such as the treatment with anti-TNF-alpha in the patients in the test-retest sample, which is in line with previous findings.[34,42] Possibly, the CRP as a traditional marker for inflammation might be insufficient to reflect the true relationship between inflammation and fatigue,[4] which might be improved through the direct measurement of pro-inflammatory cytokines such as TNF-alpha and IL-6.[7]

The associations of fatigue with the haematocrit were also low overall and only clinically significant for the CIS20R Activity subscale, which might suggest that a lower haematocrit in CD patients leads to reduced levels of activity. Romberg et al did not find a significantly higher overall level of fatigue in anaemic CD patients, but did report significant differences on the MFI-20 subscales of physical fatigue and reduced activity .[11] Jelsness-Jorgensen et al. found no association between low haemoglobin and chronic fatigue in CD.[9] Another study also found anaemia to play a minimal role when considered in the context of other factors,

such as disease activity, sleep quality and stress.[35] Overall, low haematocrit does not seem to be strongly associated to fatigue severity in CD, but further research needs to assess the role of anaemia and iron deficiency in CD, as previously suggested.[43]

Given the operating characteristics of the CIS20R in the CD population, this questionnaire could be a valuable addition to the arsenal of instruments that already exists. Previous studies in patients with inflammatory bowel disease either measured fatigue with a single question,[42] with a subscale of other questionnaires[15,36] or with other generic fatigue questionnaires which were not specifically validated for this population, such as the multidimensional fatigue inventory (MFI-20)[11,12,34,35] and the Fatigue Impact Scale (FIS).[40,44,45] Recently, the Fatigue Questionnaire (FQ) and the Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue subscale have been validated in IBD populations.[9,10] The FQ consists of 11 items and encompasses two dimensions: “physical fatigue” and “mental fatigue”. The FQ was validated as a secondary objective of a study on chronic fatigue in IBD. The FACIT-F is the 13-item, one-dimensional fatigue subscale of the multidimensional FACIT scale.[10] For the FQ, the test-retest reliability was not addressed in the original publication.[9] The validation of the FACIT-F in IBD patients was extensively done and showed good construct validity. However, no sensitivity to change was provided in this study, since it had been demonstrated in a previous study as a secondary aim.[10,36] Nevertheless, the validation study could have reproduced and confirmed the sensitivity to change.

The CIS20R is can provide broader information on several aspects of fatigue than both the FQ and the FACIT-F because of its 4 dimensions, which can be investigated as an overall score or as 4 separate subscales, without being substantially longer. Furthermore, the CIS20R has already proven to be a valid and reliable instrument in various conditions[19,21,32] and has extensively been used to assess fatigue several medical conditions, such as cancer[46] and other inflammatory diseases such as rheumatoid arthritis[33,47] and multiple sclerosis.[32,48] Recently a very large study in patients with Crohn’s Disease has been completed.[49] There have been more than 200 publications in different settings with this questionnaire and is available in several languages, also in English (see appendix A). The questionnaire is available, free of charge online. Therefore, the CIS20R can be a valuable additional instrument for the measurement of fatigue in CD in future studies.

An additional advantage of the CIS20R, are the validated cutoff scores. Cutoff scores defining the presence or absence of fatigue are poorly validated, which makes it hard to determine its prevalence.[7] Previous studies have based the cutoff arbitrarily on the 95th percentile of the score in a healthy control group[35] or, for the FQ, on a general consensus.[9]

For the CIS20R, a cutoff score for significant fatigue has previously been established and validated through a receiver operating characteristics analysis, using several subject samples (healthy and clinical) with expected differences in fatigue level.[21] The CIS20R overall score cutoff point of > 76 determines at which point the individual is "at risk" for subsequent sick leave or work disability due to fatigue.[21] Although fatigue should preferably be treated as a continuous variable when possible, such a cutoff score is particularly useful. On the one hand, cutoff values for questionnaires offer the possibility to use them as outcome parameters of a certain therapy in clinical trials to assess the effect of the studied medication or other therapeutic interventions. On the other hand, cutoff values allow to investigate the prevalence of severe fatigue or chronic fatigue in CD. In the current study, the proportion of CD patients with significant fatigue based on the cutoff score of the CIS20R, was about 63% overall, 74% in patients with active CD and 34% in patients with inactive CD. These prevalence rates were very comparable to a very recent study, also done with the CIS20R, which reported an overall prevalence of 65.7% in CD outpatients.[49] Previous studies in quiescent IBD have reported fatigue prevalence ranges from 41% to 48%[12,44] and from 27% to 41% in CD patients in remission. A study in 14 CD patients with moderate to severe disease activity found significant fatigue in 86% of the patients, which is comparable to our findings in severely active CD.[34] A recent study, measuring fatigue with the FQ, demonstrated that 52% of CD patients in remission or with mild to moderate disease activity reported substantial fatigue[9] and another recent study reported a prevalence of significant fatigue in 29% of CD patients with inactive disease and in 78% of CD patients with active disease.[35] Generally, our results were in line with these findings and significant fatigue was even more prevalent in patients with severe CD activity than in patients with mild to moderate disease activity. Jelsness-Jorgensen et al. also found that 29% of CD patients reported chronic fatigue. The overall proportion of patients with chronic fatigue in our study was somewhat higher than previously reported, especially in the subgroup of patients with mild to moderate disease activity (more than 40%). However, our study offers prevalence rates over the full range of CD activity (from inactive CD to severely active disease), both for significant fatigue and chronic fatigue. The prevalence of significant fatigue and chronic fatigue was significantly higher in women with CD, as could be expected from previous work.[40,50,51]

A number of limitations of this study and of the questionnaire should be mentioned. Methodologically, the use of a single VAS in the concurrent validity test could be mentioned as a limitation of this study. This VAS however, merely indicates the concurrences of both scales and is in no way treated as a "gold standard" (i.e. "criterion" validity), since this does not exist for the measurement of fatigue in CD, IBD or in general.[4,7] There is evidence that a single-item VAS can perform as well as longer scales in respect to sensitivity to change and

is at least as well correlated with clinical variables as longer scales, but it has slightly larger standard errors in cross-sectional analyses.[52] Since it is routinely used in clinical care, it should at least show a high correlation with a longer, multidimensional scale which measures the same concept. The concurrent validity is only a part of the validation process which indeed demonstrates that the CIS20R and the different subscales actually measure the severity of fatigue. This is obviously demonstrated by the very high correlations between the VAS on the one hand, and the multidimensional CIS20R and its subscales on the other hand. Furthermore, there is a conceptual logic to the convergence or correlation between the different subscales of the CIS20R and the VAS: the subscales and the VAS show very high correlations if they are conceptually closely related (e.g. Subjective Feeling of Fatigue which consists mainly of questions inquiring about physical fatigue) and the subscales and the VAS show lower, but still significant correlations if the conceptual link is less obvious (e.g. Concentration). Thus, we can deduce that a single question would not yield adequate information, since CD patients incorporate more in what they understand as “fatigue” than just physical fatigue. Therefore, the CIS20R probable approaches the concept of fatigue more closely than a single question can. Nevertheless, true criterion validation will most likely, never be possible.

The prevalence rates which were measured with the CIS20R in this study were relatively high. The university setting where this study has been done might account for this, which is an additional limitation. Nevertheless, a very recent study demonstrates very similar results, with the same questionnaire. It should also be noted that the cutoff which was defined, was set a level which put healthy individuals at risk for subsequent sick leave or work disability due to fatigue. Thus, this cutoff might be too strict for a population with chronic disease. On the other hand, most CD patients try to lead a “normal” life and their level of functioning should be measured accordingly. Therefore, their level of fatigue should be compared to healthy subjects and the same cutoff should be used. A further limitation to the interpretation of the aforementioned associations and is the cross-sectional nature of the data. Thus no directionality of associations can be assumed about a possible cause of fatigue.

In conclusion, the findings presented provide the CIS20R as a valid, reliable, reproducible and responsive questionnaire which can measure fatigue in 4 dimensions in CD patients, which can be assessed as an overall score or separately. Furthermore, this instrument has already been extensively used in other clinical populations and a cutoff has previously been determined and validated. Given the high prevalence of significant fatigue and chronic significant fatigue demonstrated in CD patients, it is imperative that fatigue is follow-up systematically. Thus, this relatively short questionnaire can be used as an outcome

instrument in future studies investigating the impact of interventions on fatigue and in the day to day care for CD patients who so often are burdened with this difficult symptom.

4.6 REFERENCES

1. Drossman DA, Leserman J, Li ZM, et al. The rating form of IBD patient concerns: a new measure of health status. *Psychosom Med*. 1991;53:701–12.
2. Irvine EJ. Development and subsequent refinement of the inflammatory bowel disease questionnaire: a quality-of-life instrument for adult patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 1999;28:S23–S27.
3. Stjernman H, Granno C, Jarnerot G, et al. Short health scale: a valid, reliable, and responsive instrument for subjective health assessment in Crohn's disease. *Inflamm Bowel Dis*. 2008;14:47–52.
4. Swain MG. Fatigue in chronic disease. *Clin Sci (Lond)*. 2000;99:1–8.
5. Rooy EC de, Toner BB, Maunder RG, et al. Concerns of patients with inflammatory bowel disease: results from a clinical population. *Am J Gastroenterol*. 2001;96:1816–1821.
6. Drossman DA, Patrick DL, Mitchell CM, et al. Health-related quality of life in inflammatory bowel disease. Functional status and patient worries and concerns. *Dig Dis Sci*. 1989;34:1379–1386.
7. Langenberg DR Van, Gibson PR. Systematic review: fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2010;32:131–143.
8. Czuber-Dochan W, Ream E, Norton C. Review article: Description and management of fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2013;37:505–16.
9. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is more prevalent in patients with inflammatory bowel disease than in healthy controls. *Inflamm Bowel Dis*. 2011;17:1564–72.
10. Tinsley A, Macklin EA, Korzenik JR, et al. Validation of the functional assessment of chronic illness therapy-fatigue (FACIT-F) in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2011;34:1328–36.
11. Romberg-Camps MJL, Bol Y, Dagnelie PC, et al. Fatigue and health-related quality of life in inflammatory bowel disease: results from a population-based study in the Netherlands: the IBD-South Limburg cohort. *Inflamm Bowel Dis*. 2010;16:2137–47.
12. Minderhoud IM, Oldenburg B, Dam PS van, et al. High prevalence of fatigue in quiescent inflammatory bowel disease is not related to adrenocortical insufficiency. *Am J Gastroenterol*. 2003;98:1088–1093.
13. Piche T, Ducrotte P, Sabate JM, et al. Impact of functional bowel symptoms on quality of life and fatigue in quiescent Crohn disease and irritable bowel syndrome. *Neurogastroenterol Motil*. 2010;22:626–e174.
14. Maunder R, Toner B, Rooy E De, et al. Influence of sex and disease on illness-related concerns in inflammatory bowel disease. *Can J Gastroenterol*. 1999;13:728–732.
15. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is associated with impaired health-related quality of life in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2011;33:106–14.
16. Irvine EJ, Feagan B, Rochon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Canadian Crohn's Relapse Prevention Trial Study Group. *Gastroenterology*. 1994;106:287–296.
17. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). *Gastroenterology*. 1979;77:843–846.
18. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis*. 2000;6:8–15.
19. Vercoulen JH, Swanink CM, Fennis JF, et al. Dimensional assessment of chronic fatigue syndrome. *J Psychosom Res*. 1994;38:383–392.
20. Beurskens AJ, Bültmann U, Kant I, et al. Fatigue among working people: validity of a questionnaire measure. *Occup Environ Med*. 2000;57:353–7.
21. Bultmann U, Vries M De, Beurskens AJ, et al. Measurement of prolonged fatigue in the working population: determination of a cutoff point for the checklist individual strength. *J Occup Health Psychol*. 2000;5:411–416.
22. Russel MG, Pastoor CJ, Brandon S, et al. Validation of the Dutch translation of the Inflammatory Bowel Disease Questionnaire (IBDQ): a health-related quality of life questionnaire in inflammatory bowel disease. *Digestion*. 1997;58:282–8.
23. Hlavaty T, Persoons P, Vermeire S, et al. Evaluation of short-term responsiveness and cutoff values of inflammatory bowel disease questionnaire in Crohn's disease. *Inflamm Bowel Dis*. 2006;12:199–204.
24. Jolliffe IT. *Principal Component Analysis, Second Edition*. 2nd ed. New York, NY: Springer Verlag; 2002:351.
25. Cheung WY, Garratt AM, Russell IT, et al. The UK IBDQ - A British version of the inflammatory bowel disease questionnaire: development and validation. *J Clin Epidemiol*. 2000;53:297–306.

26. Joliffe I, Morgan B. Principal component analysis and exploratory factor analysis. *Statistical Methods in Medical Research*. 1992;1:69–95.
27. Streiner DL. Starting at the beginning: an introduction to coefficient alpha and internal consistency. *Journal of personality assessment*. 2003;80:99–103.
28. Deyo RA, Diehr P, Patrick DL. Reproducibility and responsiveness of health status measures statistics and strategies for evaluation. *Controlled Clinical Trials*. 1991;12:S142–S158.
29. Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology*. 1989;96:804–810.
30. Sostegni R, Daperno M, Scaglione N, et al. Review article: Crohn's disease: monitoring disease activity. *Aliment Pharmacol Ther*. 2003;17 Suppl 2:11–7.
31. Shahid A, Shen J, Shapiro CM. Measurements of sleepiness and fatigue. *J Psychosom Res*. 2010;69:81–89.
32. Vercoulen JH, Hommes OR, Swanink CM, et al. The measurement of fatigue in patients with multiple sclerosis. A multidimensional comparison with patients with chronic fatigue syndrome and healthy subjects. *Arch Neurol*. 1996;53:642–9.
33. Hoogmoed D van, Fransen J, Bleijenberg G, et al. Physical and psychosocial correlates of severe fatigue in rheumatoid arthritis. *Rheumatology.(Oxford)*. 2010;49:1294–1302.
34. Minderhoud IM, Samsom M, Oldenburg B. Crohn's disease, fatigue, and infliximab: is there a role for cytokines in the pathogenesis of fatigue? *World J Gastroenterol*. 2007;13:2089–93.
35. Graff L a, Vincent N, Walker JR, et al. A population-based study of fatigue and sleep difficulties in inflammatory bowel disease. *Inflamm Bowel Dis*. 2011;17:1882–9.
36. Loftus E V, Feagan BG, Colombel JF, et al. Effects of adalimumab maintenance therapy on health-related quality of life of patients with Crohn's disease: patient-reported outcomes of the CHARM trial. *Am J Gastroenterol*. 2008;103:3132–3141.
37. Singh S, Blanchard A, Walker JR, et al. Common symptoms and stressors among individuals with inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2011;9:769–75.
38. Jelsness-Jørgensen L-P, Bernklev T, Henriksen M, et al. Chronic fatigue is associated with increased disease-related worries and concerns in inflammatory bowel disease. *World J Gastroenterol*. 2012;18:445–52.
39. Baars JE, Nuij VJAA, Oldenburg B, et al. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis*. 2012;18:1634–40.
40. Simrén M, Svedlund J, Posserud I, et al. Predictors of subjective fatigue in chronic gastrointestinal disease. *Aliment Pharmacol Ther*. 2008;28:638–47.
41. Persoons P, Vermeire S, Demyttenaere K, et al. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Aliment Pharmacol Ther*. 2005;22:101–10.
42. Lichtenstein GR, Bala M, Han C, et al. Infliximab improves quality of life in patients with Crohn's disease. *Inflamm Bowel Dis*. 2002;8:237–243.
43. Gasche C, Lomer MC, Cavill I, et al. Iron, anaemia, and inflammatory bowel diseases. *Gut*. 2004;53:1190–1197.
44. Bjornsson E, Simren M, Olsson R, et al. Fatigue in patients with primary sclerosing cholangitis. *Scand J Gastroenterol*. 2004;39:961–968.
45. Kalaitzakis E, Carlsson E, Josefsson A, et al. Quality of life in short-bowel syndrome: impact of fatigue and gastrointestinal symptoms. *Scand J Gastroenterol*. 2008;43:1057–1065.
46. Goedendorp MM, Gielissen MFM, Verhagen C a H, et al. Severe fatigue and related factors in cancer patients before the initiation of treatment. *Br J Cancer*. 2008;99:1408–1414.
47. Repping-Wuts H, Fransen J, Achterberg T Van, et al. Persistent severe fatigue in patients with rheumatoid arthritis. *J Clin Nurs*. 2007;16:377–383.
48. Vercoulen JHM. H, Swanink CM. M, Galama JM. M, et al. The persistence of fatigue in chronic fatigue syndrome and multiple sclerosis: development of a model. *J Psychosom Res*. 1998;45:507–17.
49. Vogelaar L, van't Spijker A, Tilburg AJP van, et al. Determinants of fatigue in Crohn's disease patients. *Eur J Gastroenterol Hepatol*. 2013;25:246–51.
50. Akerstedt T, Knutsson A, Westerholm P, et al. Mental fatigue, work and sleep. *J Psychosom Res*. 2004;57:427–433.
51. Hilsabeck RC, Hassanein TI, Perry W. Biopsychosocial predictors of fatigue in chronic hepatitis C. *J Psychosom Res*. 2005;58:173–178.
52. Wolfe F. Fatigue assessments in rheumatoid arthritis: comparative performance of visual analog scales and longer fatigue questionnaires in 7760 patients. *J Reumatol*. 2004;31:1896–902.

4.7 ADDENDUM TO CHAPTER 4

CIS20R

Checklist Individual Strength

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Instruction:

On the next page you find 20 statements. With these statements we wish to get an impression of how you have felt during the last two weeks. For example:

I feel relaxed

If you feel that this statement is entirely true, tick the left box; as follows:

I feel relaxed	**Yes**, that is true	**X**							**No**, that is not true

If you feel that this statement is not true at all, tick the right box; as follows:

I feel relaxed	**Yes**, that is true							**X**	**No**, that is not true

If you feel that this statement is neither "yes, that is true", nor "no, that is not true", tick the box that is most in accordance with how you have felt.

For example, if you feel relaxed, but not very relaxed, tick one of the boxes close to "yes, that is true": as follows:

I feel relaxed	**Yes**, that is true			**X**					**No**, that is not true

Do not skip any statement and tick each statement only once.

1.	I feel tired.	**yes, that is true**								**no, that is not true**
2.	I feel very active.	**yes, that is true**								**no, that is not true**
3.	Thinking requires effort.	**yes, that is true**								**no, that is not true**
4.	Physically I feel exhausted.	**yes, that is true**								**no, that is not true**
5.	I feel like doing lots of nice things.	**yes, that is true**								**no, that is not true**
6.	I feel fit.	**yes, that is true**								**no, that is not true**
7.	I think I do a lot in a day.	**yes, that is true**								**no, that is not true**
8.	When I am doing something, I can keep my thoughts on it. .	**yes, that is true**								**no, that is not true**
9.	I feel powerless	**yes, that is true**								**no, that is not true**
10.	I think I do very little in a day.	**yes, that is true**								**no, that is not true**
11.	I find it easy to focus my mind.	**yes, that is true**								**no, that is not true**
12.	I am rested.	**yes, that is true**								**no, that is not true**
13.	It takes a lot of effort to concentrate on things.	**yes, that is true**								**no, that is not true**
14.	Physically I feel I am in bad form.	**yes, that is true**								**no, that is not true**
15.	I have a lot of plans.	**yes, that is true**								**no, that is not true**
16.	I tire easily.	**yes, that is true**								**no, that is not true**
17.	I get little done.	**yes, that is true**								**no, that is not true**
18.	I don’t feel like doing anything.	**yes, that is true**								**no, that is not true**
19.	My thoughts easily wander.	**yes, that is true**								**no, that is not true**
20.	Physically I feel I am in an excellent condition.	**yes, that is true**								**no, that is not true**

SECTION II

IMPACT OF BIOPSYCHOSOCIAL ASPECTS ON CD

Chapter 5

5 The Impact of Major Depressive Disorder on the Short- and Long-Term Outcome of Crohn's Disease Treatment with Infliximab

Published in Alimentary Pharmacology & Therapeutics:

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The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. Aliment.Pharmacol.Ther. 2005; 22:101–110.

5.1 ABSTRACT

Background: *Major depressive disorder is the most common psychiatric diagnosis in Crohn's disease. In other chronic diseases, evidence suggests that depression influences the course of the disease. Strong evidence of such a mediating role of major depressive disorder in Crohn's disease has never been found.*

Aim: *To assess the relationship between major depressive disorder and outcome of treatment of luminal Crohn's disease with infliximab.*

Methods: *In this prospective study, 100 consecutive unselected patients underwent assessment of psychosocial, demographical disease-related biological and clinical parameters at baseline and at 4 weeks after infliximab. Major depressive disorder was diagnosed using the Patient Health Questionnaire. Subsequently, the patients were followed up clinically until the next flare or during 9 months.*

Results: *The Crohn's disease responded in 75% of the patients, and remission was achieved in 60%. The presence of major depressive disorder at baseline predicted a lower remission rate (OR = 0.166, 95%CI = 0.049–0.567, P = 0.004). At follow-up, 88% of the patients needed retreatment. At univariate regression analysis, major depressive disorder significantly decreased time to retreatment (P = 0.001). Multivariate Cox regression confirmed major depressive disorder as an independent determinant of active disease both at baseline and at re-evaluation (hazard ratio = 2.271, 95%CI: 1.36–3.79, P = 0.002).*

Conclusion: *Major depressive disorder is a risk factor for failure to achieve remission with infliximab and for earlier retreatment in patients with active luminal Crohn's disease. Assessment and management of major depressive disorder should be part of the clinical approach to patients with Crohn's disease.*

5.2 INTRODUCTION

Crohn's disease (CD) is an idiopathic, multifactorial, inflammatory, gastrointestinal disease characterized by a chronic and relapsing course. Over the past 20 years, significant advances have been made in understanding the pathophysiology and the natural history of CD and also treatment has greatly improved. The specific contribution of psychosocial factors to the evolution of the disease remains unclear and controversial. The majority of the patients and their treating physicians attribute an important role to psychosocial factors in the clinical exacerbation of symptoms or even of disease activity.[1] The presence of clinical depression may influence disease activity, aggravate disease symptoms, impact on the evolution of the disease or the response to therapy. The patient may experience enhanced morbidity, a poorer prognosis, and possibly even increased mortality.[2] Research into several chronic diseases over the past 5 years has yielded a growing body of evidence that psychological factors and depression in particular influence chronic disease in a complex manner.[3–5] In CD and ulcerative colitis (UC), the influence of stress and psychosocial factors has been investigated. A number of older studies yielded mixed results and sometimes measured only the subjective symptoms.[6–12] Four recent studies have substantiated the role of psychological distress in the exacerbation of 'objective' IBD activity. A cross-sectional study in UC showed a correlation between stressful life events, depression and disease activity.[13] The same group of investigators showed in a prospective study in 62 clinically remitted UC patients, that long-term perceived stress tripled the risk of exacerbation during the next 8 months. However, no clear association with depressive symptoms was found.[14] Bitton et al. followed 60 UC patients for 12 months and found that recent stressful events in the preceding month were associated with earlier time to relapse.[15] Finally, an 18-month prospective study in a mixed cohort of 60 clinically inactive CD and UC patients found that depressed mood and associated anxiety at baseline were independent risk factors for early clinical relapse and a higher frequency of relapses during the follow-up period.[16] However, it is possible that anxiety and depression in patients with IBD could be reactive to the disabling symptoms and to malnutrition.[17] Depression is the most common psychiatric diagnosis in CD and previous studies provide evidence supporting an association between CD and major depressive disorder (MDD).[18] Factors which are strongly associated with major depression, such as ineffective coping strategies, pessimism, low perceived control and 'catastrophizing' thoughts, are assumed to predict poor outcome of IBD.[1]

Our hypothesis was that psychosocial factors might influence the short- and long-term outcome of treatment of active CD. As therapy needs to be very effective to evaluate the importance of predictive factors, we have chosen treatment with infliximab (Remicade®) as

our experimental condition. The murine-human monoclonal chimeric antibody against tumour necrosis factor-α (TNF-α) has proven to be effective in severe, refractory luminal and fistulizing CD.[19–21] The primary objective of this prospective study was to assess if the presence of MDD at the time of treatment with infliximab had an
impact on the short-term effectiveness and the duration of response to infliximab. Moreover, we studied whether the usefulness of the instruments we use in drug trials in CD, i.e. the CDAI is influenced by the presence of MDD. As a secondary objective, we assessed the impact of other psychosocial factors, such as anxiety, perceived social support, sleep and alexithymia.

5.3 MATERIAL AND METHODS

5.3.1 Patients

Between January 2003 and July 2003, all consecutive out-patients at the Inflammatory Bowel Disease day clinic at the University Hospital Gasthuisberg (Leuven, Belgium) were asked to participate in the study. Only patients with refractory, active luminal disease treated with infliximab (5 or 10 mg/kg) were included. Patients had active CD (defined as CD Activity Index >150). Only patients previously diagnosed with CD on the basis of clinical, standard radiological or endoscopic criteria, supplemented with the typical histological appearance in a mucosal biopsy or resection specimen,[22] could enter the study. Patients younger than 18 years were excluded, as were patients who suffered from a short bowel syndrome, patients with stomas or if they participated in a clinical trial. The institutional Ethical Committee approved the study and written informed consent was obtained from all participants at inclusion.

5.3.2 Measurement of disease-related characteristics

Relevant demographic data were recorded. Clinical disease activity, assessed with the CD Activity Index (CDAI,[23]) and C-reactive protein (CRP) were assessed. Concomitant drug treatment was recorded. Established predictors of relapse of disease activity in CD such as smoking and use of non-steroidal anti-inflammatory drugs (NSAID),[24] as well as the predictors of short- and long-term response to infliximab such as concomitant use of immunosuppressive therapy, age and location of disease were recorded.[25–28] The phenotype of the CD patients was described using the Vienna classification.[29] Age at diagnosis, disease

duration, surgical history, time since previous treatment for a flare and the number of treatments for CD during the previous 12 months were recorded as well.

5.3.3 Measurement of psychosocial variables

Major depressive disorder. The presence of MDD was assessed by the self-administered Patient Health Questionnaire 9-items (PHQ-9). The PHQ-9 is the depression module of the PRIME-MD Patient Health Questionnaire and was validated in primary care patients[30] and in gastroenterological patients.[31] Applying a diagnostic algorithm allows to diagnose MDD, a categorical psychiatric diagnosis according to the Diagnostical and Statistical Manual 4th Edition (DSM-IV).[32] A summed score of all items provides a measure of severity of depression.[30]

Anxiety. The seven-item anxiety subscale of the Hospital Anxiety and Depression Scale (HADS)[33] was used to measure the intensity of anxiety symptoms. A higher summed score of the recoded items represents a higher intensity of anxiety symptoms (range 0–21). The HADS has been used in IBD populations[6] and has good reliability and validity.

Sleep. The number of hours of actual sleep per night over the past month was recorded. A Visual Analogue Scale of 100 mm measured the subjective quality of sleep. Furthermore, the patients were asked if they had sleep difficulties and a number of possible reasons for the sleep disorder were presented.

Alexithymia. In individuals with an alexithymic personality, stress finds outlet in bodily symptoms because of a deficit in mental representation of emotions.[34] It has been shown that alexithymia is higher in patients with IBD[35] and has an influence on the subjective health status of patients with IBD.[36]

The Toronto Alexithymia Scale 20 items (TAS-20) is a widely used alexithymia measure. Patients were instructed to indicate on a five-point likert scale to what extend they agreed or disagreed with 20

presented sentences. The TAS-20 has been shown to be a psychometrically sound measure of the alexithymia construct.[37]

Social support. Social support was measured with the Social Support List – Interactions (SSL-I).[38] Patients were instructed to indicate on a scale from 1 (seldom or never) to 4 (very often) how often each event happens to them. A first subscale of 34 items assessed the perceived positive interactions and a second subscale of seven items measured perceived negative interactions. A high score on the first subscale indicates a lot of perceived social support and a high score on the second subscale indicates a lot of negative interactions. The validity of the SSL-I has been demonstrated.[38]

5.3.4 Study protocol

At baseline, the clinical disease activity was assessed by physicians who were blinded for the psychological state of the patient and laboratory parameters were measured. Consequently, a single treatment with infliximab was given intravenously. Patients received self-report questionnaires assessing the psychosocial factors, which they completed just prior to the infliximab infusion. Four weeks after the infliximab infusion, the patients were reevaluated to assess the clinical and biological effects of the treatment and the same disease-related parameters were obtained. The number of patients who responded partially defined as a drop in CDAI score of ≥70, and who completely responded (remission), defined as a CDAI score <150, was recorded. Patients also completed questionnaires re-assessing relevant psychosocial factors (state variables: MDD, anxiety and sleep). After the initial treatment infusion and the assessment at 4 weeks the patients were prospectively followed for 9 months or until any treatment for a relapse of disease activity was needed. Repeated infliximab treatment was carried out only on relapse of disease activity (episodic retreatment strategy). Retreatment with infliximab or any initiation other therapy for active CD or admission to the hospital was considered as a relapse of disease activity.

5.3.5 Statistical analyses

For all statistical analyses, the Statistical Package for Social Sciences, version 12.0 for Windows (SPSS 2003, version 12.0, SPSS Inc., Chicago, USA) was used. Descriptive statistics of all relevant variables for the total group was calculated. Comparisons between groups were performed with Student's t-tests, Mann–Whitney U-tests, Fischer's exact tests, chi-squared tests and McNemar's tests, as appropriate. The Pearson's r and the Spearman's q were used to estimate univariate correlations. The determinants of the effectiveness (response and remission) of the infliximab treatment were assessed with forward stepwise logistic regression analyses. The C-statistic for the model was calculated. A higher C-statistic indicates that the logistic regression model assigns a higher probability of the correct classification of a case. The associations between the demographic, disease related and psychosocial variables and the time until the next treatment were analysed in a univariate fashion by Cox regression analysis. Subsequently, multivariate Cox regression analysis was used to identify the independent determinants of time until retreatment. Significant and moderate ($P < 0.10$) univariate determinants were entered into the model. Kaplan–Meier curves showing the retreatment-free cumulative survival of the patients with and without MDD at baseline were calculated. The curves were compared by Log Rank tests. The level of statistical significance was set at $P < 0.05$ and all P-values are two-sided.

5.4 RESULTS

A total of 109 consecutive CD patients were eligible to enter the study of whom nine patients refused to participate after reading the informed consent. There was no significant difference between the participating (n = 100) and non-participating patients for any of the baseline parameters. Table 1 shows the relevant sociodemographic, disease-related and psychosocial data of all participating patients at baseline. Sixteen patients had never been treated with infliximab (infliximab naive), while 84 patients had already received episodic infliximab previously.

All patients (n = 100) were clinically re-assessed at 4 weeks after therapy with infliximab. Seventy-eight patients (78%) had responded to treatment and 60% achieved remission. The overall CDAI score and CRP had improved significantly (drop in mean CDAI from 261 ± 100 to139 ± 96, P < 0.001 and drop in median CRP from 9.2 mg/L, IQR = 4.1–24.2 mg/L to 3.3 mg/L, IQR = 0–8.3 mg/L, P < 0.001). Eighty-eight patients (88%) required retreatment with infliximab or other rescue treatment for relapse of disease activity within 9 months. One of the 12 patients was lost to follow-up after 7 months before any retreatment. Eighty-two patients who relapsed (93%) received repeat infliximab infusion, six patients (7%) received other medications. The median number of days until retreatment for the total group was 68 days (IQR: 48–112 days).

Patients who needed retreatment had received more treatments for active CD during the previous year, in comparison to the non-retreated group (respectively median three treatments, (IQR = 2–5) treatments vs. median 1 treatment, (IQR = 0–2 treatments, P < 0.001). The median number of days since the previous treatment for active CD was also lower (not statistically significant) in the retreatment group (97 days; 95%CI: 88 to 106 days vs. 161 days; 95%CI: 86 to 236 days; Log Rank statistic = 3.3; P = 0.07). At baseline, the disease activity was significantly higher in patients who required retreatment (CDAI score ± s.d. = 267 ± 103), compared with patients who did not need retreatment (CDAI score ± s.d. = 219 ± 53, P = 0.02). This difference was not present at 4 weeks. Sixteen patients (16%) were infliximab naive at the time of inclusion. In comparison with the patients who had already received infliximab in the past, infliximab naive patients had received significantly fewer treatments for active CD in the previous year (P < 0.001) and there had been a longer time since the previous treatment for active CD (P = 0.002). However, the time until retreatment (P = 0.27) and all other baseline and prospective variables did not differ significantly. The response at 4 weeks was similar in both groups.

Table 1. Baseline description of the demographics, the disease characteristics, the psychosocial variables and the medication use for the total group

		Total group n = 100
Demographics		
% Men (n) / % Women (n)		41% (41) / 59% (59)
Age, mean years (SD)		34 (11)
Family status		
% Married – stable relationship (n)		61% (61)
% Living with family (n)		27% (27)
% Single (also divorced, widow(er)) (n)		12% (12)
% Smokers (n)		39% (39)
% Member patient association (n)		25% (25)
General course of the disease		
Age of Diagnosis, mean years (SD)		23 (8)
Duration of the disease, median years (IQR)		10 (5-16)
% Previous abdominal surgery (n)		38% (38)
Vienna Classification		
% Diagnosis < 40 years old (n)		95% (95)
Localization:	% Terminal ileum (n)	8% (8)
	% Colon (n)	32% (32)
	% Terminal ileum + colon (n)	51% (51)
	% Upper GI involvement (n)	9% (9)
Type of disease:	% Inflammatory type (n)	55% (55)
	% Penetrating type (n)	45% (45)
Treatments within the last year		
Any treatment for flare, median (IQR)		3 (2-5)
Disease Activity at inclusion		
CDAI, mean (SD)		261 (100)
CRP, median mg/L (IQR)		9.2 (4.1-24.2)
Psychosocial measures		
Depression intensity: PHQ-9 summed score (SD)		8.7 (5.7)
Anxiety intensity: HADS Anxiety (SD)		7.4 (4.4)
Alexithymia: TAS Total score (SD)		52.1 (12.7)
Social Support		
SSL Positive Interaction (SD)		78.0 (16.9)
SSL Negative Interaction(SD)		10.7 (3.6)
Sleep		
Hours of sleep (IQR)		7 (6-8)
Sleep quality (SD)		48.6 (25.4)
Pharmacological therapy		
% 5-Aminosalycilates (n)		9% (9)
% Corticosteroids (n)		9% (9)
% Immunosuppressants (n)		81%(81)
% Pain medication (n)		29% (29)
% Antibiotics (n)		4% (4)
% Vitamin supplements (n)		51% (51)
% Antidepressants (n)		17% (17)
% Sedatives (n)		12% (12)
% Hypnotics (n)		10% (10)
% Alternative treatments (n)		9% (9)

5.4.1 Major depressive disorder

Twenty-four patients (24%) were diagnosed with MDD at baseline and the mean summed depression scores were significantly higher (16.8 ± 3.7) in these patients than in non-

depressed patients (6.2 ± 3.2, P < 0.001). The demographics and the disease-related variables indicative of the disease course (in general and during the previous year) did not differ significantly between patients with and without MDD. Furthermore, the median number of days since the previous treatment for a flare of CD was not significantly different between patients with MDD (99 days; 95% CI: 85–117 days) and the patients without MDD (98 days; 95% CI: 69–127 days; Log Rank statistic <0.01; P = 0.97). Compared with non-depressed patients, significantly larger proportions of patients with MDD were treated with pain medication (25%, n = 11 vs. 44%, n = 18, P = 0.04), with vitamin supplements (43%, n = 33 vs. 75%, n = 18, P = 0.007), with antidepressants (5%, n = 4 vs. 29%, n = 7, P = 0.001), with hypnotics (7%, n = 5 vs. 21%, n = 5, P = 0.07) and with alternative therapies (4%, n = 3 vs. 25%, n = 6). The proportion of depressed patients at baseline did not differ between infliximab-naive and patients treated previously with infliximab (P = 0.76). At 4 weeks, 15 (63%) out of 24 patients still met the diagnostic criteria of MDD and one additional patient was diagnosed with MDD, resulting in a significantly smaller proportion of depressed patients (16%)

compared with baseline (24%, P = 0.021). The demographics and the disease-related variables did not differ significantly between patients still depressed at 4 weeks and non-depressed patients. Evolution of clinical disease activity and MDD The CDAI score in the 75 non-depressed patients (no MDD at baseline, nor at re-evaluation) improved significantly (243 ± 91 to 126 ± 86, P < 0.001). There was a significant positive relationship between the mean change in depression score and the mean change in CDAI in the non-depressed patients (r = 0.35, P = 0.002). At baseline, patients with MDD had significantly higher mean CDAI scores (n = 24; 322 ± 104) compared with non-depressed patients (P < 0.001). In the 15 patients with MDD at both measuring points, the CDAI score was still significantly higher at the 4 weeks re-assessment (P < 0.001), but it had also improved significantly (319 ± 104 to 223 ± 98; P = 0.009). The mean change of the CDAI score in these patients (D CDAI = 96 ± 120) did not differ from the change in non-depressed patients (P = 0.49). There was no relationship however between the mean change in depression score and the mean change in CDAI score in patients with MDD (r = -0.17, P = 0.53). The CDAI score decreased significantly in the nine patients who were depressed only at baseline (325 ± 111 to 119 ± 111; P < 0.001), and this improvement was significantly more pronounced than in non-depressed patients (P = 0.01) and than in patients with MDD at both measuring points (P = 0.01). The median CRP at baseline was significantly higher in this group (46.1 mg/L; IQR = 16.5–68.8 mg/L) in comparison with the non-depressed patients (8 mg/L; IQR = 4–21 mg/L, P = 0.003) and the patients with MDD at both timepoints (10.6 mg/L; IQR = 3.5–26.5 mg/L, P = 0.015). There was no significant difference in CRP levels between the two latter groups at

baseline (P = 0.68). There was no difference in CRP between the three groups at re-evaluation. The mean change in depression score in the patients who were only depressed at baseline was significant (14.4 ± 2.7 to 6.8 ± 2.7; P = 0.007).

Of note was the significant correlation between the depression score and well-being, one of the subjective items of the CDAI, in the total group (n = 100, r = 0.44, P < 0.001 at baseline and r = 0.58, P < 0.001 at re-evaluation). The correlation between the depression score and the CDAI score without well-being (adjusted CDAI) was significant for the non-depressed patients at baseline and at re-evaluation (respectively r = 0.23, P = 0.048 and r = 0.37, P = 0.003). However, in MDD patients, there was no correlation between the adjusted CDAI and the depression score at baseline (n = 24, r = 0.19, P = 0.38) and at re-evaluation (n = 16, r = -0.02, P = 0.95).

5.4.2 Influence of MDD on short-term therapy outcome

Univariate analysis demonstrated that MDD and the other psychological variables had no effect on response to infliximab. They were not entered in the predicting model. Forward, stepwise logistic regression identified six variables as independent determinants of failure to respond to infliximab (C-statistic = 0.85, Nagelkerke R^2 = 0.38, P < 0.001): the number of treatments for a flare in the previous year (P = 0.011), the number of days since the previous treatment (P = 0.039), previous abdominal surgery (P = 0.006), a diagnosis of CD after the age of 18 years (P = 0.011) and membership of a patient association (P = 0.042). Significantly fewer patients with MDD at baseline however achieved remission (29%) in comparison with non-depressed patients (70%, P < 0.001). Additionally, patients with possible anxiety were also significantly less likely to achieve remission (P < 0.001). A stepwise regression analysis with remission as dependent variable, adjusted for the baseline CDAI score (P = 0.006) and other significant biological variables (Nagelkerke R^2 = 0.40, P < 0.001), yielded MDD as an independent determinant of failure to achieve remission (OR = 0.166, 95%CI = 0.049–0.567, P = 0.004). Other negatively correlated determinants of remission were previous surgery (P = 0.003) and female gender (P = 0.034). The C-statistic for this model was 0.82, indicating that 82% of all possible pairs of cases in which one case was in remission and the other was not, the logistic regression model assigns a higher probability of remission to the case in remission.

5.4.3 Influence of MDD on long-term outcome of therapy

All patients with MDD at baseline needed retreatment in contrast with 73% of patients without MDD (Fischer's exact test: P = 0.04). Table 2 presents the results of the univariate Cox regression analyses for the significant (P < 0.05) and moderate (P < 0.10) psychosocial, demographic and disease-related and variables. There was a significant association between MDD and the time to retreatment. Also for anxiety, negative interaction in social support and sleep associations with time to retreatment were found. Also variables reflecting the course of the disease were moderately (P < 0.10) to significantly (P < 0.05) related to the time until the next treatment. Higher disease activity at baseline and even more at 4 weeks increased the risk of earlier retreatment, whereas a response to infliximab decreased this risk. All other variables did not show a statistically significant association with time until the next treatment for disease activity.

Table 2. Significant and moderate determinants in univariate Cox regression analyses.

	Relative Hazard	95% CI	p-value
Major Depressive Disorder (reference: no depression)	2.405	1.48-3.90	<0.001
Other Psychosocial variables			
Antidepressant (reference: no antidepressant)	1.657	.98-3.68	0.058
HADS Anxiety subscale (reference: no anxiety)			0.025
Possible anxiety disorder (HADA > 7)	1.361	.80-2.33	0.26
Probable anxiety disorder (HADA > 10)	2.038	1.22-3.42	0.007
SSL-I negative interaction	1.090 per 1 point ↑ on scale	1.03-1.16	0.006
Demographic and disease related variables			
Age (reference : quartile 4 = 42 – 59 years)			0.08
Age Quartile 1 (18 – 25 years)	1.321	.733-2.38	0.35
Age Quartile 2 (26 – 33 years)	2.054	1.12-3.76	0.02
Age Quartile 3 (34 – 41 years)	1.068	.58-1.97	0.83
Member of patient association (reference: non-member)	1.521	.94-2.45	0.09
Age of Diagnosis (reference: quartile 4 = 28 – 55 years)			0.002
Age Quartile 1 (6 – 17 years)	2.415	1.31-4.45	0.005
Age Quartile 2 (18 – 22 years)	2.963	1.61-5.47	0.001
Age Quartile 3 (23 – 27 years)	2.269	1.15-4.47	0.02
Colonic localization (reference other localisations)	1.491	.95-2.33	0.08
Any treatment for flare within the previous year	1.378 per treatment	1.23-1.55	<0.001
Number of days since the previous treatment	.996 per day	.994-.999	0.003
CDAI	1.002 per 1 point ↑ on CDAI	1.00-1.01	0.03
CDAI at re-evaluation	1.003 per 1 point ↑ on CDAI	1.00-1.01	0.008
Response at re-evaluation (reference: no response)	.554	.34-.92	0.02

Major depressive disorder and the use of antidepressants were identified by a multivariate Cox regression analysis as independent, significant predictors of time to retreatment (Table 3). Additionally, variables reflecting the course of the disease were also identified as predictors. A Kaplan–Meier survival curve demonstrates the significant difference in time until the next retreatment between patients with MDD and without MDD (Figure 1). Substituting

the baseline measurements by the measurements at re-evaluation (PHQ, HADS and CDAI at re-evaluation) and adding response to the infliximab treatment to the model did not yield different determinants. Moreover, the 16 patients with MDD at re-evaluation had a relative risk of 3.218 (95%CI = 1.712–6.051, P < 0.001) for earlier relapse, whereas post hoc analysis showed that the risk at earlier relapse was not significantly increased in the nine patients who only fulfilled the diagnostic criteria for MDD at baseline (RR = 1.282, 95%CI = 0.641–2.565, P = 0.48).

Table 3. Multivariate Cox regression with time to retreatment as dependent variable and baseline variables as predictors.

Variables	Relative Hazard	95% CI	p-value
Major Depressive Disorder (reference: no MDD)	2.271	1.36-3.79	0.002
Antidepressant (reference: no antidepressant)	2.332	1.16-4.67	0.017
Age of Diagnosis (reference: quartile 4 = 28–55 y)			0.003
Age Quartile 1 (6 – 17 years)	2.122	1.12-4.02	0.021
Age Quartile 2 (18 – 22 years)	3.062	1.61-5.82	0.001
Age Quartile 3 (23 – 27 years)	2.89	1.44-5.79	0.003
Any treatment for flare within the previous year	1.332 per treatment	1.17-1.51	<0.001
Crohn's colitis (reference: other localisations	1.825	1.14-2.94	0.013

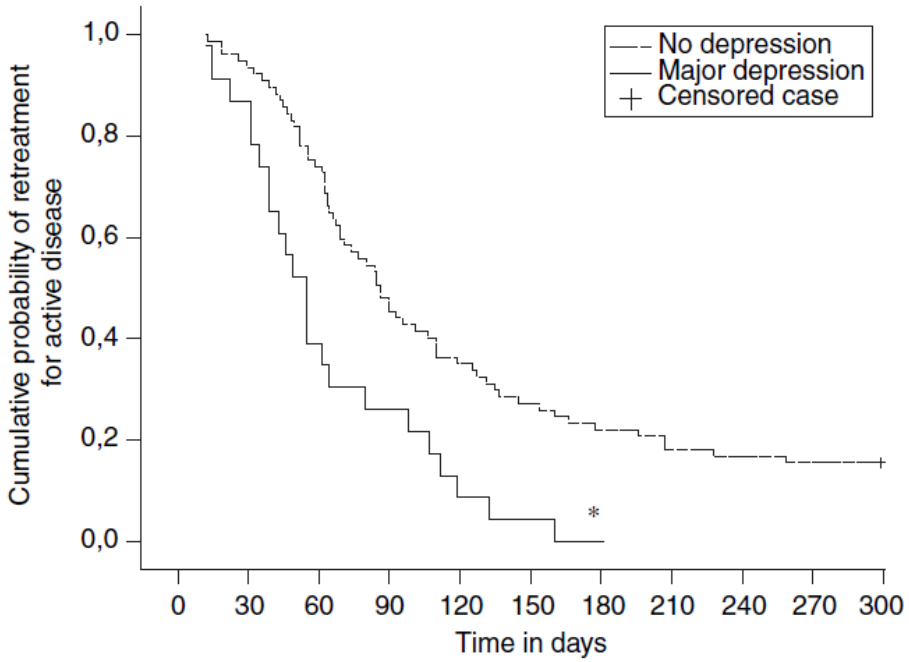

Figure 1. Patients with MDD needed retreatment for disease activity significantly earlier than patients without MDD.
*Log Rank statistic = 11.62; $P = 0.0007$.

5.5 DISCUSSION

The principal finding of this study is that MDD is a predictor of failure to reach remission after infliximab treatment for active luminal CD and of an earlier need for retreatment. Major depressive disorder is a highly relevant comorbid condition to patients with CD, given that this disorder is the most common psychological disorder in CD. Studies in IBD and other chronic medical diseases, which assessed MDD as a separate categorical diagnosis, found prevalence rates of 6–14%.[6, 39, 40] At baseline, the prevalence of depression in our cohort was higher, which could be explained by the fact that only patients with active CD were included. At re-assessment after treatment, the proportion of patients with MDD was comparable with that previously reported,[6, 39] suggesting that if MDD was diagnosed at baseline, but not at re-evaluation, this reflected a psychological reaction to the disease activity in a subgroup of patients. The higher CRP at baseline and the significantly larger improvement of disease activity in this subgroup of patients supports the hypothesis that these patients were indeed objectively more seriously ill. However, the severity of objective disease activity does not provide sufficient explanation for the presence of MDD in the 15 patients who still fulfilled the diagnostic criteria of MDD after treatment, as in these patients the disease activity had also improved significantly. Improvement of the disease activity had little effect on the depressed mood in the these patients.

Major depressive disorder and other psychosocial variables were not predictive of short-term response to infliximab. Predominantly variables reflecting a severe or refractory course of CD were associated to a failure of short-term response to infliximab treatment, as already found in previous studies.[25–28] Conversely, MDD was an independent determinant in the disease activity-adjusted prediction of short-term remission, as was female gender. In the present study, MDD and antidepressant therapy were also significant risk factors influencing the long-term outcome of CD after infliximab. It has been shown that depressed mood contributes to poorer wellbeing, lower pain thresholds and more reported symptoms, which might explain the higher CDAI scores at baseline and at re-evaluation in depressed patients.[41, 42] Furthermore, female gender influences symptom reporting, whether or not there is a psychiatric comorbidity.[43]

Of all assessed psychosocial variables, MDD was the only variable which was an independent determinant of unfavorable short-term (remission) and long-term outcome after infliximab. Besides the greater disease severity in patients with MDD, the presence of MDD might also bias the short-term outcome after treatment by its influence on the disease activity score, the CDAI. The high weight resulting from subjective well-being is a recognized

limitation in the CDAI.[44] This is illustrated in the present study by the significant relationship between the depression score and subscore of well-being on the one hand, and by the lack of relationship between the depression score in MDD patients and the adjusted CDAI score on the other hand. Thus, the presence of MDD clearly influences the CDAI score. As a consequence remission may not have been achieved, due to a higher baseline and follow-up CDAI score through the subjective item of well-being, whereas response on the CDAI score which decreases proportionally in comparison with non-depressed patients was not affected. Conversely, MDD seems to have a true effect on the long-term outcome of CD after infliximab treatment. Our results show that depression in the subgroup of 15 patients with MDD at both measuring points was unrelated to the disease course and overall severity prior to inclusion in the study. Yet, the relative risk at early retreatment was three times higher for patients with MDD after treatment with infliximab.

The presence of MDD may worsen the disease. Major depressive disorder may have a direct influence on immune deregulation associated with CD. Several studies have shown that MDD is associated with enhanced production of cytokines, such as IL-6 and TNF-α, and successful pharmacological treatment of MDD decreases the elevated cytokine levels.[45–47] Major depressive disorder might also have an indirect influence through adverse health behaviors, such as smoking and inappropriate diet, or through noncompliance to self-care regimens. A recent meta-analysis has indicated that depression is one of the main reasons of non-compliance to treatment in patients with chronic diseases.[48] Additionally, IBD patients with a comorbid psychiatric disorder present with significantly more medically unexplained symptoms and have an altered perception of disease severity.[39] Furthermore, depressed patients with chronic medical illness consult their physician more frequently.[2] These factors might ultimately lead to earlier consultation and retreatment in CD patients with MDD.

Our findings support the need for an active identification and management of MDD in CD patients instead of merely attributing depressed mood to the severity of the disease. This certainly applies to patients who still show signs of depression after an adequate treatment for active CD. This might also be the right time to screen for MDD, which is a clearly defined and well-treatable psychiatric disorder. Recent evidence has indicated that psychological support improves compliance to gluten free diet in patients with celiac disease.[49] In diabetic patients, psychological therapies result in a reduction of psychological distress and in significantly better glycemic control.[50] Furthermore, it may be important to detect MDD in patients under consideration for pivotal drug studies as patients with concomitant MDD may not be good candidates to take part in these studies. Active screening for MDD in studies and during treatment for CD can be done with the PHQ. This is a very short (nine items) and

reliable self-report questionnaire, which yields a categorical diagnosis of MDD and a severity score of depression and which has been validated in medical and gastroenterological patients.[30, 31] In this way, awareness in gastroenterologists and patients could be increased. Psycho-education of CD patients concerning psychological distress and disorders should be standard practice. Efficacious, safe medication and psychotherapy for MDD are also available. Ideally, follow-up of CD patients with MDD should be done in collaboration with a liaison psychologist or psychiatrist. Future research is needed to identify subgroups of patients at risk of developing MDD or other psychiatric disorders and to examine if interventions such as prevention strategies, psychopharmacotherapy, or psychotherapy have an effect on the outcome after treatment.

In conclusion, our results show that MDD influences the short- (remission) and long-term outcome after treatment with infliximab in CD patients. Persistent MDD after adequate treatment of CD is a risk factor for early retreatment. As there is no cure for CD, it is important to optimize the available therapy and ultimately maximize the time of remission. Therefore, adequate management of depression in the CD patient is necessary.

5.6 REFERENCES

1. Ringel Y, Drossman DA. Psychosocial aspects of Crohn's disease. Surg Clin North Am 2001; 81: 231–52.
2. Katon WJ. Clinical and health services relationships between major depression, depressive symptoms, and general medical illness. Biol Psychiatry 2003; 54: 216–26.
3. Musselman DL, Evans DL, Nemeroff CB. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. Arch Gen Psychiatry 1998; 55: 580–92.
4. Leserman J. HIV disease progression: depression, stress, and possible mechanisms. Biol Psychiatry 2003; 54: 295–306.
5. Musselman DL, Betan E, Larsen H, Phillips LS. Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment. Biol Psychiatry 2003; 54: 317–29.
6. Andrews H, Barczak P, Allan RN. Psychiatric illness in patients with inflammatory bowel disease. Gut 1987; 28: 1600–4.
7. Duffy LC, Zielezny MA, Marshall JR, et al. Lag time between stress events and risk of recurrent episodes of inflammatory bowel disease. Epidemiology 1991; 2: 141–5.
8. Duffy LC, Zielezny MA, Marshall JR, et al. Relevance of major stress events as an indicator of disease activity prevalence in inflammatory bowel disease. Behav Med 1991; 17: 101–10.
9. North CS, Alpers DH, Helzer JE, Spitznagel EL, Clouse RE. Do life events or depression exacerbate inflammatory bowel disease? A prospective study. Ann Intern Med 1991; 114: 381–6.
10. Garrett JW, Drossman DA. Health status in inflammatory bowel disease. Biological and behavioral considerations. Gastroenterology 1990; 99: 90–6.
11. von Wietersheim J, Kohler T, Feiereis H. Relapse-precipitating life events and feelings in patients with inflammatory bowel disease. Psychother Psychosom 1992; 58: 103–12.
12. Greene BR, Blanchard EB, Wan CK. Long-term monitoring of psychosocial stress and symptomatology in inflammatory bowel disease. Behav Res Ther 1994; 32: 217–26.
13. Levenstein S, Prantera C, Varvo V, et al. Psychological stress and disease activity in ulcerative colitis: a multidimensional cross-sectional study. Am J Gastroenterol 1994; 89: 1219–25.
14. Levenstein S, Prantera C, Varvo V, et al. Stress and exacerbation in ulcerative colitis: a prospective study of patients enrolled in remission. Am J Gastroenterol 2000; 95: 1213–20.
15. Bitton A, Sewitch MJ, Peppercorn MA, et al. Psychosocial determinants of relapse in ulcerative colitis: a longitudinal study. Am J Gastroenterol 2003; 98: 2203–8.
16. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. Psychosom Med 2004; 66: 79–84.

17. Addolorato G, Capristo E, Stefanini GF, Gasbarrini G. Inflammatory bowel disease: a study of the association between anxiety and depression, physical morbidity, and nutritional status. Scand J Gastroenterol 1997; 32: 1013–21.
18. North CS, Alpers DH. A review of studies of psychiatric factors in Crohn's disease: etiologic implications. Ann Clin Psychiatry 1994; 6: 117–24.
19. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med 1997; 337: 1029–35.
20. Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340: 1398–405.
21. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004; 350: 876–85.
22. Lashner BA. Clinical features, laboratory findings, and course of Crohn's disease. In: Kirsner, JB, ed. Inflammatory Bowel Disease, 5th edn. Chicago, Illinois: W.B. Saunders Company, 2000: 305–314.
23. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). Gastroenterology 1979; 77: 843–6.
24. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417–29.
25. Mortimore M, Gibson PR, Selby WS, Radford-Smith GL, Florin TH. Early Australian experience with infliximab, a chimeric antibody against tumour necrosis factor-alpha, in the treatment of Crohn's disease: is its efficacy augmented by steroid-sparing immunosuppressive therapy? The Infliximab User Group. Intern Med J 2001; 31: 146–50.
26. Parsi MA, Achkar JP, Richardson S, et al. Predictors of response to infliximab in patients with Crohn's disease. Gastroenterology 2002; 123: 707–13.
27. Vermeire S, Louis E, Carbonez A, et al. Demographic and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn's disease. Am J Gastroenterol 2002; 97: 2357–63.
28. Arnott ID, McNeill G, Satsangi J. An analysis of factors influencing short-term and sustained response to infliximab treatment for Crohn's disease. Aliment Pharmacol Ther 2003; 17: 1451–7.
29. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000; 6: 8–15.
30. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9. Validity of a brief depression severity measure. J Gen Intern Med 2001; 16: 606–13.
31. Persoons P, Luyckx K, Fischler B. Psychiatric diagnoses in gastroenterolgy: validation of a self-report instrument (PRIME-MD PHQ), epidemiology and recognition. Gastroenterology 2001; 120: 615.
32. Association AP. Diagnostic and Statistical Manual of Mental Disorders, 4th edn. Washington: APA, 1994.
33. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67: 361–70.
34. Taylor G, Bagby R, Parker J. The alexithymia construct. A potential paradigm for psychosomatic medicine. Psychosomatics 1991; 32: 153–64.
35. Porcelli P, Zaka S, Claudio L, Centonze S, Taylor G. Alexithymia in inflammatory bowel disease. Psychother Psychosom 1995; 64: 49–53.
36. Verissimo R, Mota-Cardoso R, Taylor G. Relationships between alexithymia, emotional control and quality of life in patients with inflammatory bowel disease. Psychother Psychosom 1998; 67: 75–80.
37. Bagby R, Parker J, Taylor G. The twenty-item Toronto Alexithymia Scale. II. Convergent, discriminant, and concurrent validity. J Psychosom Res 1994; 38: 33–40.
38. van Sonderen E. Sociale Steun Lijst-Interacties (SSL-I) en Sociale Steun Lijst-Discrepanties (SSL-D). Een handleiding. Groningen: NGC, Rijksuniversteit Groningen, 1993.
39. Walker EA, Gelfand MD, Gelfand AN, Creed F, Katon WJ. The relationship of current psychiatric disorder to functional disability and distress in patients with inflammatory bowel disease. Gen Hosp Psychiatry 1996; 18: 220–9.
40. Katon WJ, Ciechanowski P. Impact of major depression on chronic medical illness. J Psychosom Res 2002; 53: 859–63.
41. Guthrie E, Jackson J, Shaffer J, Thompson D, Tomenson B, Creed F. Psychological disorder and severity of inflammatory bowel disease predict health-related quality of life in ulcerative colitis and Crohn's disease. Am J Gastroenterol 2002; 97: 1994–9.
42. Bair MJ, Robinson RL, Katon W, Kroenke K. Depression and pain comorbidity: a literature review. Arch Intern Med 2003; 163: 2433–45.
43. Kroenke K, Spitzer RL. Gender differences in the reporting of physical and somatoform symptoms. Psychosom Med 1998; 60: 150–5.
44. Sostegni R, Daperno M, Scaglione N, Lavagna A, Rocca R, Pera A. Review article: Crohn's disease: monitoring disease activity. Aliment Pharmacol Ther 2003; 17: 11–7.
45. Lanquillon S, Krieg JC, Bening-Abu-Shach U, Vedder H. Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology 2000; 22: 370–9.
46. Kiecolt-Glaser JK, Glaser R. Depression and immune function: central pathways to morbidity and mortality. J Psychosom Res 2002; 53: 873–6.

47. Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E. Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. Psychopharmacology (Berl) 2003; 170: 429–33.
48. DiMatteo MR, Lepper HS, Croghan TW. Depression is a risk factor for noncompliance with medical treatment: metaanalysis of the effects of anxiety and depression on patient adherence. Arch Intern Med 2000; 160: 2101–7.
49. Addolorato G, De Lorenzi G, Abenavoli L, Leggio L, Capristo E, Gasbarrini G. Psychological support counselling improves gluten-free diet compliance in coeliac patients with affective disorders. Aliment Pharmacol Ther 2004; 20: 777–82.
50. Ismail K, Winkley K, Rabe-Hesketh S. Systematic review and meta-analysis of randomised controlled trials of psychological interventions to improve glycaemic control in patients with type 2 diabetes. Lancet 2004; 363: 1589–97.

SECTION III

IMPACT OF CROHN'S DISEASE ON PSYCHOSOCIAL FACTORS

Chapter 6

6 The Evolution of Serum Tryptophan in Crohn's Disease after Treatment with Infliximab and the Correlation with Health Related Quality of Life.

Paper to be submitted

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6.1 ABSTRACT

Background: *Decreased tryptophan (TRP) is associated with active inflammation in Crohn's Disease (CD) due to induction of Indoleamine-2,3-dioxygenase. Decreased TRP levels might be associated with psychological symptoms in inflammation. The evolution of TRP after treatment of CD with infliximab and its correlation with quality of life are unknown.*
Aim: *to assess the changes in TRP induced by treatment with infliximab in CD and study the relationship between TRP and quality of life (HRQL).*
Methods: *Consecutive CD patients were enrolled. At baseline, clinical disease activity (CDAI) and C-reactive protein (CRP) were measured and patients completed the Inflammatory Bowel Disease Questionnaire (IBDQ). Total and free TRP were determined. Patients were subsequently treated with infliximab and re-assessed 4 weeks later. Control patients with stable CD also participated.*
Results: *Overall, 39 CD patients with active disease and 9 CD controls participated. TRP levels were significantly lower at baseline in CD patients with active disease and increased significantly after treatment with infliximab. In a subgroup of 20 CD patients with normalized CRP after infliximab, there was a significant increase in total and free TRP, whereas no significant change in total or in free TRP was observed in a subgroup (n=19) with elevated CRP after infliximab. Total TRP in patients with normalized CRP after infliximab was not different from the second measurement of total TRP in control CD patients, whereas total TRP remained significantly lower in patients with elevated CRP after infliximab. Conversely, free TRP remained significantly lower in both subgroups at re-evaluation. Change (Δ_{RE-BL}) in IBDQ score correlated with Δ_{RE-BL}total and Δ_{RE-BL}free TRP score. Linear regression yielded Δ_{RE-BL}CDAI and Δ_{RE-BL}free TRP as determinants of Δ_{RE-BL}IBDQ.*
Conclusions: *Successful treatment with infliximab results in a normalization of total TRP, whereas free TRP remains significantly lower. The Δ_{RE-BL}IBDQ was correlated with the Δ_{RE-BL}TRP and this change was independently determined by the change in free TRP concentration. This might be indicative of a role of TRP in CD patients' subjective well-being.*

6.2 INTRODUCTION

Crohn's Disease (CD) is a multifactorial, inflammatory, gastrointestinal disease which has a chronic and relapsing course. Exacerbations of CD are characterized by segmental transmural gastrointestinal inflammation, variable in extent and localization. The well-controlled balance of the intestinal immune system is disturbed at all levels and antigens from commensal microbiotica induce an exaggerated inflammatory response in CD. Subsequently, the innate and the adaptive immune response are dysregulated, with a pronounced increase of immune cells (natural killer cells, effect T cells, granulocytes, macrophages) into the lamina propria and the secretion of large amounts of proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon γ (INF-γ) and tumour necrosis factor α (TNF-α). The release of cytokines leads to the attraction of more inflammatory leucocytes which contribute to tissue damage and ultimately to the vicious cycle of intestinal inflammation.[1] Additionally, these cytokines contribute to the systemic immune activation and symptoms which accompany severe exacerbations.[2]

Tryptophan (TRP) is an essential large neutral amino acid (LNAA) and the precursor of serotonin (5HT). In CD, decreased TRP availability or depletion could be caused by increased oxidative TRP catabolism along the kynurenine pathway (figure 6.1) in the presence of systemic inflammation, through the induction of the enzyme indolamine-2,3-dioxygenase (IDO), which acts as the initial and rate-limiting step.[3,4] This has recently been confirmed by Gupta et al. who demonstrated that decreased serum tryptophan (TRP) and intestinal IDO are associated with CD disease activity.[5] The IDO activity is strongly induced by proinflammatory cytokines such as Toll-like receptor (TLR) activation, IFN-γ and TNF-α, which are present during an exacerbation of CD.[5–7] IDO, which is expressed in cells of the innate immune system such as macrophages and dendritic cells, is important for immune tolerance by suppressing T-cell responses of the adaptive immune system.[3,8,9] Evidence for the activation of IDO after immune activation and its effect on serum TRP concentrations comes from in vitro experiments, experimentally cytokine-treated animals[10,11] and from measurements in patients undergoing cytokine therapy (e.g. in cancer or hepatitis C).[6,12]. Moreover, overexpression of IDO has been demonstrated in human inflammatory bowel disease[7] and a decrease of serum TRP concentrations has also been shown in other inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis and in Alzheimer's disease.[4,13,14]

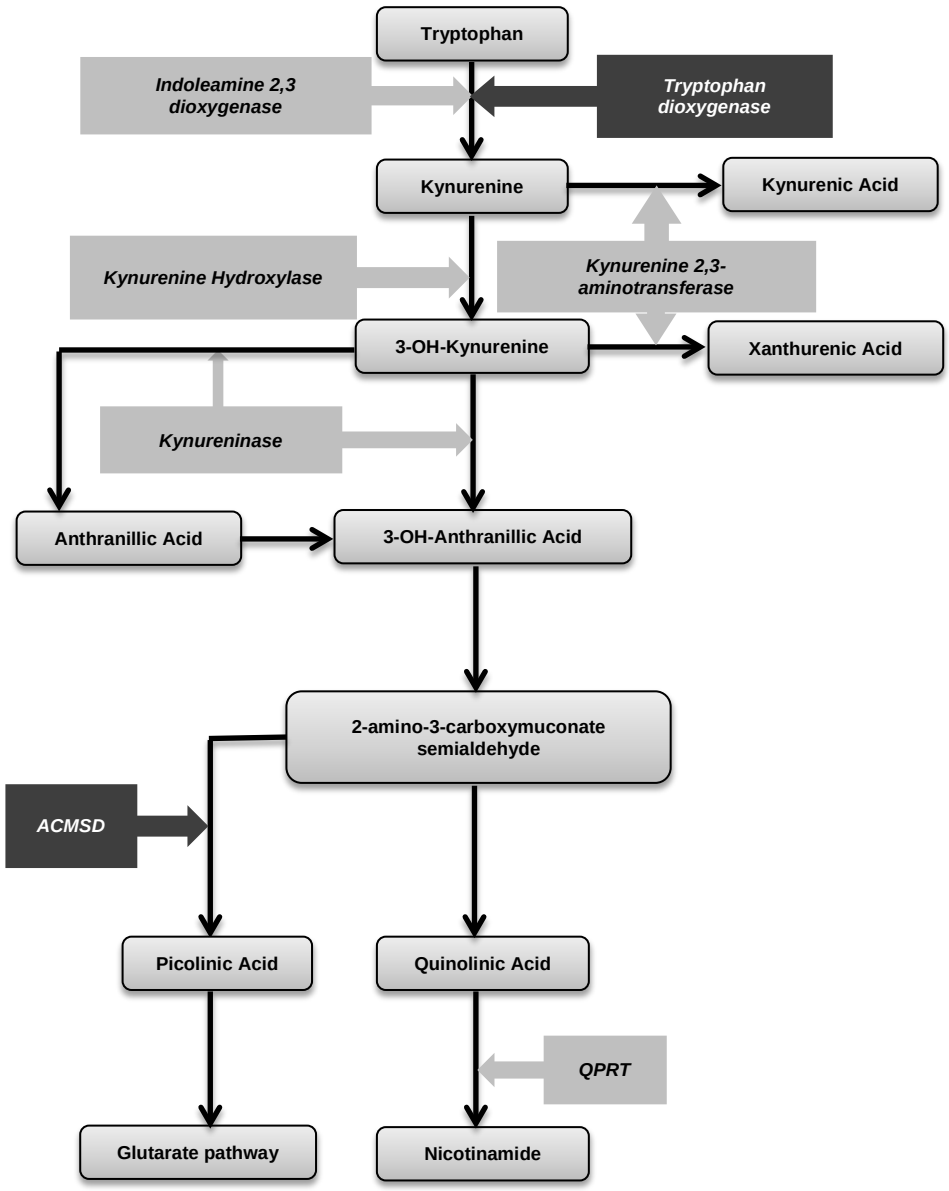

Figure 6.1 Tryptophan catabolites: kynurenine or TRYCAT pathway

Reduced TRP levels might also be relevant for the psychological status and the health related quality of life (HRQL) of the CD patient. Psychological distress, disturbed emotional well-being and psychopathology are highly prevalent in CD. Commonly, psychological symptoms are considered to be secondary to CD and they are attributed to stress, caused by the severity of the disease and the unpredictable and often aggressive disease course.[15] The more severe psychological problems and low scores on psychosocial dimensions of HRQL scales in patients with active CD in comparison with patients with stable CD is also an additional argument for this hypothesis.[16] Recently, a more direct impact of CD on the development of psychological symptoms via TRP has been suggested.[4] Serotonin (5-HT), of which TRP is the precursor, plays a key role in the current biological theories on mood regulation and on the pathophysiology of mood disorders.[17] TRP is relatively scarce

compared to the other LNAA's. The majority of the TRP is metabolized peripherally with about 1% being used to synthesize 5-HT, mainly in the gastrointestinal tract. An active, non-specific protein transports non-protein bound (free) TRP through the blood-brain barrier (BBB). At this site, there is competition between TRP and the other LNAA's. The first step (hydroxylation) of the 5-HT synthesis is rate-limiting (only 50% of the enzyme is saturated in physiological conditions).[4] A reduced availability of TRP in the central nervous system (CNS) might therefore directly compromise the 5-HT synthesis. This may lead to a change in central serotonergic neurotransmission with psychological symptoms, such as altered mood, impaired HRQL or even Major Depressive disorder.[18] The effect of active inflammation in CD on the TRP levels might increase the vulnerability of CD patients for an affected HRQL, altered mood or even Major Depressive Disorder, which might in turn affect the course of the disease. Previously, we have shown that the presence of major depressive disorder is a risk factor for failure to achieve remission after treatment with infliximab and if Major Depressive disorder is still present after treatment, these patients need earlier retreatment for active disease.[19]

There is some evidence of impaired TRP availability in CD and of increased oxidative TRP catabolism to kynurenine during an exacerbation of CD.[7,20,21] The recent study by Gupta et al has demonstrated that IDO1 expression is indeed associated with a significant decrease in total serum TRP and an increase in the ratio of kynurenine over TRP (K/T), thus suggesting the K/T ratio as a potential useful novel biomarker of CD activity.[5] However, the evolution of serum TRP concentrations after treatment of CD with infliximab and its correlation with subjective measures such as quality of life, have not yet been studied. Therefore, the aim of this study was to assess the total and free serum TRP concentrations in patients with a severe exacerbation of CD compared to CD patients in remission and the changes in TRP concentrations induced by treatment with anti-TNF-α (infliximab, IFX, Remicade®). Free TRP was assessed separately because this fraction is relevant for central 5-HT neurotransmission, since only free TRP, unbound to protein, will pass the BBB. Finally, we also studied the relationship between total and free serum TRP concentrations and the quality of life (HRQL) at different time points in patients with Crohn's disease.

6.3 METHODS

6.3.1 Patients & Procedure

Patients with CD attending the Inflammatory Bowel Disease day clinic at the University Hospital Gasthuisberg (Leuven, Belgium) enrolled prospectively in the study. Only patients previously diagnosed with CD based on clinical, standard radiological or endoscopic criteria, supplemented with the typical histological appearance in a mucosal biopsy or resection specimen,[22] could enter the study. Patients younger than 18 years were excluded, as were patients who suffered from a short bowel syndrome and patients with stomas. Furthermore, only patients with active disease refractory to steroids and (or) immunosuppression or intolerant to these drugs who were candidates to be treated with infliximab were included. Additionally, CD in remission were also recruited prospectively as a control group. The institutional Ethics Committee approved the study and written informed consent was obtained from all participants at inclusion.

At enrollment (baseline), relevant demographic data (gender and age), smoking status, age at diagnosis, Body Mass Index (BMI), disease duration, disease location according to the Vienna classification,[23] concomitant drug treatment and surgical history were recorded. Clinical Disease activity was assessed with the Crohn's Disease Activity Index (CDAI[24]). C-reactive protein (CRP) was measured. Disease specific Health Related Quality of Life (HRQL) was assessed with the disease specific Inflammatory Bowel Disease Questionnaire (IBDQ) in patients treated with IFX. The IBDQ consists of 32 items and is subdivided in 4 dimensions: bowel symptoms, systemic symptoms, emotional dysfunction symptoms and social dysfunction symptoms.[25] The IBDQ is a self-report questionnaire and patients indicate their answer on a 7-point likert scale. A lower total score is indicative of a lower HRQL (range 32-224). The IBDQ is a valid, reliable, responsive and reproducible questionnaire.[26] It has been used in numerous randomized clinical trials, including those with Infliximab. [27,28] A validated Dutch translation was used.[29]

Blood samples were obtained and centrifuged within 15 minutes during 10 minutes at 2500 rounds per minute (rpm) at 4°C, thus isolating serum to measure total TRP and free TRP (TRP unbound to protein) which was subsequently stored for later analysis at -70°C. Subsequently, a single treatment with infliximab 5mg/kg was given intravenously. Four weeks after the infliximab treatment all patients were re-evaluated in the same way to assess the clinical and biological effect of the treatment and the same disease-related

parameters were obtained. Blood samples were again obtained and processed in the same way.

6.3.2 Measurement of total and free Tryptophan concentrations

High-pressure liquid chromatography (HPLC) (Waters, Milford, MA, USA) was used to assess the total and free TRP concentrations in the serum and for the assays, the method described by Williams et al was followed,[30] however a HPLC gradient analysis was used to separate TRP from other amino acids. TRP was detected at 280 nm with a photodiode array detector. This method was first validated. L-Tryptophan was obtained from Fluka. Reagents for the buffer were from Merck and methanol, HPLC-grade, from Fisher Scientific. Other reagents were analytical grade. For total TRP, 250 µl serum was mixed with 250 µl perchloric acid 2M in Eppendorf 1.5 ml tube, centrifuged for 5 minutes at 12,000 rpm in Eppendorf centrifuge type 5451C at 4°C. Supernatants were directly injected (25 µl). For free TRP, we used a Denley thermostated centrifuge to spin the microconcentrators for 30 minutes at 2000 g. The ultrafiltrate was diluted 1:1 with 2M perchloric acid, mixed and injected directly (25 µl). An HPLC system, equipped with a photodiode array detector type Waters 996 (Waters, Milford, MA, USA) was used at 280 nm for the detection of TRP. The mobile phase buffer consisted of 100 mM citrate, 100 mM acetate and 30 nM disodium EDTA, at pH 8.50. Mobile phase A (10% MeOH: 90% v/v) ran isocratic for 5 minutes, changing to gradient mode during 4 minutes, reaching 100% mobile phase B (40%) MeOH: 60% buffer v/v). After 1 minute at 100% B, reequilibration started with solvent A during 12 minutes. The pH of both mobile phases was set at 8.50 at 25°C using an Orion research pH-meter. The mobile phases were degassed by filtration on a Millipore Filtration Apparatus using a Durapore 0.45 µm filter membrane. The flow-rate was set at 1 ml/minute. A Nucleosil 100 RP-C18 cartridge (125 mm long and 4 mm in diameter) was used at 25°C (Macherey-Nagel, Düren, Germany). The cartridge was rinsed daily during 30 minutes with methanol:water 70:30 v/v. Peak identification and data acquisition were realized using a Millennium 32 software (Waters). L-TRP was used as external standard at 1, 5, 10, 20, 50 and 100 µmol/l in MilliQ-grade water. Standards were diluted 1:1 with 2M perchloric acid just before injection of 25 µl. Calibration curves for free TRP (1 to 50 µmol/l and total TRP (1 to 100 µmol/l) were used to calculate concentrations.

6.3.3 Statistical Analyses

For all statistical analyses the Statistical Package for Social Sciences, version 16.0 for Windows (SPSS 2010, version 16.0, SPSS Inc, Chicago) was used. Descriptive statistics of all relevant variables for the total group were calculated. Comparisons between groups were performed with Student's t-tests, Fisher exact tests and χ^2 tests as appropriate. Pearson correlations were used to estimate univariate correlations. A linear stepwise regression was performed to assess the significant determinants of the change in HRQL. The value of the CRP was logarithmically transformed for the correlation calculations and for the stepwise linear regression. The level of statistical significance was set at $p < 0.05$ and all p-values are two-sided.

6.4 RESULTS

6.4.1 Baseline results

The demographic and baseline disease characteristics of the 39 patients with active CD and the 9 control patients who entered the study are shown in table 1.

Table 1. Description of the baseline characteristics of the CD patients with active disease (n = 39) and the control CD patients in remission (n = 9).

Variable	CD patients before treatment	CD patients in remission	p-value
N Participating	39	9	-
Mean age (SD)	34 (11)	41 (11)	0.11
Men/Women	18/21	3/6	0.71
Localisation of disease			
Terminal ileum (n/total)	2/39	1/9	0.34
Colon (n/total)	13/39	0/9	
Ileo-colonic (n/total)	24/39	8/9	
Type of disease			
Inflammatory	28/39	3/9	0.051
Fistulizing	11/39	6/9	
Medication			
5-ASA	16/39	5/9	0.48
Corticosteroids	12/39	3/9	0.88
Azathioprine	20/39	5/9	0.82
Methotrexate	8/39	0/9	0.32
Smokers	11/39	2/9	0.72
Disease Activity			
BL CRP median mg/L (IQR)	30.60 (9.8-42.4)	5.5 (1.3-12.4)	0.001
BL CDAI (SD)	258 (111.6)	82 ()	<0.001

There were no significant differences in age, gender, medication use and smoking status between both groups. In both groups, the disease was located in the ileum and the colon in the majority of the patients. A slightly larger proportion of patients in the control group

suffered from a fistulizing type of CD. The baseline CRP and CDAI score were significantly higher in CD patients with active disease than in the control CD patients. At baseline, total and free TRP concentration were significantly lower in patients with active CD than total and free TRP concentration in the control CD patients (table 2a and 2b).

Table 2a. Comparison of the total TRP concentration between the total group of CD patients before (baseline) and after (re-evaluation) treatment with IFX and control CD patients in remission

	CD Patients		
Total TRP concentration (µmol/L)	*Treated patients (n = 39)*	*Control Patients in remission (n = 9)*	*p-value between groups*
Baseline (SD)	39.01 (11.97)	48.56 (6.85)	0.004
Re-evaluation (SD)	41.87 (12.64)	49.40 (12.46)	0.11

Table 2b. Comparison of the free TRP concentration between the total group of CD patients before (baseline) and after (re-evaluation) treatment with IFX and control CD patients in remission

	CD Patients		
Free TRP concentration (µmol/L)	*Treated patients (n = 39)*	*Control Patients in remission (n = 9)*	*p-value between groups*
Baseline (SD)	5.22 (2.22)	12.94 (1.41)	<0.001
Re-evaluation (SD)	6.12 (2.84)	13.04 (1.34)	<0.001

6.4.2 Evolution of TRP after treatment with infliximab (IFX)

CD patients with active disease (n = 39) were treated with IFX and after 4 weeks they were re-evaluated. The median CRP decreased significantly from 30.6 mg/L (IQR = 9.8 to 42.4 mg/L) before treatment to 3.3 mg/L (IQR = 1.5 to 23.9 mg/L; p = 0.001) after treatment with IFX and the mean CDAI scores decreased significantly (from 258 ± 112 to 157 ± 106; p < 0.001), indicating a lower level of disease activity. The mean IBDQ scores (145 ± 32 to 171 ± 28; p < 0.001) increased significantly after treatment with IFX, indicating an improved HRQL. Figure 2 and 3 illustrate the statistically significant increase of both total and free TRP concentrations after IFX treatment in the 39 CD patients with active disease.

In the control CD patients in remission, there were no significant differences in the CDAI nor in the levels of CRP (p = 0.50), CDAI (p = 0.57), total TRP concentration (p = 0.75) and free TRP concentration (p = 0.98) between the 2 sampling time points. Both total and free TRP increased significantly after treatment with IFX (figure 1 & 2). The comparison at the re-evaluation moment yielded a total TRP concentration in the 39 CD patients with active disease after treatment with IFX, which was still lower, although not significantly, than in the control CD patients in remission (table 2a). The free TRP concentration remained significantly lower in the 39 CD patients with active disease after treatment with IFX (table 2b) than in the control CD patients in remission.

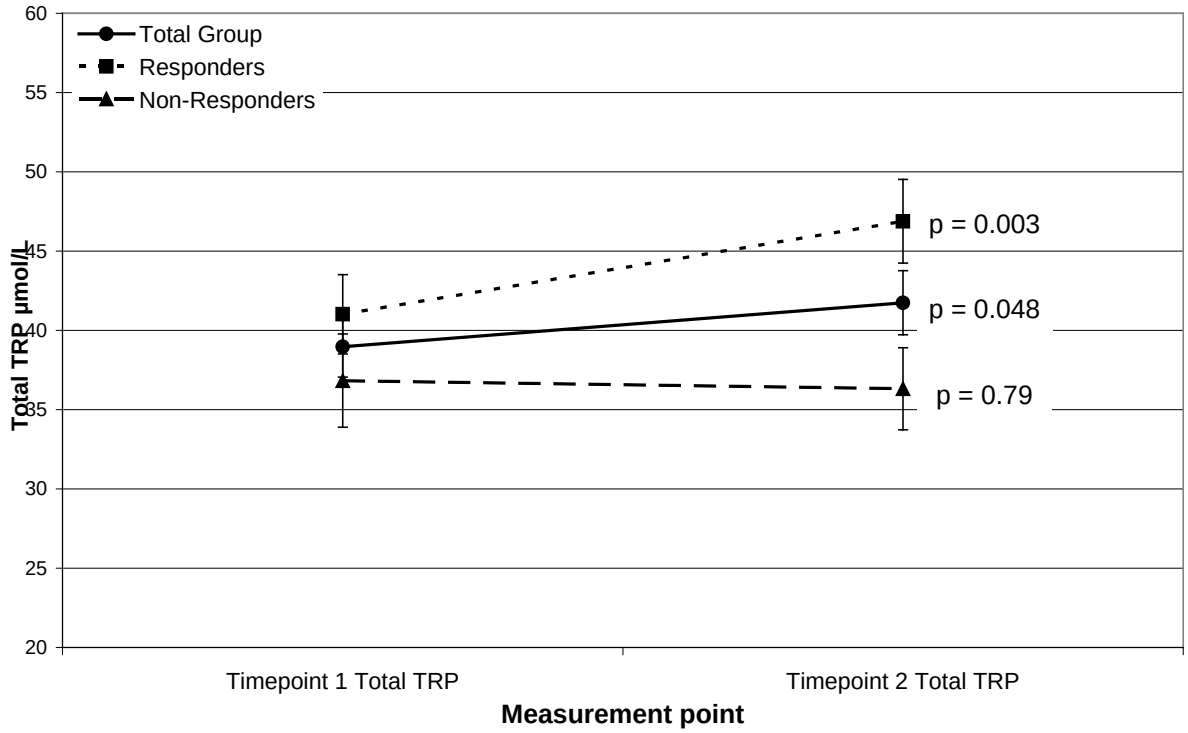

Figure 1. Evolution of the total TRP concentrations (value ± standard deviation) for all CD patients treated with IFX (circles), for the patients without elevated CRP after IFX treatment (squares) and for the patients with elevated CRP after IFX treatment (triangles). P-values reflect the significance of change from time point 1 to time point 2.

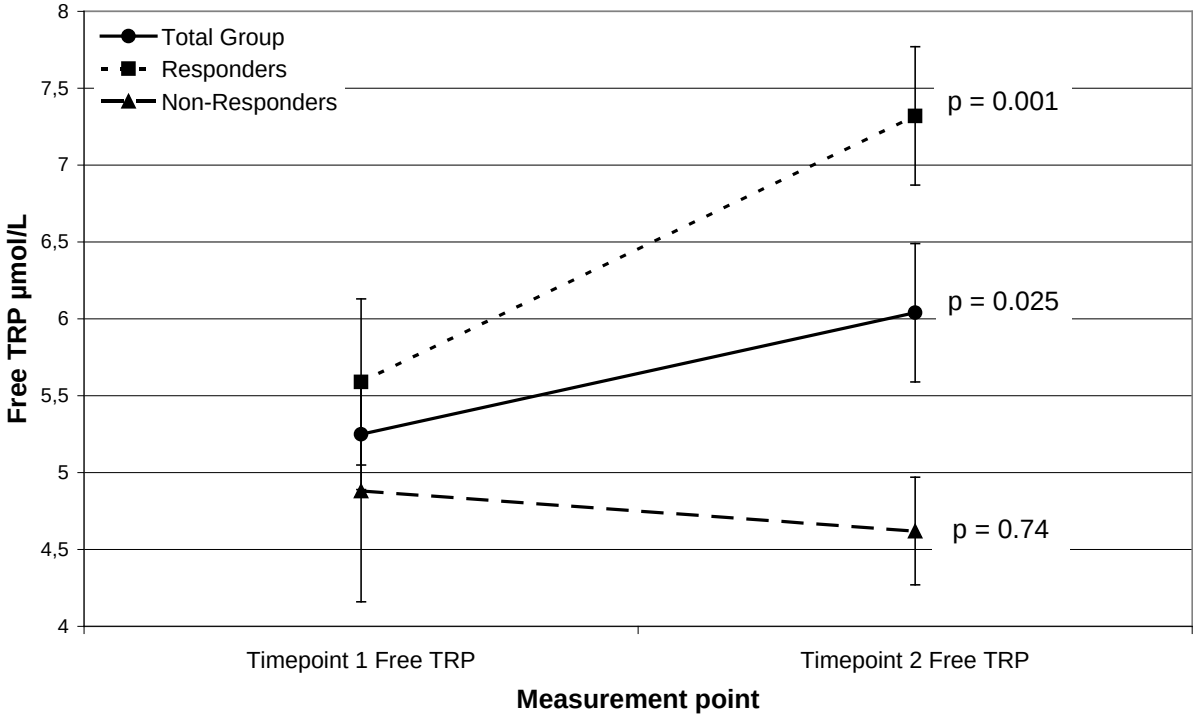

Figure 2. Evolution of the Free TRP concentration (value ± standard deviation) for all CD patients treated with IFX (circles), for the patients without elevated CRP after IFX treatment (squares) and for the patients with elevated CRP after IFX treatment (triangles). P-values reflect the significance of change from time point 1 to time point 2.

6.4.2.1 Subgroup analyses

Total and free TRP concentration and the respective changes over the measurement points did not differ between men (n = 18) and women (n = 20), between smokers (n = 11) and non-smokers (n = 28) and between patients who had previous surgery (n = 16) and patients without previous surgery (n = 23). Furthermore, no associations were found with age, concomitant pharmacological treatment, CD localization and CD behaviour. In 24 out of 39 CD patients with active disease, treatment with IFX resulted in clinical remission with CDAI score < 150 or a decrease in CDAI score of more than 70 points at the re-evaluation point after 4 weeks. The baseline total and free TRP concentrations (respectively p = 0.34 and p = 0.81) and the free TRP concentration at re-evaluation (p = 0.55) did not differ significantly between these 24 clinically remitted patients and the 15 patients with persistent clinically active disease. There was a trend of a higher total TRP concentration after IFX treatment in the 24 clinically remitted patients (44.90 µmol/L ± 11.80 µmol/L) compared to the 15 patients who did not achieve clinical remission (37.01 µmol/L ± 12.80 µmol/L, p = 0.057).

In 20 CD patients with active disease, CRP concentrations decreased below 3.0 mg/L after IFX treatment, which is indicative of the absence of systemic inflammation. In 19 patients some degree of CRP elevation remained detectable at re-evaluation: these patients were defined as patients with elevated CRP after IFX treatment or persistent systemic inflammation in the current study. The 2 groups did not differ in age (p = 0.65), in gender (p = 0.26) and in disease related variables. At baseline, patients with normalized CRP after IFX treatment and patients with elevated CRP after IFX treatment did not differ significantly in CRP (respective median CRP values 26.6 mg/L, IQR 6.7 mg/L – 42.1 mg/L vs. 30.6 mg/L, IQR 12.4 mg/L – 79.0 mg/L; p = 0.71), in CDAI score (respective CDAI scores 233 ± 114 vs. 285 ± 105; p = 0.15) and in IBDQ score (respective IBDQ scores 152 ± 31 vs. 138 ± 33; p = 0.17).

At baseline, there was no statistically significant difference between the patients with normalized CPR after IFX treatment and patients with elevated CRP after IFX treatment for total TRP concentration (p = 0.28, figure 1) and for free TRP concentration (p = 0.32, figure 2). At baseline, the total and free TRP concentration was significantly lower in both subgroups compared to CD patients in remission (table 3a and 3b).

After treatment in with IFX, the patients with normalized CRP after IFX treatment had a significantly lower CDAI score (93 ± 65; p < 0.001) and higher IBDQ score (183 ± 27; p < 0.001), in comparison to the baseline scores. The mean improvement of the CDAI was also

clinically significant, with mean clinical remission in this group (mean improvement > 70 points and mean score < 150). The patients with elevated CRP after IFX treatment had significantly lower CDAI scores (233 ± 101; p 0.017) and higher IBDQ scores (155 ± 24; p = 0.033) in comparison to the baseline scores.

Figure 1 and 2 demonstrate the evolution of total and free TRP after treatment with IFX in both subgroups. In patients with normalized CRP after IFX treatment there was a significant increase in total and free TRP concentration after treatment with IFX, whereas no significant change in total or in free TRP concentration was observed in patients with elevated CRP after IFX treatment (figure 1 and 2). Moreover, patients with normalized CRP after IFX treatment had significantly higher total TRP concentration (p = 0.007) and free TRP concentration (p = 0.003) after IFX treatment than patients with elevated CRP after IFX treatment.

After IFX treatment, total TRP concentration in patients with normalized CRP after IFX treatment was not different from the second measurement of total TRP concentration in control CD patients in remission, whereas the total TRP concentration remained significantly lower in patients with elevated CRP after IFX treatment (table 3a and 3b). Conversely, free TRP concentration remained significantly lower in both subgroups at re-evaluation after IFX treatment (table 3a and 3b).

Table 3a. Comparison of the total TRP concentration between CD patients in remission and the subgroups (patients without residual biological inflammation (normal CRP) after IFX treatment and patients with persistent biological inflammation after IFX treatment) before (baseline) and after (re-evaluation) treatment with IFX.

	Control group	**Treatment CD patients**			
Total TRP concentration (µmol/L)	Patients in remission (n = 9)	normalized CRP (n = 20)	p-value*	persistent CRP elevation (n = 19)	p- value*
Baseline (SD)	48.56 (6.85)	41.10 (11.05)	0.035	36.81 (12.80)	0.004
Re-evaluation (SD)	49.40 (12.46)	47.25 (11.70)	0.61	36.20 (11.26)	0.01

* p-value for comparison between CD patients in remission and the respective subgroup at baseline and at re-evaluation

Table 3b. Comparison of the free TRP concentration between CD patients in remission and the subgroups (patients without residual inflammation (normal CRP) after IFX treatment and patients with persistent biological inflammation after IFX treatment) before (baseline) and after (re-evaluation) treatment with IFX.

	Control group	**Treatment CD patients**			
Free TRP concentration (µmol/L)	Patients in remission (n = 9)	normalized CRP (n = 20)	p-value*	persistent CRP elevation (n = 19)	p- value*
Baseline (SD)	12.94 (1.41)	5.58 (2.45)	<0.001	4.83 (1.95)	<0.001
Re-evaluation (SD)	13.04 (1.34)	7.48 (3.17)	<0.001	4.69 (1.47)	<0.001

* p-value for comparison between CD patients in remission and the respective subgroup at baseline and at re-evaluation

6.4.3 Correlations of TRP with disease activity parameters and quality of life in the total group

At baseline, total TRP concentration correlated negatively with the logarithmically adjusted CRP (logCRP) value ($r = -0.57$, $p < 0.001$) and with the CDAI score ($r = -0.35$, $p = 0.029$). Total TRP concentration was not significantly correlated with the total IBDQ score ($r = 0.25$, $p = 0.13$), nor with the bowel symptoms ($r = 0.16$, $p = 0.34$), systemic symptoms ($r = 0.29$, $p = 0.08$) and emotional dysfunction ($r = 0.10$, $p = 0.55$) subscales. There was a significant although low correlation between total TRP concentration and the IBDQ social dysfunction subscale ($r = 0.35$, $p = 0.031$). At baseline, there were no statistically significant correlations between free TRP concentration and the different measured parameters: logCRP ($r = -0.23$, $p = 0.16$), CDAI score ($r = 0.24$, $p = 0.15$), IBDQ total score ($r = 0.31$, $p = 0.058$) and its subscales of bowel symptoms ($r = 0.24$, $p = 0.15$), systemic symptoms ($r = 0.23$, $p = 0.16$), emotional dysfunction ($r = 0.28$, $p = 0.09$) and social dysfunction ($r = 0.26$, $p = 0.11$).

At re-evaluation, the total TRP concentration correlated negatively and significantly with the logCRP value ($r = -0.48$, $p = 0.002$), but not significantly with the CDAI score ($r = -0.29$, $p = 0.08$), the IBDQ total score ($r = 0.14$, $p = 0.41$) and the IBDQ subscales of bowel symptoms ($r = 0.17$, $p = 0.31$), systemic symptoms ($r = 0.01$, $p = 0.96$), emotional dysfunction ($r = -0.03$, $p = 0.87$) and social dysfunction ($r = 0.27$, $p = 0.09$). In contrast with the baseline correlations, the free TRP concentration was significantly negatively correlated with the logCRP value (-0.44, $p = 0.008$), however not significantly with the CDAI score (-0.30, $p = 0.07$). There was a significant positive correlation between free TRP concentration and the total IBDQ score ($r = 0.32$, $p = 0.05$) and with 2 out of 4 subscales: bowel symptoms ($r = 0.36$, $p = 0.023$) and the social dysfunction subscale ($r = 0.32$, $p = 0.05$) but not the systemic symptoms ($r = 0.27$, $p = 0.10$) and the emotional subscale ($r = 0.14$, $p = 0.39$).

The correlations of the *difference (Δ) between baseline (BL) and re-evaluation (RE)* (= $\Delta_{RE - BL}$) of the total TRP concentration with the $\Delta_{RE - BL}$ logCRP value, the $\Delta_{RE - BL}$ CDAI score and the $\Delta_{RE - BL}$ IBDQ and its subscales are shown in table 4. All correlations were statistically significant except for the association with the $\Delta_{RE - BL}$ IBDQ systemic symptoms subscore, which was a trend. Similar results were found for the correlation of the $\Delta_{RE - BL}$ free TRP concentration with the $\Delta_{RE - BL}$ logCRP value, the $\Delta_{RE - BL}$ CDAI score and the $\Delta_{RE - BL}$ IBDQ and its subscales (table 4). The highest correlation for the $\Delta_{RE - BL}$ free TRP concentration was with the $\Delta_{RE - BL}$ IBDQ social dysfunction subscale. Controlling for gender, age and BMI did not yield different results.

Table 4. Correlations of the *difference (Δ) between baseline (BL) and re-evaluation (RE)* (= $\Delta_{RE - BL}$) of the total TRP concentration and the $\Delta_{RE - BL}$ free TRP concentration on the one hand with the $\Delta_{RE - BL}$ logCRP value, the $\Delta_{RE - BL}$ CDAI score, the $\Delta_{RE - BL}$ IBDQ score and its subscales on the other hand, for the total group of CD patients treated with Infliximab.

Correlates	$\Delta_{RE - BL}$ Total TRP	p-value	$\Delta_{RE - BL}$ Free TRP	p-value
$\Delta_{RE - BL}$ LogCRP	r = -0.55	<0.001	r = -0.52	0.001
$\Delta_{RE - BL}$ CDAI	r = -0.46	0.005	r = -0.46	0.005
$\Delta_{RE - BL}$ IBDQ	r = 0.52	0.001	r = 0.59	< 0.001
$\Delta_{RE - BL}$ IBDQ Bowel	r = 0.48	0.003	r = 0.53	0.001
$\Delta_{RE - BL}$ IBDQ Systemic	r = 0.32	0.071	r = 0.32	0.077
$\Delta_{RE - BL}$ IBDQ Emotional	r = 0.51	0.001	r = 0.54	0.001
$\Delta_{RE - BL}$ IBDQ Social	r = 0.42	0.010	r = 0.66	< 0.001

6.4.4 Prediction of evolution of Quality of life

An exploratory linear stepwise regression analysis for the total group with $\Delta_{RE - BL}$ IBDQ as dependent variable and $\Delta_{RE - BL}$ logCRP, $\Delta_{RE - BL}$ total TRP concentration and $\Delta_{RE - BL}$ free TRP concentration as independent variables, was performed. An adjusted $\Delta_{RE - BL}$ CDAI score was also used as an independent variable, without the heavily weighed "*well-being*" item, since this item also assesses HRQL. The analysis yielded the adjusted $\Delta_{RE - BL}$ CDAI score (β = -0.37; p = 0.022) and the $\Delta_{RE - BL}$ free TRP concentration (β = 0.34; p = 0.03) as significant, independent determinants of the $\Delta_{RE - BL}$ IBDQ score. This model predicted 34% of the variability (adjusted R square = 0.34). The unique variance (i.e., the squared, semi partial correlation) contributed by the predictors was 10% for the adjusted $\Delta_{RE - BL}$ CDAI score and 9% for $\Delta_{RE - BL}$ free TRP concentration.

6.5 DISCUSSION

TRP is an essential amino acid which is actively metabolized along the kynurenine pathway by the immunomodulatory enzyme IDO1 in inflammatory conditions induced by cytokines. The results of this study confirm that total and free serum TRP levels were decreased in CD patients with active inflammatory disease before IFX treatment in contrast to the control CD patients. After treatment with IFX, a significant increase of total and free TRP concentrations was observed. Decreased serum TRP levels and inflammation can further be linked since no significant changes in the total and free TRP concentrations were observed in the subgroup with persistent elevated CRP after IFX treatment which indicates that only the subgroup of successfully treated CD patients accounted for the increase of TRP concentrations in the total group. An association between decreased TRP levels and the presence of systemic inflammation in CD was further reflected by the significant inverse correlations between the

TRP concentrations and CRP at both evaluation points and by the correlation between the change in TRP concentrations and the change in CRP. Our results support previous findings that active CD is associated with significantly decreased serum TRP levels which is a substrate of IDO1 and an increased K/T ratio. Additionally, Gupta et al also demonstrated a significant correlation between CRP, CD activity and the K/T ratio. The changes in tryptophan levels were mostly responsible for the changes in K/T ratio since kynurenine levels did not differ significantly between groups and controls.[5] An early study in CD also reported lower TRP levels in 13 out of 32 patients.[21] It has been shown that a Th-1 immune response induces the enzyme IDO1 by the release of proinflammatory cytokines. The increased IDO activity catabolises TRP to kynurenine and kynurenic acid through oxidation.[6] Forrest et al did not find a difference for total TRP levels between CD patients with mild disease activity and a normal control group.[20] However, the patients in our sample had active luminal disease refractory to standard therapies requiring treatment with IFX. Forrest et al did find increased levels of systemic kynurenine and kynurenic acid,[20] which suggests an increased TRP catabolism in CD patients with mild disease activity. Furthermore, IDO-induction and an increase of kynurenine and the K/T ratio has previously been demonstrated in biopsies of inflamed bowel in CD patients.[7] Additionally, they also found a nearly normal IDO staining pattern in tissue samples from patients who had successfully responded to IFX, whereas no difference before and after treatment was found in patients without clinical response.[7] These results correspond with the difference in evolution of TRP concentrations between patients with and without detectable inflammation after treatment in our study, indicating that the shutting off of local and systemic inflammation has a favourable influence on systemic total serum TRP concentrations.

The free serum TRP concentration remained significantly lower in all treated CD patients compared to the control CD patients, regardless of the success of the treatment. The free TRP levels of the control CD patients in remission were similar to those measured in a control group of normal control subjects (results not shown) and were in range of previously published reference TRP values.[4] Residual IDO-activity due to residual local inflammation might be responsible for the significantly lower free TRP levels after successful treatment with infliximab. Possibly, the time period between treatment and re-evaluation (4 weeks) was not enough to recover. Additionally, a reduction of TRP availability could be influenced by a poor nutritional status which is common in CD, especially during an exacerbation and extensive bowel resections. This can be due to a decrease or change in food intake and malabsorption or increased loss of nutrients.[2,31] Previous research has confirmed significantly more depressed mood in CD patients with a poor nutritional status[32] and this compromised free TRP availability might especially be relevant for mood regulation and

HRQL in CD patients since only TRP which is not bound to protein will cross the BBB to be metabolized into 5-HT.[4]

The analyses yielded a significant association between the evolution of TRP levels and the evolution of HRQL in the treated CD patients. HRQL is always severely affected in CD patients with active disease.[25,27,28,33] Although the severity of disease activity is primarily responsible for the decrease in HRQL, previous research has demonstrated that the variance in HRQL scores is only partially explained by disease activity.[15,34] Other underlying mechanisms probably play a role and TRP has been proposed as a possible contributing intermediate between inflammation and psychosocial dysfunction in other diseases.[4] Previous research in cancer demonstrated a significant correlation between decreased TRP levels, HRQL and pro-inflammatory cytokines.[35] Thus, the associations found in the present study might be an indication of the modulation of HRQL in CD by TRP. The fact that the change in the free TRP concentration was an independent determinant of the HRQL measure adds an argument in favour of this hypothesis. Furthermore, this finding was also in line with the results of Huang et al who demonstrated that serum TRP was an independent predictor of HRQL.[35] Although these associations in no way establish cause and effect, they might suggest a role for inflammation-mediated TRP reduction in the change of HRQL in CD patients.

This strong association between free TRP and HRQL might be explained by the fact that the free TRP fraction seems to be of higher importance to brain serotonin (5-HT) function. The majority of the available TRP (50% - 80%) is bound to albumin[4] and it has been argued that only free TRP is able to cross the blood brain barrier (BBB), where it is metabolized into 5HT. The transport of TRP across the BBB is not selective. Thus, TRP has to compete with other large neutral amino acids (LNAA). In the case of depletion of TRP, the ratio TRP/LNAA reduces and less TRP will enter the CNS. Since the oxidation of TRP into 5-hydroxy-TRP is a rate limiting step, this will impair the synthesis of 5HT. TRP depletion studies in patients with a history of mood disorders, in relatives of these patients and even in healthy control subjects, have demonstrated that rapid experimental depletion of TRP can temporarily induce symptoms associated with mood disorders and impaired cognition.[18,36–40] Mood disorders are recognized side effects of cytokine therapy (e.g. in cancer or hepatitis C).[6,41] Since TRP levels are also significantly decreased in CD patients with active disease, this might have consequences for their 5HT availability in the CNS and consequently for their psychosocial functioning. Thus, inflammation might induce a state of “serotonergic vulnerability” in these CD patients through the relative depletion of free TRP. This is highly relevant give the hypothesis that patients with CD are prone to develop psychopathology

more frequently[42] and the possible interaction between psychosocial stress, psychopathology and the course of the disease.[19,43–45]

There are some limitations to this study. First, we did not measure kynurenine and thus we were not able to determine the K/T ratio, which has been proposed as the best marker of IDO1 activity by immune activation limiting the variability seen when TRP alone is used.[46] TRP concentrations might be somewhat variable due to dietary variations. However, in the study of Gupta et al, kynurenine levels did not significantly differ between controls and across groups and only TRP levels were responsible for the change in K/T ratio.[5] Secondly, cytokines, such as IL-6 or TNF-α were not directly measured and no correlation could be calculated between TRP levels and cytokines. Thirdly, the IBDQ is limited to use as a psychosocial measure which makes it difficult to extrapolate conclusions concerning mood disorders. Furthermore, the associations found in this study do not demonstrate causality.

In conclusion, we have shown that inflammation in CD is associated with decreased total and free TRP and that successful treatment of CD with IFX results in a normalization of total TRP within four weeks, whereas free TRP remains significantly lower. Furthermore, the change in quality of life was correlated with the change in TRP concentrations and this change was independently determined by the change in free TRP concentration. This might be indicative of a role of TRP concentrations in the subjective well-being of CD patients. Further research is needed to explore a possible role of TRP as a mediator of mood in CD and thus explain in part the increased prevalence of mood disorders in CD. Further research should measure TRP, given that it is a good biomarker for inflammation, and correlate this with the measurement of mood.

6.6 REFERENCES

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet*. 2007;369:1627–1640.
2. Podolsky DK. Inflammatory bowel disease. *N Engl J Med*. 2002;347:417–29.
3. Cherayil BJ. Indoleamine 2,3-dioxygenase in intestinal immunity and inflammation. *Inflamm Bowel Dis*. 2009;15:1391–6.
4. Russo S, Kema IP, Fokkema MR, et al. Tryptophan as a Link between Psychopathology and Somatic States. *Psychosom Med*. 2003;65:665–671.
5. Gupta NK, Thaker AI, Kanuri N, et al. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis*. 2012;18:1214–20.
6. Capuron L, Neurauter G, Musselman DL, et al. Interferon-alpha–induced changes in tryptophan metabolism. *Biol Psychiatry*. 2003;54:906–914.
7. Wolf AM, Wolf D, Rumpold H, et al. Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. *Clin Immunol*. 2004;113:47–55.
8. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004;4:762–74.
9. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol*. 2012.
10. Carlin JM, Borden EC, Byrne GI. Interferon-induced indoleamine 2,3-dioxygenase activity inhibits Chlamydia psittaci replication in human macrophages. *J Interferon Res*. 1989;9:329–37.

11. Lestage J, Verrier D, Palin K, et al. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun*. 2002;16:596–601.
12. Capuron L, Ravaud A, Neveu PJ, et al. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry*. 2002;7:468–73.
13. Widner B, Leblhuber F, Walli J, et al. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm*. 2000;107:343–53.
14. Schroecksnadel K, Winkler C, Duftner C, et al. Tryptophan degradation increases with stage in patients with rheumatoid arthritis. *Clin Rheumatol*. 2006;25:334–7.
15. Ringel Y, Drossman DA. Psychosocial aspects of Crohn's disease. *Surg Clin North Am*. 2001;81:231–52, x.
16. Drossman DA, Ringel Y. Psychosocial factors in ulcerative colitis and Crohn's disease. In: Sartor RB, Sandborn W, eds. *Kirsner's Inflammatory Bowel Disease*.Vol 6th. Philadelphia: W.B. Saunders Co; 2004:340–356.
17. Stahl S. Chemical neurotransmission as the mediator of disease actions. In: *Essential psychopharmacology: neuroscientific basis and practical applications*. 2nd ed. Cambridge: Cambridge University Press; 2000:99–135.
18. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry*. 2001;178:399–405.
19. Persoons P, Vermeire S, Demyttenaere K, et al. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Aliment Pharmacol Ther*. 2005;22:101–10.
20. Forrest CM, Youd P, Kennedy A, et al. Purine, kynurenine, neopterin and lipid peroxidation levels in inflammatory bowel disease. *J Biomed Sci*. 2002;9:436–442.
21. Beeken WL. Serum tryptophan in Crohn's disease. *Scand J Gastroenterol*. 1976;11:735–40.
22. Lashner BA. Clinical features, laboratory findings, and course of Crohn's disease. In: Kirsner JB, ed. *Inflammatory Bowel Disease*.Vol 5. Philadelphia, Pennsylvania: W.B. Saunders Company; 2000:305–314.
23. Vermeire S, Assche G Van, Rutgeerts P. Classification of inflammatory bowel disease: the old and the new. *Curr Opin Gastroenterol*. 2012.
24. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). *Gastroenterology*. 1979;77:843–846.
25. Irvine EJ, Feagan B, Rochon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Canadian Crohn's Relapse Prevention Trial Study Group. *Gastroenterology*. 1994;106:287–296.
26. Irvine EJ. Quality of life issues in patients with inflammatory bowel disease. *Am J Gastroenterol*. 1997;92:18S–24S.
27. Lichtenstein GR, Bala M, Han C, et al. Infliximab improves quality of life in patients with Crohn's disease. *Inflamm Bowel Dis*. 2002;8:237–243.
28. Feagan BG, Yan S, Bala M, et al. The effects of infliximab maintenance therapy on health-related quality of life. *Am J Gastroenterol*. 2003;98:2232–8.
29. Russel MG, Pastoor CJ, Brandon S, et al. Validation of the Dutch translation of the Inflammatory Bowel Disease Questionnaire (IBDQ): a health-related quality of life questionnaire in inflammatory bowel disease. *Digestion*. 1997;58:282–8.
30. Williams WA, Shoaf SE, Hommer D, et al. Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5-Hydroxyindoleacetic Acid in Normal Volunteers. *J Neurochem*. 2001;72:1641–1647.
31. Sitrin MD. Nutrition and Inflammatory Bowel Disease. In: Kirsner JB, ed. *Inflammatory Bowel Disease*. 5th ed. Philadelphia, Pennsylvania: Saunders; 2000:598–610.
32. Addolorato G, Capristo E, Stefanini GF, et al. Inflammatory bowel disease: a study of the association between anxiety and depression, physical morbidity, and nutritional status. *Scand J Gastroenterol*. 1997;32:1013–1021.
33. Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology*. 1989;96:804–810.
34. Sostegni R, Daperno M, Scaglione N, et al. Review article: Crohn's disease: monitoring disease activity. *Aliment Pharmacol Ther*. 2003;17 Suppl 2:11–7.
35. Huang A, Fuchs D, Widner B, et al. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. *Br J Cancer*. 2002;86:1691 – 1696.
36. Young SN, Smith SE, Pihl RO, et al. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)*. 1985;87:173–7.
37. Delgado PL, Charney DS, Price LH, et al. Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry*. 1990;47:411–8.
38. Does AJ Van der. The effects of tryptophan depletion on mood and psychiatric symptoms. *J Affect Disord*. 2001;64:107–19.

39. Lam RW, Bowering TA, Tam EM, et al. Effects of rapid tryptophan depletion in patients with seasonal affective disorder in natural summer remission. *Psychol Med*. 2000;30:79–87.
40. Leyton M, Young SN, Pihl RO, et al. Effects on mood of acute phenylalanine/tyrosine depletion in healthy women. *Neuropsychopharmacology*. 2000;22:52–63.
41. Capuron L, Gumnick JF, Musselman DL, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology*. 2002;26:643–52.
42. Sajadinejad MS, Asgari K, Molavi H, et al. Psychological issues in inflammatory bowel disease: an overview. *Gastroenterol Res Pract*. 2012;2012:106502.
43. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. *Psychosom Med*. 2004;66:79–84.
44. Bitton A, Dobkin PL, Edwardes MD, et al. Predicting relapse in Crohn's disease: a biopsychosocial model. *Gut*. 2008;57:1386–1392.
45. Maunder RG, Levenstein S. The role of stress in the development and clinical course of inflammatory bowel disease: epidemiological evidence. *Curr Mol Med*. 2008;8:247–52.
46. Schröcksnadel K, Wirleitner B, Winkler C, et al. Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta*. 2006;364:82–90.

Chapter 7

7 Acute Tryptophan Depletion in Crohn's Disease: Investigating a Possible Biological Vulnerability for Depression

Paper to be submitted

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7.1 ABSTRACT

Background: *psychological symptoms and psychopathology are common in Crohn's Disease (CD). This might be linked to decreased peripheral tryptophan levels due to increased inflammation-induced catabolism in active CD, causing "serotonergic (5-HT) vulnerability" in the central nervous system. This can be assessed with acute TRP depletion (ATD).*
Aims: *to investigate if ATD can induce a temporary, acute, clinically significant change in mood, fatigue and anxiety in CD patients.*
Methods: *CD patients enrolled in a randomized, controlled, double blind, crossover protocol with an ATD and a control arm. Mood, fatigue and anxiety ratings, as well as total and free serum TRP concentrations were measured.*
Results: *Nine consecutive patients with quiescent CD participated. Total and free serum TRP concentrations decreased significantly after ATD, whereas these concentrations increased in the control condition. The change in mood scores, fatigue scores and anxiety scores did not differ significantly between the ATD and the control condition.*
Conclusions: *the results do not support the hypothesis of a 5-HT vulnerability due to inflammation-induced TRP reduction in CD patients with quiescent disease, which can be elicited by an ATD paradigm. Most likely, the inflammation-induced psychological symptoms or psychopathology are not associated to the drastic decrease of TRP bioavailability, but rather to other mechanisms related to high levels of pro-inflammatory cytokines.*

7.2 INTRODUCTION

Crohn's Disease (CD) is characterized by a relapsing and remitting course of transmural inflammation of the gastrointestinal mucosa which can affect the whole gastrointestinal tract, but predominantly affects the small bowel and the colon. Complications are very common in CD and patients frequently develop fistulas, abscesses and strictures.[1]The well-controlled balance of the intestinal immune system is disturbed at all levels. The exaggerated inflammatory response in active CD most likely stems from the false recognition of luminal antigens from the commensal microbiotica by the innate immune system. This will lead to an activation of the production of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12, IL-23 and others and a further inflammatory cascade promoting the differentiation of T cells in effector T cells (Th1 cells and Th17 cells of the adaptive immune system).[2] These inflammatory cells will further secrete proinflammatory cytokines, which will attract more inflammatory leucocytes which contribute to tissue damage and ultimately to the vicious cycle of intestinal inflammation.[2,3] The localized inflammation will cause local symptoms, but will also have a broader, systemic effect with symptoms of general malaise and illness. The variability in disease activity and the presenting symptoms between individuals is high and there is often a low correlation with the extend of the disease and the "objective" disease parameters which might be partly due to the mediating role of psychosocial factors.[4]

An association between CD and psychological disorders has been suggested in previous research.[5,6] It is often assumed that the higher prevalence of psychological disorders, such as depression or anxiety, in CD patients than in the healthy population, is a component of the illness (e.g. due to the distress caused by the severe, aggressive and unpredictable disease course of CD) which influences the clinical symptoms and the health seeking behavior.[7] Other researchers, however, have observed that depression might be predictive of early relapse[8,9] and in chapter 5 our results suggested that major depressive disorder was predictive of failure to achieve remission and early relapse after treatment with infliximab.[10] Furthermore, numerous studies found an association between psychiatric disorders and CD.[11–13] North and Alpers examined the specificity of such a link in a systematic review and concluded that CD patients appeared to have a higher life time burden of psychiatric disorders than UC patients.[14] A number of studies have established a link between active CD and psychological factors.[5,15,16] It has been proposed that underlying biological mechanisms contribute to the development of psychological symptoms and psychiatric disorders in inflammatory medical illness, such as CD. These mechanisms are related to the direct and indirect effects of pro-inflammatory cytokines on the central nervous system.[17] One of these proposed mechanisms is an increased catabolism and drastic decrease of tryptophan (TRP)

levels by the induction of the indoleamine 2,3 dioxygenase-1 (IDO) enzyme.[17,18] Our research hypothesis was derived from this putative biological mechanism.

Toll-like receptor activation and pro-inflammatory cytokines which are associated with active, inflammatory CD, such as interferon-γ (IFN-γ), IFN-α and TNF-α induce the enzyme indoleamine 2,3 dioxygenase-1 (IDO).[19–21] IDO is expressed in the cells (macrophages, monocytes, dendrites) of the innate immune system and can essentially be found in all tissues, including the intestine.[22] IDO has immunoregulatory properties by suppressing T-cell responses of the adaptive immune system, which is highly important for the intestinal immune tolerance.[19,22] Together with Tryptophan 2,3-dioxygenase (mainly expressed in hepatocytes), IDO catalyzes the rate-limiting step of the oxidative tryptophan (TRP) catabolism along the kynurenine pathway (see chapter 6 – figure 6.1).[18,22] Evidence for the activation of IDO after immune activation and its effect on serum TRP concentrations comes from in vitro experiments, experimentally cytokine-treated animals[23,24] and from measurements in patients undergoing cytokine therapy (e.g. in cancer or hepatitis C).[25,26] A decrease of serum TRP concentrations has also been shown in other inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis and in Alzheimer's disease.[18,27,28] Previous studies have shown increased IDO expression in active CD[29] and its association with significantly decreased TRP levels,[19] which we also confirmed in the research presented in chapter 6 of this dissertation.

TRP is an essential, large, neutral amino-acid (LNAA) which is absorbed from the intestine. TRP is relatively scarce and there are no tissue reserves. TRP is the precursor of serotonin (5-hydroxytryptamine, 5-HT), which is a neurotransmitter in the central nervous system (CNS) with a central role in the current theories on mood regulation, cognition, behavior, and the pathophysiology of depression and anxiety.[30] About 1% of the total TRP is converted to 5-HT, primarily in the intestine. A small proportion of the TRP passes through the blood-brain barrier (BBB) via a non-specific, active, transport protein, in competition with other LNAA's. In the CNS, TRP is hydroxylized into 5-hydroxyTRP, which is decarboxylized into serotonin (5-HT). This hydroxylation is rate-limiting and a decrease in TRP levels also leads to a decrease in 5-HT synthesis and to a change in central 5-HT availability,[31] and possibly a change in central serotonergic neurotransmission.[32] Therefore, it is possible that the inflammation in active CD, which is associated with decreased TRP levels due to increased catabolism might contribute to the pathogenesis of psychological symptoms or even psychopathology in CD, via impaired 5-HT synthesis and a dysfunctioning central serotonergic neurotransmission, or "serotonergic vulnerability".[33] A non-invasive way to investigate the vulnerability of the central 5-HT neurotransmission in CD patients is the technique of the "Acute TRP Depletion" (ATD).

Acute TRP depletion (ATD) was developed to investigate the response of behavior, mood and cognitive functions in humans to a pharmacological challenge of the central serotonergic neurotransmission. The oral administration of a mixture of essential, large, neutral amino acids (LNAA) without TRP, induces a significant reduction of TRP concentrations (80% within 5 to 7 hours), due to acceleration of protein synthesis in the liver which consumes the circulating TRP.[34] This will lead to a reduced 5-HT synthesis in the CNS because the excess LNAA which have been administered orally, compete with the remaining TRP to pass through the BBB. Thus, less TRP passes into the CNS.[32,34–36] Animal research has demonstrated that this technique results in a significant depletion of TRP and in a decrease of 5-HT synthesis and 5-HT release in the CNS.[37–40] Functional imaging studies in human subjects have shown altered metabolism after ATD in brain regions which are linked to the pathogenesis of depression.[32,41] Numerous ATD studies have found significant deterioration in mood scores in patients who had been successfully treated with an antidepressant for a mood disorder,[41,42] in patients with a history of mood disorders,[43–45] in patients with a family history of mood disorders[46] and in patients with seasonal affective disorder.[47–51]

The findings in chapter 6 have demonstrated that TRP levels decrease in patients with active, inflammatory CD and the results suggested that TRP levels modulate health related quality of life in CD, which was in line with previous findings in cancer research.[52] Even when CD patients seem to be in clinical remission, it is difficult to ascertain if there is no more inflammation present and the majority of patients with CD in clinical remission still have mucosal inflammation.[53] In chapter 6, the free serum TRP concentration remained significantly lower in the successfully treated CD patients compared to the control CD patients. This was either due to the residual, local inflammation with IDO activation or it might be due to malabsorption or increased loss of nutrients, which has been implicated in mood disorders in CD patients.[16,54]

The hypothesis for this study was that patients with CD are prone to psychological symptoms and even psychopathology due to a “serotonergic vulnerability” which might be induced by an increased TRP catabolism due to inflammation. For this first intervention study, both patients in remission or patients with active CD were allowed to enter the study. The primary aim of this study was to investigate if ATD can induce a temporary, acute, clinically significant mood reduction in patients with CD. The secondary aim of this study was to assess the effect of ATD on the level of anxiety and fatigue of patients with CD and to investigate if an acute, clinically significant increase of these levels can be induced.

7.3 METHODS

7.3.1 Subjects

The study was done between April 2004 and December 2004 at the university hospital Gasthuisberg, Leuven, Belgium and was approved by the institutional ethical committee. CD patients were recruited at the outpatient clinic in this hospital. Patients with confirmed CD, on the basis of clinical, radiological, endoscopic and histological findings, could enter the study. Women in the follicular phase of the menstrual cycle and preferably using contraceptives, and men between the ages of 18 and 65 years could participate. Patients were excluded if they were involved in any other study, if they were pregnant or breast feeding, if they suffered from an infectious syndrome or an illness which was unrelated to CD, if they had short-bowel syndrome, if they had a stoma, if they had a malignant disease, if they suffered from diabetes mellitus, if urgent surgery was needed, if they were being treated with corticoids, if they were being treated with motility regulating drugs with 5-HT receptor activity (e.g. ondansetron) or if they received additional liquid food or were dependent of a liquid diet. Additional exclusion criteria were the presence of a lifetime psychiatric disorder, including alcohol or drug abuse, or the presence of a psychiatric diagnosis at inclusion, a first degree relative with a mood disorder, and the current or past use of psychopharmacological medication. Patients received a compensation for their participation and all participants gave written informed consent.

7.3.2 Study design

The study used a randomized, controlled, double blind, crossover design (figure 1). The patients were their own control subjects. On day 1 of the study, the patients were assigned randomly to either the “active” condition, when they received the amino acid (AA) mixture without L-TRP, or to the control condition, when they received the AA-mixture with L-TRP. On day 2, the patient participated in the other condition of the study. The 2 test days were spaced 1 to 2 weeks apart. The randomization was done by the pharmacist responsible for the preparation of the AA-mixtures. In order to avoid possible bias from premenstrual symptoms, all women were tested in the follicular phase of the menstrual cycle.[55]

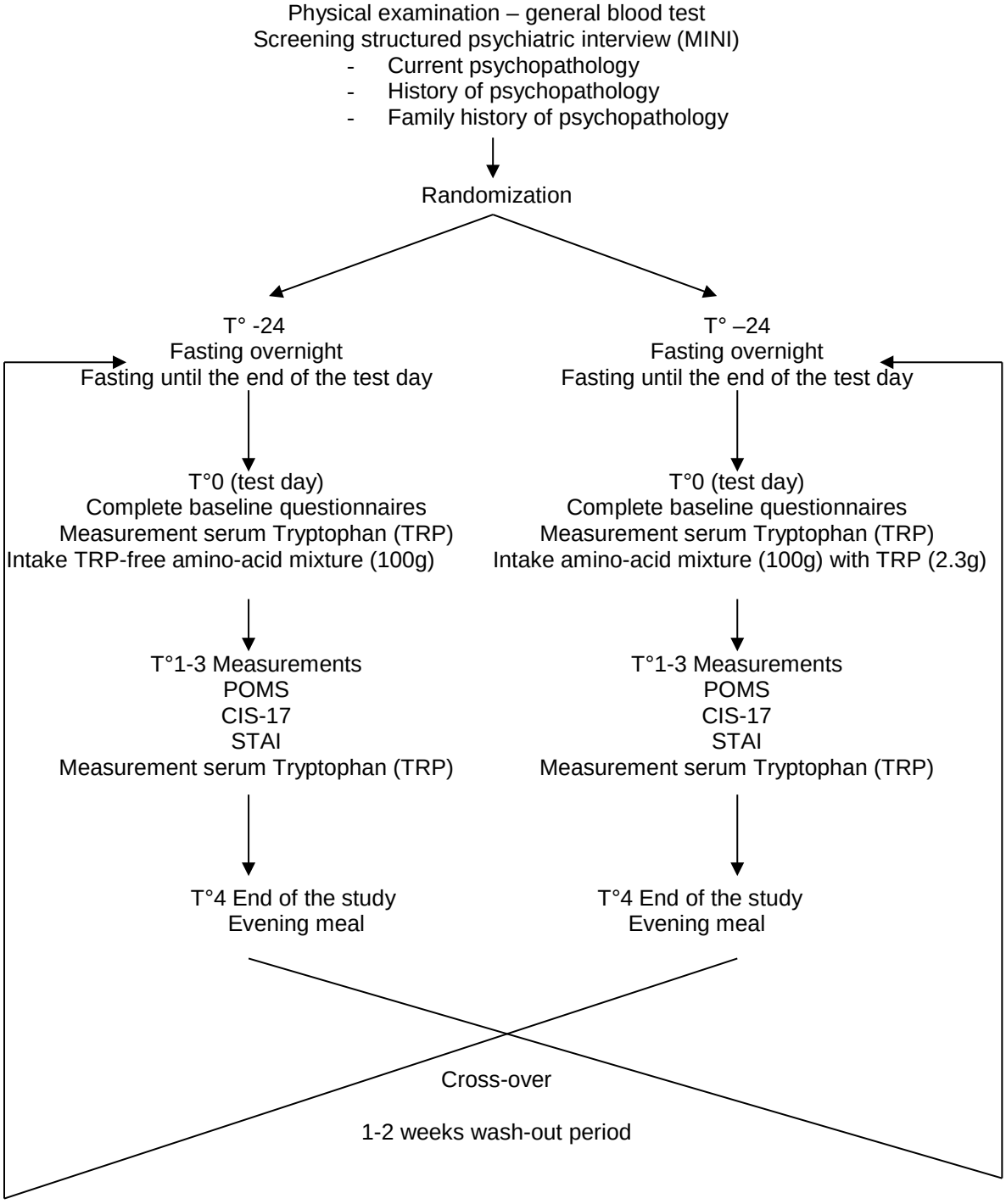
7.3.3 Composition of the amino-acid mixture

The composition of the TRP deficient AA-mixture administered to the patients, which was used in this study, was first described by Young et al.[56] It consisted of 100 g of 15 essential amino-acids: L-Alanine (5.5 g), L-Lysine (8.9 g), L-Threonine (6.5 g), L-Cystene (2.7 g), L-

Leucine (13.5 g), L-Serine (6.9 g), L-Arginine (4.9 g), Glycine (3.2 g), L-Histidine (3.2 g), L-Tyrosine (6.9 g), L-Phenylalanine (5.7 g), L-Methionine (3.0 g), L-Isoleucine (8.0 g), L-Proline (12.2 g) and L-Valine (8.9 g). At the beginning of the test day, the AA-mixture was dissolved in 250 ml water. Sugar (30g) was added to provide caloric energy and patients were offered nose pinchers to mask the unpleasant taste and odor of the mixture. They were encouraged to drink the AA-mixture within 10 minutes. In the control condition, 2.3 g L-tryptophan was added to the AA-mixture, as used by Young and colleagues.[56]However, this was a called a control condition and not a "placebo" condition on the basis of previous research where either 2.3g or 4.6g of TRP was added to the AA-mixture and where subsequently the ratio of TRP/∑LNAA (sum of LNAA tyrosine, leucine, isoleucine, valine and phenylalanine) was assessed. It is hypothesized that the TRP/∑LNAA ratio is an estimation of the TRP uptake in the CNS, given the competition at the BBB.[35]Weltzin et al. demonstrated that adding 2.3g of TRP to the mixture still led to a significant 45% decrease in brain uptake of TRP, whereas adding 4.6g of TRP led to a maximum of 154% increase in uptake of TRP after 3 hours, which would be an acute TRP increase.[57] An intermediate amount of 3g TRP has shown to lead to a slight decrease of 11% in the TRP/∑LNAA ratio.[58] Thus, our procedure aimed at a maximal reduction of TRP levels, compared with a 40% to 50% reduction, as recommended by Van der Does.[35]

7.3.4 Study procedure

Screening After considering the in- and exclusion criteria, the patient was asked to participate in the study. All patients gave informed consent before entering the study. Figure 1 illustrates the study procedure upon entry. The patients were physically examined by a gastroenterologist to exclude possible health issues and to establish a baseline disease activity index by calculating the Crohn's Disease Activity Index (CDAI,[59]). A structured psychiatric interview, the Mini International Neuropsychiatric Interview-plus (MINI-plus[60,61]) was administered to all patients to exclude current and previous psychiatric disorders according to DSM-IV criteria. The 17-item Hamilton Depression Rating Scale (HDRS) was also administered. Additionally a family history was done to exclude psychiatric disorders in first degree relatives. As an extra check on the presence of psychopathology and psychological symptoms, all subjects also completed the Patient Health Questionnaire[62] and the Hospital Anxiety and Depression Scale.[63]



° T: Time T1: after 3 hours
T2: after 5 hours
T3: after 7 hours
* GI: gastrointestinal

Figure 1. Study design

Test Day On the test day, the patients arrived at 8:00 A.M (T_{-1}) (all time points $T_{(-)x}$ refer to a time x hours before or after T_0) after an overnight fast for 12 hours before the start of the experiment (drinking water was allowed). Upon arrival, the patient was examined medically, the clinical information to establish the disease activity with the CDAI was obtained. To ensure patients did not develop a depressive disorder between the screening period and the study or between the two test days, the HDRS was repeated at $T_{-0.25}$. At 9:00 A.M. (T_0), the patients drank the AA mixture within 10 minutes. Blood samples were taken at $T_{-0.25}$, T_3, T_5 and T_7 to measure total and free serum TRP concentrations. At $T_{-0.25}$, the C-reactive protein (CRP) was also measured to assess inflammation, together with hemoglobin, hematocrit and the white blood cell count. Mood, anxiety and fatigue were assessed immediately before the intake of the AA-mixture, at 8:45 A.M., at T_3, T_5 and T_7.

7.3.5 Assessments

Relevant demographic variables, such as gender, age, civil status and work status were gathered. Clinical disease activity was assessed with the Crohn's Disease Activity Index (CDAI).[59] A CDAI disease score of ≥ 150 is considered as clinically active CD.

Mood was assessed with the **Profile of Mood States (POMS)**.[64] The abbreviated Dutch translation of the POMS which was used in this study and which was previously used in other TRP depletion studies,[65,66] consists of 32 bimodal sets of mood related adjectives and measures 5 mood dimensions: depression, anger, fatigue, tension and vigor, of which the scores can be calculated separately. A total score can also be calculated by summing the scores for the five subscales after changing the polarity of the vigor subscale.

Fatigue was assessed with the **Checklist of Individual Strength 20 items(CIS20R)**.[67] It is a multi-dimensional questionnaire developed to assess the severity of subjective fatigue, which we validated for CD patients in this dissertation (chapter 4). The answers are checked on a 7-point Likert scale. In the original questionnaire, the subject is asked to consider each item for the previous 2 weeks. For this study, we adapted the instructions and asked the subjects to consider the items "at this time", since repeated measures were needed. The CIS20R has four measured dimensions which are Subjective Feeling of Fatigue (8 items), reduction in Concentration (5 items), reduction in Motivation (4 items) and reduction in Activity (3 items). The Activity dimension consists of 3 items which cannot be assessed momentarily ("I think I do a lot in 1 day"; "I think I do little in 1 day"; "I achieve almost nothing") and this dimension was therefore omitted from the questionnaire for this study.

The state of anxiety was assessed with the state version of the 20-item self-report **State and Trait Anxiety Inventory Form Y (STAI-Y1)**. The scale measures the degree of anxiety and includes items such as: "I am tense; I am worried" and "I feel calm; I feel secure". Patients

rated the items on a 4-point Likert scale from “not at all” to “very much”. Higher scores indicated a higher degree of anxiety.

7.3.6 Measurement of total and free Tryptophan concentrations

Serum was taken by venipuncture in lithium-heparine tubes and immediately centrifuged within 30 minutes (2400 rpm, 10 minutes at 4 degrees) and stored at -80°C. High-pressure liquid chromatography (HPLC) (Waters, Milford, MA, USA) was used to assess the total and free TRP concentrations in the serum and for the assays, the method described by Williams et al was followed,[31] however a HPLC gradient analysis was used to separate TRP from other amino acids. TRP was detected at 280 nm with a photodiode array detector. This method was first validated. L-Tryptophan was obtained from Fluka. Reagents for the buffer were from Merck and methanol, HPLC-grade, from Fisher Scientific. Other reagents were analytical grade. For total TRP, 250 µl serum was mixed with 250 µl perchloric acid 2M in Eppendorf 1.5 ml tube, centrifuged for 5 minutes at 12,000 rpm in Eppendorf centrifuge type 5451C at 4°C. Supernatants were directly injected (25 µl). For free TRP, we used a Denleythermostated centrifuge to spin the microconcentrators for 30 minutes at 2000 g. The ultrafiltrate was diluted 1:1 with 2M perchloric acid, mixed and injected directly (25 µl). An HPLC system, equipped with a photodiode array detector type Waters 996 (Waters, Milford, MA, USA) was used at 280 nm for the detection of TRP. The mobile phase buffer consisted of 100 mM citrate, 100 mM acetate and 30 nM disodium EDTA, at pH 8.50. Mobile phase A (10% MeOH: 90% v/v) ran isocratic for 5 minutes, changing to gradient mode during 4 minutes, reaching 100% mobile phase B (40%) MeOH: 60% buffer v/v). After 1 minute at 100% B, reequilibration started with solvent A during 12 minutes. The pH of both mobile phases was set at 8.50 at 25°C using an Orion research pH-meter. The mobile phases were degassed by filtration on a Millipore Filtration Apparatus using a Durapore 0.45 µm filter membrane. The flow-rate was set at 1 ml/minute. A Nucleosil 100 RP-C18 cartridge (125 mm long and 4 mm in diameter) was used at 25°C (Macherey-Nagel, Düren, Germany). The cartridge was rinsed daily during 30 minutes with methanol:water 70:30 v/v. Peak identification and data acquisition were realized using a Millennium 32 software (Waters). L-TRP was used as external standard at 1, 5, 10, 20, 50 and 100 µmol/l in MilliQ-grade water. Standards were diluted 1:1 with 2M perchloric acid just before injection of 25 µl. Calibration curves for free TRP (1 to 50 µmol/l and total TRP (1 to 100 µmol/l) were used to calculate concentrations.

7.3.7 Statistics

Statistical analyses were done with SPSS 16.0. Explorative statistics were done to assess the normal distribution of variables and additional tests (Shapiro-Wilk) of normality and visual inspections of the normality plots were done. The mean and the standard deviation (SD) are presented, unless indicated otherwise. Paired samples t-tests and Wilcoxon Signed Rank tests were used when appropriate to compare two related variables. Outcome variables were analyzed using General Linear Model (GLM) for repeated measures. The within-subjects factors for TRP concentrations and for the assessments of mood, anxiety and fatigue were "treatment" (two levels: ATD, control) and "time" (4 levels: T_0, T_3, T_5 and T_7). In case of violation of the assumption of sphericity, the Greenhouse-Geisser correction was applied. Differences in baseline values between test sessions were assessed using GLM and when no differences were found, baseline values were left out of analyses. Bonferroni corrections were used to correct for multiple testing, if post hoc analyses were performed. A $p < 0.05$ was considered as statistically significant.

7.4 RESULTS

7.4.1 Patients

Nine patients participated in the study. One patient who had been screened and who was eligible, did not show up for the experiment without giving notice. The relevant characteristics of the participating patients at the screening visit are presented in table 1. Six of the participating patients were married or living with a partner and the 3 other participants were still living with their parents. Four participants were working full time, 4 participants were on sick leave and 1 participant was a student. The screening blood tests yielded no abnormalities for thyroid hormones, iron reserves, white blood cell count, folate and vitamin B12. Three patients started the study by receiving the LNAA mixture without TRP (test condition) whereas 6 patients received the LNAA mixture with TRP (control condition) on the first test day.

Table 1. Characteristics of the participating patients

Male/Female	4/5
Age (SD)	41 (11.4)
Body Mass Index (SD)	26.3 (5.9)
Age of diagnosis, median (IQR)	21 (18 – 30)
Location of CD	
Terminal Ileum	1/9
Ileocolonic	8/9
Behavior of CD	
Inflammatory type	3/9
Penetrating type	6/9

The comparisons of the relevant patient characteristics between the depletion condition and the control condition on both test days are illustrated in table 2. The clinical disease activity, measured with the CDAI and the CRP, indicative of the severity of inflammation did not differ significantly between the 2 test days. The white blood count was somewhat lower on the day of the ATD, but this difference was not clinically significant. There was no difference between the total and the free serum TRP concentrations at baseline between the two test days.

Table 2. Disease Characteristics before the TRP depletion and before the control condition

	ATD	Control	p-value
CDAI score (SD)	82.4 (66.3)	95.11 (63.9)	0.58
CRP, median (IQR)	5.3 (0.8 – 17.7)	5.5 (0.6 – 12.4)	0.40
Haemoglobin (SD)	13.7 (0.7)	13.8 (1.0)	0.55
Hematocrit (SD)	0.41 (0.02)	0.41 (0.03)	0.39
White Blood Cell Count (SD)	5.53 (1.94)	6.09 (2.29)	0.03
Total Serum TRP (SD)	49,4 (12,5)	48,6 (6,9)	0.75
Free Serum TRP (SD)	13.3 (1.7)	13.3 (2.1)	0.98

ATD: Acute Tryptophan Depletion
Control: control condition

7.4.2 Evolution of TRP after depletion and in the control condition

The evolution of total and free serum TRP after depletion and in the control condition are shown in figure 1 and 2, respectively. Both total and free serum TRP concentrations did not differ at baseline between the test conditions but differed significantly between the depletion condition in comparison with the control condition (figure 2 and 3).

After ATD, the total serum TRP concentration decreased significantly over time ($F_{1.201,\ 9.612}$ = 85.979; $p < 0.0001$) in comparison with the baseline measurement (T0 = 49.40 µmol/L ± 12.46 µmol/L, figure 1) and remained significantly lower 3 hours (16.46 µmol/L ± 5.05 µmol/L; $p < 0.0001$), 5 hours (12.11 µmol/L ± 4.79 µmol/L; $p < 0.0001$) and 7 hours (13.01 µmol/L ± 5.10 µmol/L; $p < 0.0001$) after the intake of the LNAA mixture without TRP, whereas the total serum TRP concentration in the control condition ($F_{1.706,\ 13.652}$ = 64.898; $p < 0.0001$) was significantly higher 3 hours (137.85 µmol/L ± 34.54 µmol/L; $p < 0.0001$) and 5 hours (83.30 µmol/L ± 24.08 µmol/L; $p = 0.007$) after intake of the LNAA mixture with TRP than at baseline (48.56 µmol/L ± 6.85 µmol/L; figure 1). Seven hours after drinking the TRP enriched LNAA mixture, the total serum TRP concentration was not significantly different from the baseline measurement (50.88 µmol/L ± 13.12 µmol/L; $p = 0.99$).

Figure 2 demonstrates that the free serum TRP concentration also decreased significantly over time ($F_{3,\ 24}$ = 39,7333; $p < 0.0001$) in comparison with the baseline measurement (T_0 =

13.26 µmol/L ± 1.68 µmol/L; figure 2) and remained significantly lower 3 hours (5.02 µmol/L ± 1.32 µmol/L; $p < 0.0001$), 5 hours (4.83 µmol/L ± 3.07; $p < 0.0001$) and 7 hours (5.80 µmol/L ± 3.02 µmol/L; $p = 0.001$) after the ATD with the TRP free LNAA mixture. In the control condition, the free TRP concentration increased significantly after the intake of the LNAA mixture with TRP ($F_{3, 24}$ =71.546; $p < 0.0001$), compared to the baseline measurement (13.28 µmol/L ± 2.12 µmol/L; figure 2): after 3 hours (40.74 µmol/L ± 8.86 µmol/L; $p < 0.0001$) and after 5 hours (23.30 µmol/L ± 8.28 µmol/L; $p = 0.025$). Seven hours after drinking the TRP enriched LNAA mixture, the free serum TRP concentration was not significantly different from the baseline measurement (16.36 µmol/L ± 5.27 µmol/L; $p = 0.32$).

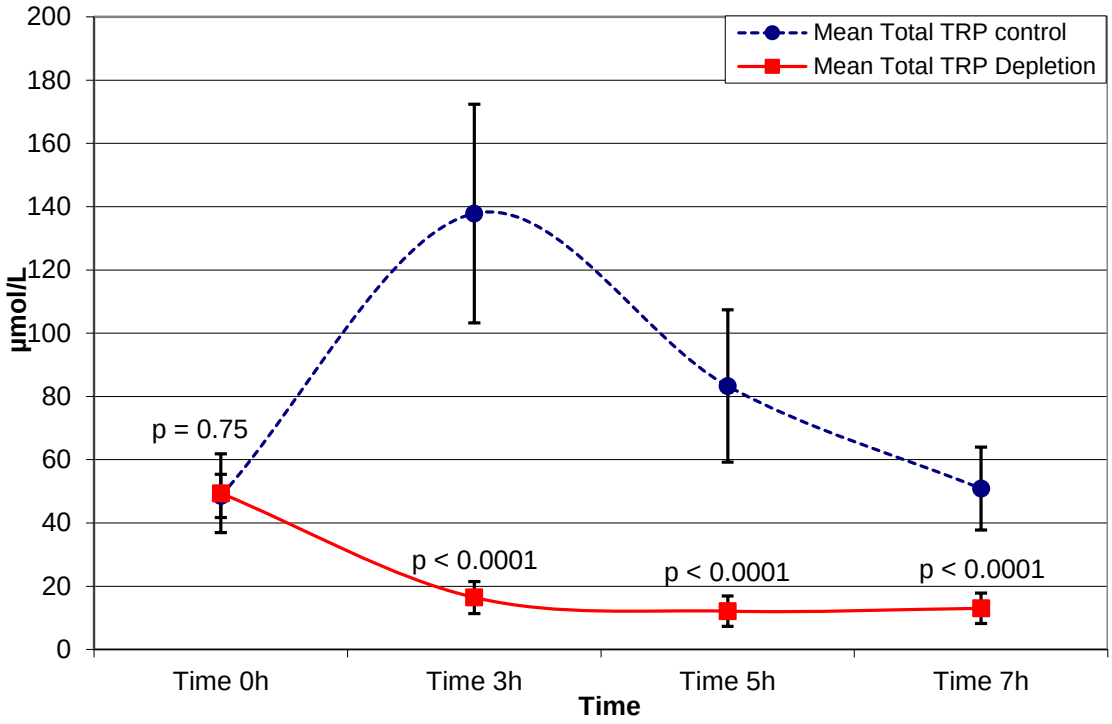


Figure 2. Evolution of mean total serum TRP (standard deviation) indicating a marked difference between the ATD and the control condition ($F_{1.705, 13.643}$ = 78.421; $p < 0.0001$). P-values are for the post hoc analyses of the individual differences between the mean total TRP concentrations of the control condition and the acute tryptophan depletion condition after Bonferroni correction.

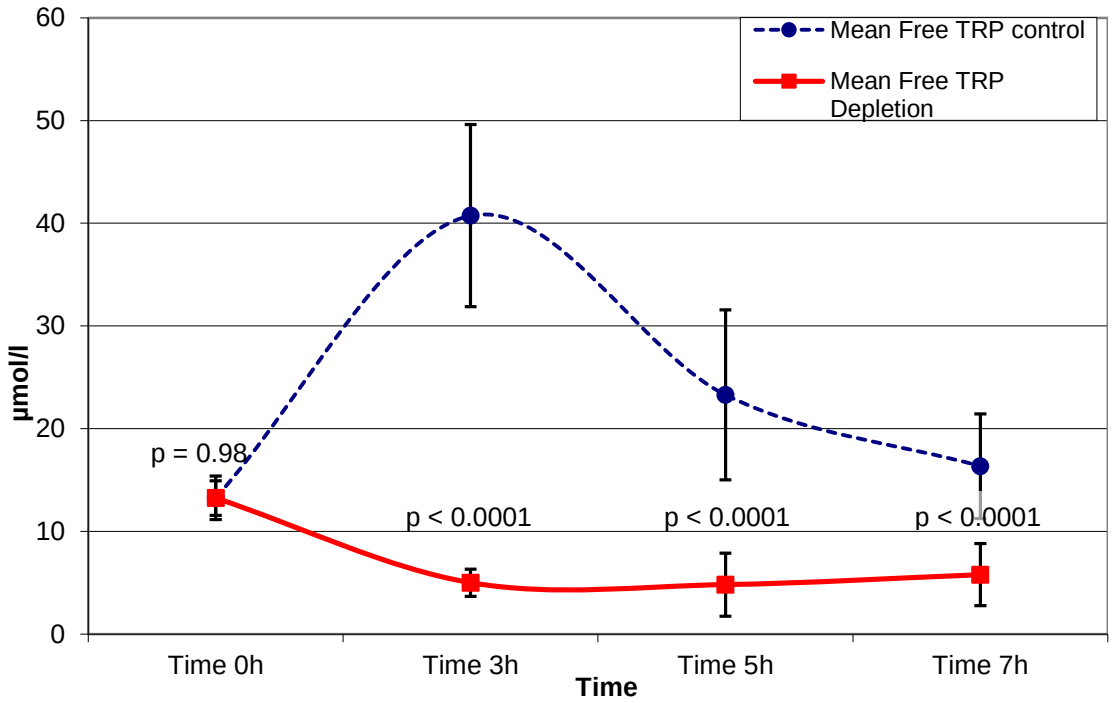


Figure 3. Evolution of mean free serum TRP (standard deviation) indicating a significant difference between the ATD and the control condition ($F_{3, 24} = 92.891$; $p < 0.0001$); P-values are for the post hoc analyses of the individual differences between the mean free TRP concentrations of the control condition and the acute tryptophan depletion condition after Bonferroni correction.

7.4.3 Evolution of the mood, fatigue and anxiety scales

At baseline (T_0), the POMS scores did not differ significantly between the ATD condition and the control condition. There were also no significant differences between the ATD condition and the control condition for the overall POMS Mood score and the depression, anger, fatigue, tension and vigor subscores (treatment x time interaction, table 3).

After controlling for the baseline values, there were no significant differences between the ATD condition and the control condition for the CIS-17 overall fatigue score and its subscales. The overall CIS-17 fatigue score and its subscales did not change significantly after ATD or in the control condition.

There were no significant differences in baseline STAI-Y1 score between the ATD and the control condition. There was no significant difference between the evolution of the STAI-Y1 score in the ATD condition and in the control condition (table 3).

Table 3. Evolution of the mood, fatigue and anxiety scores in the ATD and the control condition

		T0h	T3h	T5h	T7h	Treatment x Time	P
POMS							
Overall Mood	*ATD*	0.66 (23.31)	28.65 (38.63)	30.40 (36.58)	20.66 (38.94)	$F_{1.421,11.372} = 1.283$	0.30
	Control	1.52 (41.09)	18.94 (42.99)	33.67 (60.48)	48.32 (73.00)		
Depression	*ATD*	1.94 (5.60)	7.71 (9.91)	8.68 (10.01)	6.05 (9.03)	$F_{1.463,11.702} = 2.003$	0.18
	Control	2.92 (8.07)	6.88 (10.63)	9.89 (14.13)	12.86 (15.69)		
Anger	*ATD*	3.33 (9.74)	8.25 (10.86)	8.89 (11.25)	7.54 (11.70)	$F_{1.289,10.311} = 0.878$	0.40
	Control	4.21 (11.14)	8.18 (13.16)	10.00 (14.18)	13.25 (16.46)		
Tension	*ATD*	0.24 (8.99)	9.26 (11.02)	10.46 (10.54)	8.89 (11.77)	$F_{1.604,12.836} = 1.185$	0.33
	Control	-0.56 (12.37)	7.32 (11.26)	11.48 (12.75)	14.72 (15.90)		
Fatigue	*ATD*	-3.06 (4.71)	1.48 (6.15)	1.48 (4.14)	0.19 (8.50)	$F_{3,24} = 0.628$	0.60
	Control	-3.52 (8.82)	-0.93 (7.61)	1.30 (11.50)	4.26 (14.80)		
Vigor	*ATD*	1.81 (3.60)	-1.94 (5.34)	-0.89 (4.54)	2.00 (4.42)	$F_{3,24} = 1.684$	0.20
	Control	1.53 (6.70)	2.50 (6.79)	-0.89 (11.90)	-3.22 (14.13)		
CIS-17							
Total Fatigue	*ATD*	56.11 (22.05)	57.44 (23.37)	54.89 (19.43)	59.33 (21.23)	$F_{3,24} = 0.417$	0.74
	Control	66.33 (16.26)	66.11 (21.60)	65.78 (20.04)	62.89 (22.67)		
STAI-Y1							
	ATD	34.2 (11.2)	30.7 (8.3)	29.1 (6.0)	28.9 (6.4)	$F_{1.512,12.093} = 0.448$	0.60
	Control	37.1 (9.6)	34.4 (8.1)	31.2 (7.0)	30.3 (7.3)		

7.5 DISCUSSION

The aim of this study was to test the hypothesis of the possible central 5-HT vulnerability due to inflammation-induced TRP catabolism in CD patients with a double-blind, controlled cross-over ATD paradigm. To our knowledge, this is the first study in CD patients to test this hypothesis with the ATD paradigm. Total and Free TRP concentrations decreased significantly following the experimental ATD condition, which indicates an adequate acute TRP depletion. In the control condition, the peripheral total and free TRP concentrations increased significantly at the two initial measurement points after intake of the AA-mixture and gradually decreased.

The results showed no significant differences between the ATD condition and the control condition in any of the measured variables: mood and its subscales (depression, tension, fatigue, anger, vigor), fatigue and anxiety. There were also no discernible effects for the different variables in individual participants. From the interaction model, it was clear that the condition did not play a role, but time did play a role in the evolution of the variables. ATD did not induce a significant mood reduction in this CD patient population, without severe inflammation or disease activity and without a history of psychiatric problems. Thus, the results from this study do not support the hypothesis of a serotonergic vulnerability in all CD patients as an explanation for a possible role of TRP depletion in the development of psychological symptoms. These results are in line with earlier negative studies in healthy volunteers, without a personal or family history of a psychiatric disorder.[35]

In contrast to the patients in chapter 6, the participating patients in the current study did not present with active, inflammatory CD. The baseline TRP levels in this population, were also not significantly different from normal control samples (data not shown) and significantly higher when compared with the TRP levels of patients with severe, chronic inflammation in chapter 6. The participants of this study did not suffer from clinically active CD, since the mean CDAI was < 150. Furthermore, significant inflammation was only present in 2 patients on both test days, but in neither patient a clear decrease of mood was present in the ATD condition.

Active inflammation in CD is characterized by cell-mediated immune activation and the production of pro-inflammatory cytokines such as TNF-α, IL-2 and IL-1β. These cytokines induce the enzyme IDO which will lead to a significant decrease in peripheral TRP levels by increasing its oxidative catabolism.[19] The activation of the TRP degrading IDO, with the drastic fall in plasma or serum TRP levels has been proposed by several authors as a possible depressogenic mechanism of action of cytokines, also in studies researching TRP

in CD.[17,19] Furthermore, our findings in chapter 6 could also be interpreted as a possible role for inflammation-mediated TRP reduction in the change of HRQL. Thus, it is possible that ATD in CD patients with mildly to moderately active CD might have produced a different result because the initial TRP levels are lower in this population of CD patients, and might have had an influence on mood. On the other hand, it is unclear whether the reduced availability of peripheral TRP due to inflammation will, by definition, lead to a reduced central availability of TRP, an altered central serotonergic neurotransmission and an effect on psychological symptoms.

The alterations of brain serotonergic neurotransmission has long been one of the main hypotheses in the pathogenesis of inflammation-associated depression, through a drastic fall in TRP bioavailability by the activation of IDO, which would decrease the biosynthesis of serotonin in the central nervous system. This was suggested by observations of decreased plasma TRP levels in patients with inflammatory disorders and in patients who received cytokine immunotherapy.[18,68] The finding of a linear relationship between decreased peripheral TRP levels and depression scores in cancer patients after 3 weeks of immunotherapy by Capuron et al. was very suggestive of such a causal relationship.[25] Thus, IDO activation might play a crucial role in the transition from cytokine-induced sickness behavior symptoms (a core of neurovegetative symptoms including fatigue, listlessness, pain, decreased appetite, and sleep disorders rapidly emerging in most patients[69]) to depression, with a clear temporal dissociation.[68] These results were in support of our initial hypothesis in CD patients and previous studies have demonstrated that IDO is induced due to the increased inflammatory cytokines in active CD, which is associated with significantly decreased levels of serum TRP.[19,29]
Animal experiments were done in which either the inducible expression of cytokines (and thus the induction of IDO) was blocked or the activation of IDO was directly blocked. Blocking the expression of cytokines resulted in the absence of both sickness behavior and depressive-like behavior in test animals. When only the activation of IDO was blocked, the sickness behavior remained intact, whereas the depressive-like behavior did not appear. These studies have confirmed that inflammation-associated depression develops on a background of sickness behavior, but does not confound it and that it is dependent on the activation of IDO.[17]

More recently, however, both animal and clinical human studies have rendered the hypothesis of a decreased serotonergic neurotransmission as a central mechanism for inflammation-associated depression less probable and have led to a shift in the hypothesis from TRP depletion towards other possible mechanisms, such as the detrimental effects of

the TRP metabolites. O'Conner et al. found activation of IDO by administering lipopolysaccharide (LPS) to mice, which resulted in decreased TRP levels and increased the levels of its metabolite of the oxidative pathway, kynurenine, with an increased kynurenine to TRP ratio.[70] In the brain, only an increase of kynurenine levels was observed, without a concomitant decrease of TRP levels. LPS paradoxically increased brain TRP and the turnover of brain 5-HT. Furthermore, when the LPS-induced changes were blocked, either by blocking the expression of cytokines or by directly blocking IDO, the depressive-like behavior disappeared, and the LPS-increases in the kynurenine to TRP ratio in the plasma and the brain were attenuated. There was, however, no effect on the LPS-induced increases in brain TRP and 5-HT turnover.[70]

Clinical studies in humans confirm the findings in animal studies. A recent study in interferon-alpha (IFN-α) treated patients demonstrated that central TRP levels (measured in the central spinal fluid) did not change significantly, despite the significantly lowered peripheral blood TRP levels.[71] Wichers et al. found an increased kynurenine/TRP ratio, together with increased symptoms of depression in patients with chronic hepatitis C, treated with IFN-α for up to 24 weeks. The peripheral TRP/∑LNAA (indication of the uptake of TRP at the BBB), however did not change despite significantly lower plasma TRP levels.[72] These findings also suggest a depletion of peripheral TRP is not sufficient as an explanation for the development of depressive symptoms. Most likely, there is a compensation mechanism of the brain - which still needs to be elucidated - for a decrease in circulating TRP induced by acute or chronic inflammation.[17] Thus, it is possible that an ATD protocol cannot yield positive results in CD patients without a history of mood disorder, since a different mechanism is likely responsible for inflammation-associated mood changes or depression. This is a possible explanation for the negative results of this study.

Other hypotheses for the mechanism of inflammation-induced depression have been proposed to account for the depressogenic action of cytokines, such as the development of glucocorticoid resistance, the induction of extrahypothalamic CRF and vasopressin and increased expression of the 5-HT transporter.[17,73] Possibly, TRP metabolites might play a role, independently of 5-HT through their neurotropic action. IDO degrades TRP into kynurenine which is inactive, but kynurenine is further degraded into several other metabolites, dependent of the cell type in which it was produced or transported.[17] In the brain, the metabolites of kynurenine can be neurotoxic (3-hydroxykynurenine and quinolinic acid) due to their ability to generate oxidative radicals and to act as agonists of the NMDA receptor. Kynurenic acid, another metabolite, is neuroprotective by antagonizing the NMDA and α-7 nicotinic acetylcholine receptor. Picolinic acid is also neuroprotective. It has been hypothesized that inflammation-induced IDO activation might switch the metabolism of the

kynurenine pathway toward the production of neurotoxic metabolites and away from kynurenic acid.[74] Clinical studies have found correlations between increased depressive symptoms and evidence of such a shift, away from neuroprotective metabolites (kynureninic acid), toward neurotoxic metabolites of kynurenine (quinolinic acid) in the presence of inflammation.[71,72] It is possible that this mechanism also plays a role in the development of depressive symptoms or syndrome in patients with active CD. A proportion of the depressive disorders which are diagnosed in CD patients could therefore be inflammation-associated depression, rather than simply caused by the severity of the disease. This should be further explored in research in CD which focusses on the activation of IDO, the metabolites of the kynurenine pathways and their effects, the difference between subjects who become depressed and those who remain depression-free and the possible effects of anti-inflammatory therapeutics such as infliximab. At this point, it is unclear if a further pursuit of the ATD paradigm, can contribute to a better understanding of the association between inflammation in CD and mood.

An important limitation to this study was the small sample size. Thus, it might be possible that the negative result is the result of a type 2 error. Furthermore, this made it impossible to compare subgroups, such as the different phenotypes, or patients with active inflammation and no inflammation. The patients in this sample had only inactive or mildly active CD. The initial protocol included a group of patients with moderately active CD. Although the ATD protocol is not invasive, it was difficult to recruit patients with gastrointestinal illness, especially while they had active symptoms. This might be one of the explanations why most patients in this sample did not have active CD. Another limitation is the absence of an appropriate control group. It was therefore impossible to compare between groups effects. The measurements of the biological parameters were also somewhat limited. There was no measurement of TRP/∑LNAA ratio. This ratio is important to have an indication of the TRP availability at the BBB and thus the availability in the CNS.[35] This would have been of particular interest, since Wichers et al. found no change of this ratio in IFN-alpha treated hepatitis C patients, despite a decrease in TRP levels.[72] Furthermore, without the TRP/∑LNAA ratio, the effect of the control condition on the central availability of TRP remains unclear. There was also no measurement of kynurenine or kynurenic acid, which is important to assess the IDO activity. This was, however, not the aim of this study.
Only mood, fatigue and anxiety were measured in this study. There was no measurement of gastrointestinal (GI) symptoms. Studies in irritable bowel syndrome have demonstrated a clear effect of changes in TRP load (both ATD and acute TRP loading) on GI symptoms.[75,76] ATD probably also modulates the 5-HT metabolism in the intestine and might influence the intestinal functionality and the visceral perception. Since the brain-gut axis consists of

bidirectional neurohumoral pathways, any intervention at the systemic, GI or CNS level, will probably have an effect on the symptoms of the patients. Altered sensory processing has also been described in CD, although not necessarily similar to IBS.[77] The ATD paradigm might have been an interesting additional way to explore the brain-gut axis.

In conclusion, this study does not support the hypothesis of a 5-HT vulnerability due to inflammation-induced TRP reduction, which can be elicited by an ATD paradigm. Most likely, the inflammation-induced psychological symptoms, such as depressive symptoms, or psychopathology, such as depressive disorder, are not associated to the drastic decrease of TRP bioavailability, but rather to other mechanisms which are influenced by the presence of high levels of pro-inflammatory cytokines. One such mechanism, inflammation-induced IDO activation, might switch the kynurenine pathway which degrades TRP from neuroprotective to neurotoxic, which is correlated with depressive symptoms. This possible mechanism cannot be demonstrated with ATD, however.

7.6 REFERENCES

1. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet*. 2007;369:1641–1657.
2. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet*. 2007;369:1627–1640.
3. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med*. 2009;361:2066–2078.
4. Ringel Y, Drossman DA. Psychosocial aspects of Crohn's disease. *Surg Clin North Am*. 2001;81:231–52.
5. Mikocka-Walus AA, Turnbull DA, Moulding NT, et al. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflamm Bowel Dis*. 2007;13:225–34.
6. Sajadinejad MS, Asgari K, Molavi H, et al. Psychological issues in inflammatory bowel disease: an overview. *Gastroenterol Res Pract*. 2012;2012:106502.
7. Drossman DA, Ringel Y. Psychosocial factors in ulcerative colitis and Crohn's disease. In: Sartor RB, Sandborn W, eds. *Kirsner's Inflammatory Bowel Disease*.Vol 6th. Philadelphia: W.B. Saunders Co; 2004:340–356.
8. Mardini HE, Kip KE, Wilson JW. Crohn's disease: a two-year prospective study of the association between psychological distress and disease activity. *Dig Dis Sci*. 2004;49:492–497.
9. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. *Psychosom Med*. 2004;66:79–84.
10. Persoons P, Vermeire S, Demyttenaere K, et al. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Aliment Pharmacol Ther*. 2005;22:101–10.
11. Andrews H, Barczak P, Allan RN. Psychiatric illness in patients with inflammatory bowel disease. *Gut*. 1987;28:1600–1604.
12. Schwartz RA, Schwartz IK. Psychiatric disorders associated with Crohn's disease. *Int J Psychiatry Med*. 1982;12:67–73.
13. Helzer JE, Chammas S, Norland CC, et al. A study of the association between Crohn's disease and psychiatric illness. *Gastroenterology*. 1984;86:324–330.
14. North CS, Alpers DH. A review of studies of psychiatric factors in Crohn's disease: etiologic implications. *Ann.Clin.Psychiatry*. 1994;6:117–124.
15. Nahon S, Lahmek P, Durance C, et al. Risk factors of anxiety and depression in inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:2086–91.
16. Addolorato G, Capristo E, Stefanini GF, et al. Inflammatory bowel disease: a study of the association between anxiety and depression, physical morbidity, and nutritional status. *Scand J Gastroenterol*. 1997;32:1013–1021.
17. Dantzer R, O'Connor JC, Lawson MA, et al. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology*. 2011;36:426–36.
18. Russo S, Kema IP, Fokkema MR, et al. Tryptophan as a Link between Psychopathology and Somatic States. *Psychosom Med*. 2003;65:665–671.

19. Gupta NK, Thaker AI, Kanuri N, et al. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis*. 2012;18:1214–20.
20. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol*. 2012.
21. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004;4:762–74.
22. Cherayil BJ. Indoleamine 2,3-dioxygenase in intestinal immunity and inflammation. *Inflamm Bowel Dis*. 2009;15:1391–6.
23. Carlin JM, Borden EC, Byrne GI. Interferon-induced indoleamine 2,3-dioxygenase activity inhibits Chlamydia psittaci replication in human macrophages. *J Interferon Res*. 1989;9:329–37.
24. Lestage J, Verrier D, Palin K, et al. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun*. 2002;16:596–601.
25. Capuron L, Ravaud A, Neveu PJ, et al. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry*. 2002;7:468–73.
26. Capuron L, Neurauter G, Musselman DL, et al. Interferon-alpha–induced changes in tryptophan metabolism. *Biol Psychiatry*. 2003;54:906–914.
27. Widner B, Leblhuber F, Walli J, et al. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm*. 2000;107:343–53.
28. Schroecksnadel K, Winkler C, Duftner C, et al. Tryptophan degradation increases with stage in patients with rheumatoid arthritis. *Clin Rheumatol*. 2006;25:334–7.
29. Wolf AM, Wolf D, Rumpold H, et al. Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. *Clin Immunol*. 2004;113:47–55.
30. Stahl S. Chemical neurotransmission as the mediator of disease actions. In: *Essential psychopharmacology: neuroscientific basis and practical applications*. 2nd ed. Cambridge: Cambridge University Press; 2000:99–135.
31. Williams WA, Shoaf SE, Hommer D, et al. Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5-Hydroxyindoleacetic Acid in Normal Volunteers. *J Neurochem*. 2001;72:1641–1647.
32. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry*. 2001;178:399–405.
33. Wichers M, Maes M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. *Int J Neuropsychopharmacol*. 2002;5:375–88.
34. Reilly JG, McTavish SF, Young AH. Rapid depletion of plasma tryptophan: a review of studies and experimental methodology. *J Psychopharmacol*. 1997;11:381–92.
35. Does AJ Van der. The effects of tryptophan depletion on mood and psychiatric symptoms. *J Affect Disord*. 2001;64:107–19.
36. Neumeister A, Praschak-Rieder N, Besselmann B, et al. Effects of tryptophan depletion on drug-free patients with seasonal affective disorder during a stable response to bright light therapy. *Arch Gen Psychiatry*. 1997;54:133–8.
37. Young SN, Ervin FR, Pihl RO, et al. Biochemical aspects of tryptophan depletion in primates. *Psychopharmacology (Berl)*. 1989;98:508–11.
38. Moja EA, Cipolla P, Castoldi D, et al. Dose-response decrease in plasma tryptophan and in brain tryptophan and serotonin after tryptophan-free amino acid mixtures in rats. *Life Sci*. 1989;44:971–6.
39. Gartside SE, Cowen PJ, Sharp T. Effect of 5-hydroxy-L-tryptophan on the release of 5-HT in rat hypothalamus in vivo as measured by microdialysis. *Neuropharmacology*. 1992;31:9–14.
40. Gartside SE, Cowen PJ, Sharp T. Evidence that the large neutral amino acid L-valine decreases electrically-evoked release of 5-HT in rat hippocampus in vivo. *Psychopharmacology (Berl)*. 1992;109:251–3.
41. Bremner JD, Innis RB, Salomon RM, et al. Positron emission tomography measurement of cerebral metabolic correlates of tryptophan depletion-induced depressive relapse. *Arch Gen Psychiatry*. 1997;54:364–74.
42. Aberg-Wistedt A, Hasselmark L, Stain-Malmgren R, et al. Serotonergic "vulnerability" in affective disorder: a study of the tryptophan depletion test and relationships between peripheral and central serotonin indexes in citalopram-responders. *Acta Psychiatr Scand*. 1998;97:374–80.
43. Smith KA, Fairburn CG, Cowen PJ. Relapse of depression after rapid depletion of tryptophan. *Lancet*. 1997;349:915–9.
44. Leyton M, Young SN, Benkelfat C. Relapse of depression after rapid depletion of tryptophan. *Lancet*. 1997;349:1840–1.
45. Moreno FA, Gelenberg AJ, Heninger GR, et al. Tryptophan depletion and depressive vulnerability. *Biol Psychiatry*. 1999;46:498–505.
46. Benkelfat C, Ellenbogen MA, Dean P, et al. Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry*. 1994;51:687–97.
47. Lam RW, Zis AP, Grewal A, et al. Effects of rapid tryptophan depletion in patients with seasonal affective disorder in remission after light therapy. *Arch Gen Psychiatry*. 1996;53:41–4.

48. Lam RW, Bowering TA, Tam EM, et al. Effects of rapid tryptophan depletion in patients with seasonal affective disorder in natural summer remission. *Psychol Med*. 2000;30:79–87.
49. Neumeister A, Praschak-Rieder N, Hesselmann B, et al. Rapid tryptophan depletion in drug-free depressed patients with seasonal affective disorder. *Am J Psychiatry*. 1997;154:1153–5.
50. Neumeister A, Habeler A, Praschak-Rieder N, et al. Tryptophan depletion: a predictor of future depressive episodes in seasonal affective disorder? *Int Clin Psychopharmacol*. 1999;14:313–5.
51. Neumeister A, Konstantinidis A, Praschak-Rieder N, et al. Monoaminergic function in the pathogenesis of seasonal affective disorder. *Int J Neuropsychopharmacol*. 2001;4:409–20.
52. Huang A, Fuchs D, Widner B, et al. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. *Br J Cancer*. 2002;86:1691 – 1696.
53. Baars JE, Nuij VJAA, Oldenburg B, et al. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis*. 2012;18:1634–40.
54. Sitrin MD. Nutrition and Inflammatory Bowel Disease. In: Kirsner JB, ed. *Inflammatory Bowel Disease*. 5th ed. Philadelphia, Pennsylvania: Saunders; 2000:598–610.
55. Menkes DB, Coates DC, Fawcett JP. Acute tryptophan depletion aggravates premenstrual syndrome. *J Affect Disord*. 1994;32:37–44.
56. Young SN, Smith SE, Pihl RO, et al. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)*. 1985;87:173–7.
57. Weltzin TE, Fernstrom JD, McConaha C, et al. Acute tryptophan depletion in bulimia: effects on large neutral amino acids. *Biol Psychiatry*. 1994;35:388–97.
58. Klaassen T, Riedel WJ, Someren A van, et al. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiatry*. 1999;46:489–97.
59. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). *Gastroenterology*. 1979;77:843–846.
60. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59 Suppl 2:22–33.
61. Vliet IM van, Beurs E de. [The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders]. *Tijdschr Psychiatr*. 2007;49:393–7.
62. Persoons P, Luyckx K, Desloovere C, et al. Anxiety and mood disorders in otorhinolaryngology outpatients presenting with dizziness: validation of the self-administered PRIME-MD Patient Health Questionnaire and epidemiology. *Gen Hosp Psychiatry*. 2003;25:316–323.
63. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67:361–370.
64. McNair DM, Lorr M, Droppleman LF. *Profile of Mood States (POMS)*. San Diego, CA: Educational and Industrial Testing Services; 1971.
65. Wald FD, Mellenbergh GJ. De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). [The shortened version of the Dutch translation of the Profile of Mood States (POMS).]. *Nederlands Tijdschrift voor de Psychologie en haar Grensgebieden*. 1990;45:86–90.
66. Sobczak S, Honig A, Nicolson N a, et al. Effects of acute tryptophan depletion on mood and cortisol release in first-degree relatives of type I and type II bipolar patients and healthy matched controls. *Neuropsychopharmacology*. 2002;27:834–42.
67. Vercoulen JH, Swanink CM, Fennis JF, et al. Dimensional assessment of chronic fatigue syndrome. *J Psychosom Res*. 1994;38:383–392.
68. Capuron L, Dantzer R. Cytokines and depression: The need for a new paradigm. *Brain Behav Immun*. 2003;17:119–124.
69. Dantzer R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North Am*. 2009;29:247–64.
70. O'Connor JC, Lawson MA, André C, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*. 2009;14:511–22.
71. Raison CL, Dantzer R, Kelley KW, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry*. 2010;15:393–403.
72. Wichers MC, Koek GH, Robaeys G, et al. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol Psychiatry*. 2005;10:538–44.
73. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry*. 2012;83:495–502.
74. Maes M, Leonard BE, Myint AM, et al. The new "5-HT" hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011;35:702–21.
75. Kilkens TOC, Honig A, Nieuwenhoven MA van, et al. Acute tryptophan depletion affects brain-gut responses in irritable bowel syndrome patients and controls. *Gut*. 2004;53:1794–800.
76. Shufflebotham J, Hood S, Hendry J, et al. Acute tryptophan depletion alters gastrointestinal and anxiety symptoms in irritable bowel syndrome. *Am J Gastroenterol*. 2006;101:2582–7.
77. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory bowel disease. *Gastroenterology*. 2013;144:36–49.

Chapter 8

8 General Discussion

The aim of this part is to discuss the major findings of the 3 sections of this thesis. Their relationship and the results will be confronted with the current literature. The focus will be on the bidirectional relationship between psychosocial factors and Crohn's disease (CD), rather than on cause and effect. The limitations of this thesis will be discussed and finally a number of suggestions with regard to future research are given.

8.1 THE IMPORTANCE OF VALIDATED QUESIONNAIRES - DISCUSSION OF SECTION 1 – METHODOLOGICAL STUDIES

Methodological issues are often at the basis of the heterogeneity in the results concerning the association between psychosocial factors and CD. This can lead to confusion and ultimately to controversies, which will make it impossible to draw firm conclusions. The focus of this section was on the validation of two questionnaires which were used in the doctoral project. The two studies differed in the type of validation which was done and they each have their specific uses for research purposes. For each instrument their possible usefulness in clinical practice will be assessed.

In the *first study*, presented in chapter 3, we **contributed to the validation process of the Inflammatory Bowel Disease Questionnaire (IBDQ)** to measure health related quality of life (HRQL) in CD patients and described the **responsiveness of the IBDQ** to rapid changes in a CD patient's condition after treatment. Furthermore, **cutoff values for remission and for a meaningful clinical improvement** were also proposed. All four dimensions of the IBDQ responded quickly to a change in a patient's clinical condition after treatment focused on induction of remission. This validation data added to the considerable literature of construct validity and reproducibility of the IBDQ in CD patients.[1] The results from this validation study confirmed that the IBDQ could be used as an outcome parameter in short-term studies in this doctoral project, reflecting change in HRQL of CD patients after an intervention.

The IBDQ is a disease specific, multi-dimensional questionnaire and is currently the undisputed gold standard to measure HRQL in IBD patients. Other instruments measuring HRQL are global assessment instruments, such as visual analogue scales or graded scales, which provide a summary of an overall score of HRQL, and generic instruments which are developed in and for the general population, and which reflect multiple relevant dimensions, independent of gender, age and disease (e.g. SF-36).[2] Both global assessment instruments and generic HRQL instruments are very well suited to compare different populations, but often lack the ability to detect important clinical changes. The IBDQ was developed for IBD patients and should be able to detect changes over time and significant effects of interventions. Since a longitudinal study design and intervention studies, rather than cross-sectional studies with a control population, were best suited to study the association between psychosocial factors and CD, a disease specific HRQL such as the IBDQ was preferable. Nevertheless, it is important to remain vigilant for the possible shortcomings of disease specific questionnaires. An overlap between the measured disease activity and the disease specific HRQL, assessed with the IBDQ is possible, which could magnify the correlation between both factors. Therefore, it might have been useful to include a short generic HRQL questionnaire and this should be mentioned as a limitation.

The convergent and construct validity, as well as the reliability of the IBDQ have been studied extensively and have been demonstrated previously.[2] This study assessed a specific aspect of the instrument property, i.e. **the responsiveness** of the IBDQ, specifically in CD patients, to rapid changes. It was important to add to this aspect of the validation process, given the specific studies in which this questionnaire was applied within this doctoral project. Nevertheless, it should be stressed that a validation is an ongoing process which is never complete.

HRQL and psychosocial factors are closely related and several studies in IBD have demonstrated that psychological factors predict HRQL.[2,3] Overall, HRQL is an important gauge to measure the impact of the CD illness and of the therapeutic interventions on a patient. The cutoff values of the IBDQ, determined in our study, can therefore be meaningful additional indicators of the effect of any treatment, since they give an insight if a clinical improvement of symptoms also indicates an improvement of the patient's overall well-being and functioning. An additional interesting aspect of the association between HRQL and psychosocial factors in IBD in general and CD in particular, is the inconsistency in the correlation between disease activity and HRQL. Results from earlier studies demonstrate that psychosocial factors play an important role in determining HRQL in CD.[4,5] Furthermore, psychiatric disorders are an independent risk factor of poor HRQL in patients with CD.[6,7] Zhang et al recently demonstrated an important role for depression in the prediction of

HRQL. Their study showed that depressive symptoms may be a stronger risk factor for poor HRQL than disease activity alone.[8] These findings stress the importance of psychiatric disorders in general and depression in particular, in relation to the HRQL in patients with CD.

The *second study* of this section, which is described in chapter 4, presented **the validation of the generic Checklist of Individual Strength 20 items (CIS-20R), specifically for CD patients and the prevalence of fatigue in CD patients with the previously established cut-off of the CIS-20R for significant fatigue**. We demonstrated that the CIS-20R is a reliable and valid instrument to measure fatigue in patients with CD. Furthermore, the CIS-20R remains stable over time in CD patients who did not report any change in their disease activity, yet the CIS-20R is also able to detect clinically significant changes in fatigue in CD patients who report a clinically significant change in disease activity. These findings are important for the usefulness of this scale in longitudinal research and as an outcome parameter in intervention studies (see chapter 7).

Although a validation process is never completed, the **validation procedure** of the CIS-20R in CD patients was done as **comprehensive** as possible by determining the underlying dimensions, the internal consistency, the reliability, the reproducibility, the responsiveness, the concurrent and the convergent validity. Determining criterion validity for the CIS-20R is impossible because of the lack of an accepted “gold standard” measure of fatigue. This would inherently require a thorough understanding and universally accepted definition of “fatigue”, which does not yet exists. The relative recent interest for fatigue in IBD-related research adds to the confusion and the heterogeneity in the concepts and terminology which is used by different authors. There is an urgent need for further exploration and conceptualization of the symptom fatigue in IBD. Figure 8.1 demonstrates the different aspects of fatigue and in general the multidimensional character is confirmed in the literature.[9,10]

With the CIS-20R, we provided a multidimensional questionnaire which can reflect the type of fatigue, its effect on the functioning of the patients and its severity. To ascertain the duration of fatigue, it remains necessary to ask additional questions. Furthermore, the precise impact for the individual is unclear without qualitative information, which is currently the case for all assessment tools.

Since there is no universally accepted definition of “fatigue”, there are obviously no accepted diagnostic criteria to determine a “diagnosis” of “significant fatigue”. Nevertheless, prevalence data are omnipresent in the literature and in most cases they are based on an arbitrary cut-off for whichever questionnaire has been used.[9,10] Since fatigue is a subjective,

multidimensional and ill-defined concept, prevalence rates are difficult to compare between studies and they lead to controversies. Although fatigue is not a disorder as such, it is not merely a symptom of Crohn's disease or part of the illness behavior complex. Because fatigue seems to exist as a unique entity with significant consequences for the patient's functioning and quality of life, it is interesting to determine which patients might be at risk due to the severity of fatigue. The CIS-20R provides a well validated and well defined cut-off score, which has been determined as the *fatigue level that puts the individual "at risk" for subsequent sick leave or work disability*.[11] This cut-off should not however, be considered as a "diagnosis" of fatigue, but can be described as the level at which the functional impairment due to fatigue becomes clinically and socially significant for the individual patient. Thus, it is possible to identify CD patients who are at risk as a result of the impact of their fatigue. Further research on the validity of this cut-off in CD patients or IBD patients in general is warranted. In chronic disease, fatigue is almost never exclusively determined by the activities which someone performed. Several studies have shown that disease related factors play a role, but insufficiently explain fatigue.[9] Other factors have been identified and suggest that there might be several subgroups within CD patients with severe fatigue. Further research into the character of fatigue which is present in CD patients is necessary.

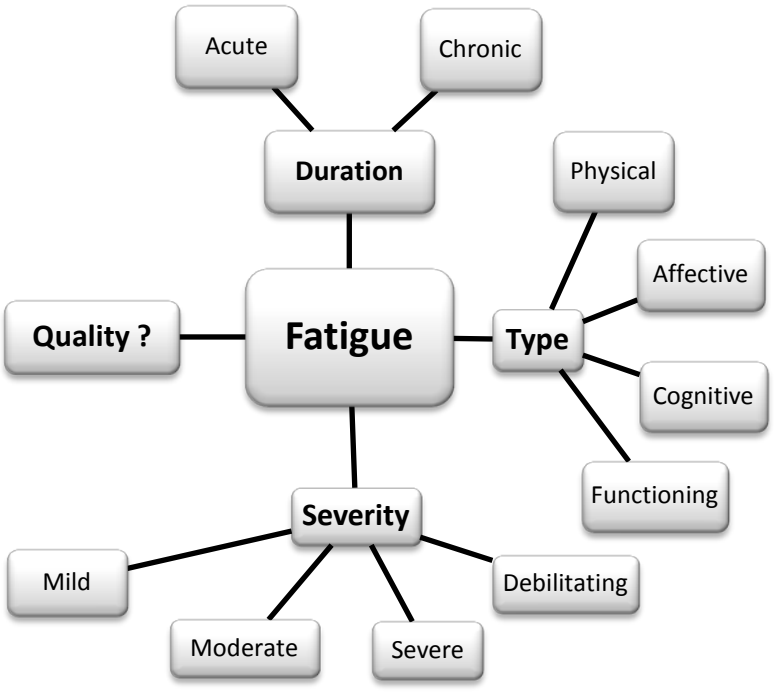


Figure 8.1 Theoretical aspects of fatigue

8.2 DISCUSSION PART 2 – PSYCHO – SOMA: THE ROLE OF DEPRESSION IN THE ASSOCIATION BETWEEN PSYCHOSOCIAL FACTORS AND CROHN'S DISEASE

The aim of the second section was to investigate the role of psychological factors in the pathophysiology of CD. The study, presented in chapter 6, assessed **the influence of major depressive disorder (MDD) on the short- and long-term outcome of treatment with infliximab**. Biological and psychosocial parameters were assessed in patients with active CD at the time of treatment with infliximab. They were re-evaluated 4 weeks after treatment and subsequently followed up until the moment they needed to be treated again due to relapse of CD activity. We found that patients with MDD at baseline had a lower chance to achieve short-term remission after treatment with infliximab, but MDD did not affect short-term clinical response. Additionally, we found that the presence of MDD at the time of treatment predicted an earlier retreatment in these patients. These findings illustrate the highly complex relationship between psychological factors in general, and MDD in particular, on the one hand and CD on the other hand, especially when investigating the association with the disease activity and the disease course.

8.2.1 Discussion of the effect of MDD on the short-term outcome of treatment with infliximab

The data on the short-term effect of MDD on the outcome of infliximab treatment could either be interpreted as a true interaction between psychopathology and the effectiveness of the medication and/or the course of the disease. However, the definition of clinical remission of CD was only based on the score of the CDAI (defined as CDAI score < 150), rather than on inflammatory markers or other parameters which could be assessed objectively. Furthermore, there was no influence of MDD on the short-term response (defined as a decrease of ≥ 70 point on the CDAI) after infliximab treatment which decreased proportionally in comparison with non-depressed patients. "General well-being" and "intensity of abdominal pain" are two subjective items of the CDAI which weigh heavily on the total score (x 7 and x 5, respectively) and which can be influenced by the presence of MDD.[5,12,13] The relationship between the "general well-being" item and depressive symptoms was also demonstrated by our results. Thus, based on our data, we cannot conclude that infliximab was less effective in patients with MDD after 4 weeks. This rather seemed to be an illustration of the association between psychological factors, i.e. MDD, and physical symptoms. Previous studies have demonstrated a relationship between psychological factors and physical symptoms in CD.[14]

8.2.2 Discussion of the effect of MDD on the long-term outcome of treatment with infliximab

At long-term, patients with MDD had a significantly higher risk at earlier relapse, which was reflected by an earlier need of retreatment. These results can be interpreted as a true association between MDD and the course of CD after treatment. Our results are in line with the findings in other studies, which demonstrated a specific association between depressive symptoms and CD.[15,16] The results concerning the effect of stressors, perceived stress and coping with stress on the course of CD remain inconsistent and the evidence is not as strong as in patients with UC. In contrast to MDD or depressive symptoms, it is often necessary to look at specific subgroups or post-hoc analyses when studying stress in CD, as illustrated by the recent study by Bitton and colleagues which demonstrated an effect of perceived stress and of "avoidance coping", together with their interaction, in the multivariate time-dependent model.[17] Furthermore, the recent results from the Swiss IBD Cohort Study Group demonstrated that the association between perceived stress and the exacerbation of CD was fully attributable to the mood components, specifically depressive and anxiety symptoms.[18] Our findings, together with the findings from the other studies in CD, seem to support a relatively specific association between depression on the one hand, and the course of and disease outcome in CD and inflammation, on the other hand.

Although the results from this study seem to support the hypothesis that MDD might influence the long-term outcome of CD, it remains unclear how and it is impossible to deduce the possible mechanisms directly from this study. However, since this study was done, the understanding of the brain-gut axis and the psychoneuroimmune modulation, which we proposed as the possible framework of the interaction between psychological processes and the biological factors of CD, has increased. A number of possible psychoneuroimmunological mechanisms have been implicated in **how depression might modulate the brain-gut axis and inflammatory processes in CD**.

- The *hypothalamic-pituitary-adrenal (HPA)-axis* modulates and moderates inflammation through the release of cortisol. There is ample evidence for HPA-axis dysregulation in patients with MDD, albeit not straightforward. HPA-hyperactivity with increased cortisol and exaggerated responses to psychological stress is often found in severely depressed patients.[19,20] In a subpopulation of patients with depression however, a hypoactive HPA-axis functioning has been demonstrated. It has been suggested that a hypoactive HPA axis may facilitate or maintain inflammatory diseases by contributing to cytokine imbalances. Although the HPA axis reactivity in CD patients is still unclear, there is some evidence from human and animal research (Lewis rats) for a link between stress, a hypoactive HPA-axis functioning and inflammation.[19] Thus, the blunted HPA axis function which may accompany MDD in a subpopulation of patients, might influence (i.e. facilitate

or maintain) the inflammatory processes in CD, after treatment with infliximab. Further research is necessary to investigate the functioning of the HPA axis in patients with CD in general and in CD patients with MDD, with other psychiatric disorders or with psychological symptoms. Of note is also the treatment history of these patients, since they often received high doses of systemic corticosteroids. There are currently no data on the mutual influence of the HPA axis dysregulation in psychopathology and in inflammatory processes in IBD.

- Depression is associated with hypoactivity of the prefrontal cortex (PFC) and a decreased inhibitory control of the limbic structures, such as the amygdala, by the PFC. The HPA-axis is associated with the activity of the PFC and the amygdala. There is evidence for a negative feedback control of the HPA axis by the PFC and it modulates the efferent outflow of the vagal nerve, thus controlling the parasympathetic tone.[21] The amygdala is implicated in the control of the HPA-axis and receives afferent inputs from the vagal nerve.[22] The dysregulation of the delicate balance of the PFC-amygdaloid complex in depression, might induce an imbalance between the HPA axis and the autonomic nerve system, causing autonomic, neuroendocrine disturbances and other visceral dysfunctions.[20,22] Consequently, a proinflammatory condition might arise due to decreased parasympathetic activity.[22]

- A causal link between depression and immunologic activation with inflammation has been suggested, with hypersecretion of *proinflammatory cytokines*, such as IL-6 and TNF-α and other inflammatory parameters. In patients with MDD, significantly increased levels of IL-6, TNF-α and IL-1 have been demonstrated, in the absence of a medical condition.[19,22,23] Animal research has demonstrated increased susceptibility to experimental colitis in mouse models after experimentally sustained depression. The colitis was reduced by treatment with antidepressants.[22] Thus, depression might induce the increased production of cytokines or might result in an increased number of proinflammatory producing immune cells, which could further modulate the inflammatory processes of CD. Further research is needed to assess if MDD or depressive symptoms have an effect on the levels of cytokines in CD and if this might be relevant to the local, intestinal disease process. Currently, there are no data on the inflammatory biomarkers in CD patients with MDD in comparison to CD without MDD. Additionally, the effects of antidepressants and psychotherapy should be further researched rigorously and prospectively, since they can both influence the function of the HPA axis and the cytokine levels.[24] These effects on specific inflammatory biomarkers, but also on symptom reduction in CD should be further investigated, because they can indirectly improve the understanding of the relationship between depression and the course of CD.

- MDD might modulate the course of CD through the brain-gut axis, specifically through the activation of their end effectors, *mast cells*, which are in close contact with the sympathetic nerve system (SNS) and the nervus vagus (NV), favoring neurogenic inflammation by releasing several proinflammatory mediators which can induce hyperpermeability and activate the mucosal immune cascade.[22] Evidence from animal research has suggested an association between stress, increased colonic mast cells and increased intestinal permeability. The precise role and the importance of mast cells in the course of CD in human patients should be the subject of further research. One study has found that experimental stress activates mast cells, resulting in an inflammatory response which was more pronounced in IBD patients and another study reported correlations between the number of intestinal mast cells and psychological functioning in IBD.[19] However, it is currently unknown if the presence of MDD or depressive symptoms have an effect on the mast cells in CD and this should be further pursued in research.

- *Substance P* is another possible mediator which might play a role in the association between depression and the course of CD, since substance P is significantly higher in depression and it enhances the inflammatory response.[19] Further research should determine if the increased substance P in depression and IBD are related.

8.2.3 Discussion of the short-term change in MDD prevalence

It remains controversial if MDD in severely active CD should be considered as simply "reactive", i.e. as a psychological reaction to the stress which is a consequence of the CD illness, or if MDD is associated – possibly in a subgroup of patients – with the high levels of pro-inflammatory cytokines which accompany a severe flare of CD. This would be *"inflammation-associated depression"*, a concept which has recently garnered increasing attention in the literature, and which has been demonstrated in patients receiving recombinant human cytokine therapy.[23,25,26] Of note is the clear temporal dissociation between the rapid onset of the symptoms of *sickness behavior*, characterized by neurovegetative symptoms (e.g. malaise, fatigue, listlessness, loss of appetite), which come on within hours after the increase in the levels of pro-inflammatory cytokines, and the symptoms of the inflammation-associated depression (e.g. depressed mood, guilt, feelings of worthlessness and even suicidality), which take weeks to culminate in a MDD.[25]

First, from the data in this study we could demonstrate that for the variables which were assessed, the overall severity of the CD illness, over the overall course of the disease or the previous year, was not worse in patients with MDD than in patients without MDD. This

suggests that the severity of the CD illness prior to the study did not seem to be a determining factor in the presence of MDD.
Secondly, the prevalence of MDD was higher at baseline than after treatment with infliximab, because of a subgroup of patients diagnosed with MDD at baseline, but not at re-evaluation (short-term). After treatment, the disease activity and the inflammation improved significantly more in this subgroup with MDD before, but not after infliximab than in patients who remained depressed. In the discussion of chapter 6 we initially hypothesized that the depressive disorder in these patients might have been "reactive" because they were more seriously ill and we based this conclusion on the significantly higher baseline levels of CRP. However, the **alternative interpretation** should certainly be considered: *the MDD in this subpopulation of patients was associated with the severe inflammatory condition (i.e. induced by the high levels of proinflammatory cytokines), i.e. these CD patients suffered from inflammation-associated depression.*

The treatment with infliximab in this subgroup, not only improved the intestinal inflammation, *but also the depressive symptoms, through the improvement of the systemic inflammatory processes*. There is evidence from other intervention studies which supports this interpretation. The depressive symptoms in patients with moderate to severe psoriasis improved significantly after treatment with the TNF-α etanercept, independent of the improvement of pain.[25] Additionally, a recent study showed the favorable effect of infliximab over placebo in patients with refractory MDD without a medical, inflammatory condition, but only in those patients with high baseline inflammatory biomarkers. In the subgroup with pretreatment high sensitivity CRP (hs-CRP) levels above 5 mg/L, the reduction of depression scores was significantly greater after blockade of peripheral TNF by infliximab compared with placebo.[27] The group of patients with MDD at baseline but not after treatment with infliximab in our study, had significantly higher CRP levels at baseline. Thus, it might be possible that the anti-inflammatory response after infliximab in our study was accompanied with antidepressant effects in this subgroup of patients and that this explains the difference in prevalence of MDD between baseline and re-evaluation. A major limitation of this interpretation is of course the fact that the study was not designed to assess the effect of infliximab on MDD in CD patients. There is also no control group of patients receiving placebo. Furthermore, there is no data on the cytokine levels available in these patients.

The finding of a possible subgroup of patients with inflammation-associated depression in CD and the evolution of MDD after the administration of infliximab is intriguing and should be further explored in future research. Most research on inflammation-associated depression has been done in patients receiving cytokine immunotherapy, which induces drastic immune changes due to the massive doses of cytokines which are administered. However, mood

changes have also been demonstrated in healthy volunteers after induction of inflammation, due to the increased production of proinflammatory cytokines.[25] Further research should ascertain if inflammation-associated depression can be differentiated in CD as a conceptual entity and a clear distinction should be made from sickness behavior. It could be interesting to differentiate between CD patients with severely active CD and high levels of pro-inflammatory cytokines on the one hand, and CD patients with chronically active CD with low levels of pro-inflammatory cytokines on the other hand, to assess the different effects on psychological symptoms. This is highly relevant, since studies have shown that the majority of CD patients in clinical remission still have mucosal inflammation[28] and it is currently unknown if this low grade, localized, chronic inflammation might modulate psychosocial functioning. From the cytokine immunotherapy studies, we know that categorical evaluation of psychopathology should be complemented with longitudinal, dimensional assessment of symptoms (degree of intensity). Furthermore, it is important to determine the risk factors in CD for developing inflammation-induced depression (or psychopathology), since only a subgroup of patients seems to be at risk. In patients receiving cytokine therapy, greater depressed mood at baseline, sleep disorder, low social support and high IL-6 were examples of independent risk factors of subsequent inflammation-induced depression.[25] The effects of anti-inflammatory drugs, such as anti-TNF-α, on cognitive and psychological functioning should be investigated in CD patients. Conversely, the effects of antidepressants in CD patients with inflammation-induced depression, both on the depression and on the course of the disease (e.g. the levels of cytokines, the symptoms) might be interesting, given the bidirectional relationship mediated by several psychoneuroimmunologic mechanisms.[19,24]

Ultimately, further research into the association between depression and inflammation in CD may contribute to a better understanding of the possible mechanisms. The investigation of the latter mechanism was the subject of the third part of this doctoral project.

8.3 DISCUSSION PART 3 – SOMA – PSYCHE

The aim of the third section was to investigate the possible role of biological factors, specifically inflammation, in the development of psychological symptoms and mood disorders in Crohn's disease. There are several proposed mechanisms underlying the depressogenic action of pro-inflammatory cytokines, e.g. the induction of extrahypothalamic corticotropin-releasing factor (CRF) and vasopressin, development of glucocorticoid resistance, increased expression of the serotonin transporter (SERT) and increased tryptophan catabolism by the induction of the enzyme indoleamine 2,3 dioxygenase (IDO).[25] This latter mechanism, i.e. a drastic decrease in peripheral tryptophan levels due to the induction of the tryptophan

metabolizing indoleamine-2,3-dioxygenase (IDO), was further explored in CD patients in chapter 6 and 7.

In the first study of this section, presented in *chapter 6*, we described the **evolution of total and free tryptophan after treatment with infliximab and the association between tryptophan and HRQL**. The tryptophan concentrations and the HRQL were measured before treatment with infliximab in patients with active CD and 4 weeks later. These measurements were compared to control patients with stable CD. We confirmed that total and free tryptophan concentrations were significantly decreased in CD patients with active inflammatory disease in comparison with control CD patients. The tryptophan concentrations were also significantly and inversely correlated with the degree of inflammation. Both total and free tryptophan concentrations increased significantly after treatment with infliximab, but only in the treated CD patients without elevated CRP at re-evaluation. In patients with elevated CRP after infliximab treatment, the tryptophan concentrations did not change significantly. Furthermore, free tryptophan concentrations also remained significantly lower in CD patients with normalized CRP after infliximab in comparison with control CD patients. The evolution of tryptophan concentrations and the evolution of HRQL were significantly correlated and both disease activity and the change in free tryptophan concentration were significant determinants of the change in HRQL.

The results from this study confirmed an association between tryptophan levels and inflammation in CD. In CD with active inflammation, the cell-mediated immune activation and the production of pro-inflammatory cytokines such as TNF-α, IL-2 and IL-1β induce IDO which will lead to a significant decrease in peripheral tryptophan levels as a consequence of its increased oxidative catabolism.[29] This catabolic route for tryptophan is called the kynurenine pathway (figure 8.2) and the first step is (tryptophan → kynurenine) is rate limiting. Various other metabolites than kynurenine are derived from tryptophan, such as kynurenic acid, xanthurenic acid and quinolinic acid, also called tryptophan catabolites (TRYCATs). These TRYCATs have significant physiological effects, the kynurenine pathway has alternatively been labeled as the TRYCAT pathway.[30] In CD, intestinal IDO is immunomodulatory and it has potent suppressive effects on T-cell proliferation. Since tryptophan concentrations increased in patients who were successfully treated with infliximab (i.e. no detectable systemic inflammation), this suggests that IDO expression was no longer increased in these patients, or at least less active, since free TRP levels were still significantly lower in CD patients with normalized CRP after infliximab treatment. This could be indicative of some remaining IDO activity after successful treatment with infliximab, although further research should clarify this issue.

From the finding that the change in tryptophan was associated with the change in HRQL and that the change in free tryptophan was a significant determinant of the change in HRQL, we hypothesized that the level of peripheral tryptophan might influence HRQL in CD patients and that the role of decreased peripheral tryptophan levels as a possible mechanism for the development of psychological symptoms in CD patients warranted further research. As described in the introduction, tryptophan is the precursor of serotonin (5-hydroxytryptamine, 5-HT), which is a neurotransmitter in the central nervous system (CNS) with a central role in the current theories on mood regulation, cognition, behavior, and the pathophysiology of depression and anxiety.[31] Tryptophan passes through the blood-brain barrier (BBB) via a non-specific, active, transport protein, in competition with other large neutral amino acids (LNAA's). In the CNS, tryptophan is hydroxylized into 5-hydroxytryptophan, which is decarboxylized into serotonin (5-HT). This hydroxylation is rate-limiting and a decrease in tryptophan levels also leads to a decrease in 5-HT synthesis and to a change in central 5-HT availability,[32] and possibly a change in central serotonergic neurotransmission.[33]

In inflammation-associated depression, such a relationship between decreased tryptophan concentrations and depression has been observed in other inflammatory disorders and has extensively been researched in patients who received cytokine therapy.[25,34] Lowered tryptophan has also consistently been observed in patients with MDD, where it is associated with a positive response to serotonergic antidepressants, which suggests that treatment with such medication might compensate for a deficit of the central serotonergic neurotransmission caused by lowered tryptophan availability. These lowered tryptophan levels in MDD might be (in part) the result of induction of IDO through cell-medicated immune activation via pro-inflammatory cytokines, such as IFN-γ, TNF-α and possibly IL-2.[30] To investigate the response of behavior, mood and cognitive functions in humans to a pharmacological challenge of the central serotonergic neurotransmission, the technique of acute tryptophan depletion (ATD) has been used extensively. ATD can reduce the tryptophan levels and studies in several subpopulations of remitted depressed patients have consistently shown that this is associated with significant changes in mood.[35] Thus, decreased peripheral tryptophan concentrations seemed to be linked to impaired central, presynaptic 5-HT neurotransmission, or "serotonergic vulnerability" which might be associated to psychological symptoms and psychopathology. With the technique of ATD (chapter 8) we investigated if this mechanism could be responsible for inflammation-associated psychological mood changes.

The second study of this section, described in *chapter 7*, **assessed the effect of acute tryptophan depletion (ATD) on mood, fatigue and anxiety in Crohn's disease patients, in comparison with a control condition, using a double blind, cross-over protocol**. We found significant tryptophan depletion after ATD in comparison with the control condition.

However, mood, fatigue and anxiety did not differ significantly in the ATD condition in CD patients in comparison with the control condition.

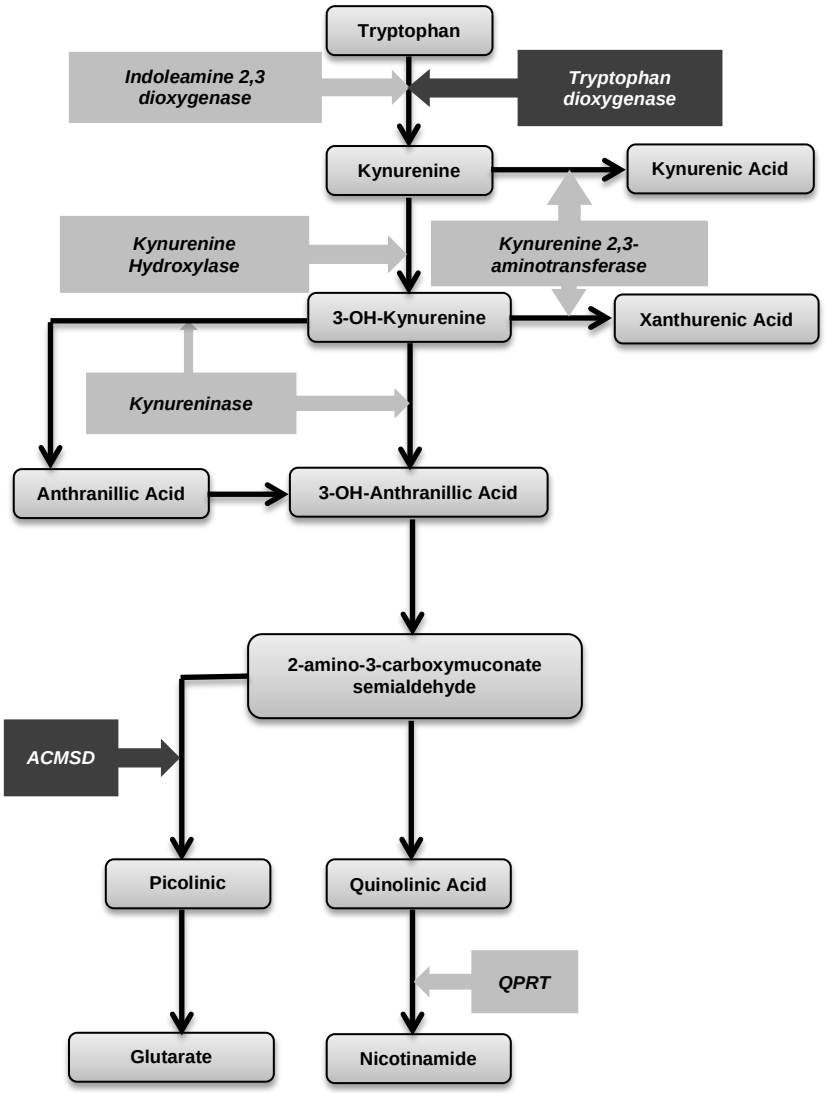


Figure 8.2 Tryptophan catabolites: kynurenine or TRYCAT pathway

The negative results of this study could be interpreted in two ways:

[1] First, in contrast to the CD patients in chapter 6, the patients in chapter 7 did not present with severely active, inflammatory CD and it is possible that ATD did not affect their mood. Furthermore, there was no evidence of decreased peripheral tryptophan levels at the start of the study in the patients of chapter 7, so it is possible that the central serotonergic neurotransmission actually was not affected in this study population. These patients had also specifically been selected to have no psychiatric history and no first degree relatives with a psychiatric disorder.

[2] Second, the results may be interpreted as unsupportive of the hypothesized “serotonergic vulnerability” associated with the inflammation-induced depletion of tryptophan in CD patients, since ATD provoked adequate tryptophan depletion without any effect on the psychological measures. This interpretation is supported by recent evidence from animal and

clinical human studies, which demonstrated that a decrease of peripheral tryptophan and the consequent change in central serotonin synthesis were insufficient to explain the complexity of the effects of inflammation and IDO activation on the development of psychological symptoms, thus rendering the hypothesis of a deficit of the serotonergic neurotransmission as a central mechanism for inflammation-associated depression untenable.[25,30] This has led to a shift in the hypothesis from tryptophan depletion towards other possible mechanisms, such as the detrimental effects of some TRYCATs as a result of IDO activation, and their role in the development of psychological symptoms and depression.

The interpretation and the discussion of the results of both chapter 6 and 7 is significantly restricted because **only peripheral free and total tryptophan, together with CRP were measured**, which is **a major limitation of this doctoral project**. The peripheral free and total tryptophan concentrations do not adequately reflect how much tryptophan crosses the blood-brain barrier or the availability of tryptophan in the CNS. Ideally, kynurenine and possibly other metabolites of tryptophan (see figure 8.2), the competing large neutral amino acids (LNAA) and cytokines should also have been measured. The kynurenine/tryptophan ratio better reflects the IDO activity and the tryptophan/LNAA ratio reflects the availability of tryptophan to the brain. Despite these limitations, chapter 6 and 7 should be discussed in relation to the recent literature, especially since this recently proposed paradigm shift towards the detrimental effects of some TRYCATs as a result of IDO activation in the development of inflammation-associated depression might be of clinical relevance in CD and should be the subject of further research in IBD.

The possible role of IDO and the detrimental effects of TRYCATs in inflammation associated depression in CD

Gupta et al. showed an increased kynurenine/tryptophan ratio, which reflects IDO activity, and a positive correlation with inflammation in CD patients.[29] This was in line with our findings in chapter 7 and with the findings of studies in patients treated with interferon (IFN)-α.[30] Initially, studies in patients receiving INF-α therapy, showed that this also lowered peripheral tryptophan availability, which was correlated with the development and severity of depressive symptoms.[36] Treatment with a selective serotonin reuptake inhibitor blocked the behavioral consequences of IFN-α induced tryptophan depletion, without an effect on the levels of its metabolites.[37] Thus, we can conclude that both in CD and in INF-α therapy IDO is induced by inflammation, which is reflected by decreased peripheral tryptophan levels and an increased kynurenine/tryptophan ratio. Furthermore, the studies in patients receiving cytokine therapy seemed to confirm our initial hypothesis that the lowered tryptophan availability affected the central serotonergic neurotransmission, given the correlations between peripheral tryptophan levels and mood.[25,38] Other studies however, did not

unequivocally confirm this association between peripheral tryptophan levels and depressive symptoms, but did find significant associations between the kynurenine/tryptophan ratio and the development of depressive symptoms.[39]

Several studies have further investigated the mechanism of the transition from cytokine-induced sickness behavior to depression and the role of IDO, with its effects on tryptophan and its metabolism.[25,40] The prevailing hypothesis for the mechanism of inflammation-induced depression in the literature was based on reduced serotonergic neurotransmission as a result of decreased tryptophan bioavailability, due to increased catabolism by IDO. Our acute tryptophan depletion study in CD patients (chapter 7) is actually the only one to test the impact of decreased peripherally circulating tryptophan levels due to inflammation on brain tryptophan and 5-HT neurotransmission by assessing mood after a dietary intervention. As mentioned before, the negative result could be interpreted as an indication that the decreased peripheral tryptophan levels might not affect the central serotonergic neurotransmission. Evidence supporting this conclusion mainly comes from animal research and from research in IFN-α treated patients. Activation of peripheral IDO as a result of inflammation resulted in mice in decreased tryptophan levels and in increased kynurenine levels with an increased kynurenine/tryptophan ratio. In the brain however, only increased kynurenine levels were observed, without a concomitant decrease of tryptophan levels.[41] The administration of lipopolysaccharide (LPS) paradoxically *increased* brain tryptophan and the turnover of brain 5-HT in mice. Furthermore, when the LPS-induced changes were blocked, either by blocking the expression of cytokines or by directly blocking IDO, the depressive-like behavior, usually observed as a result of cytokine-activation in mice, disappeared, and the LPS-increases in the kynurenine to tryptophan ratio in the plasma and the brain were attenuated. There was, however, *no effect on the LPS-induced increases in brain tryptophan and 5-HT turnover*.[41] In human patients, Raison et al recently found significantly decreased peripheral tryptophan levels in patients treated with IFN-α, while the tryptophan levels in the cerebrospinal fluid remained stable.[42] A study in hepatitis C patients treated with IFN-α for up to 24 weeks, also found an increased peripheral kynurenine/tryptophan ratio, reflecting increased IDO activity, but no association between peripheral tryptophan levels and depressive symptoms. The peripheral tryptophan levels decreased as a result of the IFN-α treatment, but there was no decrease of the tryptophan/competing LNAA, which reflects the availability of tryptophan to the brain.

It is well established that the induction of systemic immune activation by pro-inflammatory cytokines (both therapeutically and experimentally) may induce depressive symptoms. By extension, we can assume that chronic exposure to high levels of pro-inflammatory cytokines as is often the case in IBD, can have the same result.[30] The development of these

depressive symptoms seem to be more related to IDO activation than to the tryptophan levels, as illustrated by animal and human studies. On the basis of these findings, the hypothesis shift was proposed that the production of TRYCATs as a consequence of IDO activation due to inflammation, rather than decreased tryptophan levels, were associated to the development of inflammation-associated depressive symptoms.[25,30] This hypothesis cannot be supported by the results in chapter 6 and 7, but there is evidence from other populations and on the basis of these findings, further research in CD and IBD into the possible role of IDO, TRP and TRYCATS in the development of psychological symptoms could be given direction.

Future research investigating the association between biological factors such as pro-inflammatory cytokines, IDO and the TRYCAT pathway on the one hand, and psychological symptoms or inflammation-associated psychopathology on the other hand, should take the above-described hypothesis shift into account and should also measure tryptophan metabolites with neurotropic properties, since they might play a role in the development of psychological symptoms independent of serotonin. It has been hypothesized that inflammation-induced IDO activation might switch the metabolism of the kynurenine pathway toward the production of neurotoxic metabolites and away from kynurenic acid.[30] Clinical studies in patients treated with IFN-α have found correlations between increased depressive symptoms and evidence of such a shift, away from neuroprotective metabolites (kynureninic acid), toward neurotoxic metabolites of kynurenine (quinolinic acid) in the presence of inflammation.[42,43] Kynurenine is a precursor of neurotoxic metabolites of tryptophan and it is able to pass the blood-brain barrier (BBB), where it is metabolized into 3-hydroxykynurenine and quinolinic acid, which are neurotoxic (figure 8.2).[25,30] Thus, the increase of the kynurenine/kynurenic acid ratio reflected an increase in neurotoxic potential through the increased production of neurotoxic TRYCATs as a result of IDO induction. It is possible that this mechanism also plays a role in the development of depressive symptoms or syndrome in patients with active CD. A proportion of the depressive disorders which are diagnosed in CD patients could therefore be inflammation-associated depression, rather than simply caused by the severity of the disease. This should be further explored in research in CD which focuses on the activation of IDO, the metabolites of the kynurenine pathways and their effects, the difference between subjects who become depressed and those who remain depression-free and the possible effects of anti-inflammatory therapeutics such as infliximab.

8.4 CROHN'S DISEASE: A SEGUE BETWEEN DEPRESSION, PSYCHOSOCIAL FACTORS AND INFLAMMATION IN FUTURE RESEARCH?

Since the macrophage hypothesis of depression by Smith,[44] there is ample evidence from preclinical and clinical research for a central role of the association between inflammation and psychiatric illness, particularly major depressive disorder (MDD). It is important to note that the findings of an association between inflammation and depression do not necessarily indicate a causal relationship. MDD in otherwise medically healthy patients, is currently not considered as an inflammatory disorder, but there is evidence to suggest that inflammatory processes may be involved in the pathophysiology of MDD through bidirectional interactions with other systems (e.g. HPA axis, neurotransmitters, neuronal activity, etc.).[23,26,30] Even in MDD patients without medical illness, evidence of the activation of the inflammatory response system is observed.[26] In approximately one-third of the patients with MDD, increased levels of inflammatory markers are found, compared with the non-depressed population. Several pro-inflammatory cytokines, such as TNF-α and interleukin (IL)-6, and acute phase reactant proteins, such as CRP, are consistently increased in depression, but there is significant heterogeneity and a definite biomarker for inflammation in depression has yet to be identified.[23,26] However, systemic inflammation is difficult to assess in clinical, psychiatric populations since the increases of the inflammatory markers are not at all in the range of inflammatory or infectious diseases. As such, the association between inflammation and depression has been easier to observe in other populations. Patients receiving high doses of systemic cytokine therapy with interferon α or interleukin-2 for e.g. hepatitis C or cancer, are such a population, since they are at increased risk of developing inflammation-associated depression.[25] Additionally, the association between depression and inflammation has been investigated in groups of medical patients, such as patients with coronary heart disease and associations between biomarkers of inflammation and intensity of depressive symptoms have been found during aging. [25]

From the results of this doctoral project, we might conclude that future research in patients with Crohn's disease, a paradigmatic chronic, inflammatory medical illness accompanied by high levels of pro-inflammatory cytokines, might provide interesting insights in the association between psychological symptoms, psychiatric illnesses, particularly MDD, and inflammatory processes. The field of psychoneuroimmunology provides a bidirectional framework for this type of research and the research within CD is still limited.

Future research is necessary on the possible association between inflammation in CD and psychological symptoms and psychopathology, especially inflammation-associated depression. The underlying mechanisms should be explored. It is especially interesting to

investigate the effects of known, severe inflammatory processes, the mechanisms which are responsible for the possible depressogenic action of cytokines and their effects on the brain. Since the inflammatory pathophysiological processes are relatively well known in CD, this might be helpful in identifying inflammatory markers for MDD. Research in MDD has suggested that even modest, chronic increases in inflammatory markers can have significant consequences over time. This could also be further explored in CD, since this disease is characterized by chronic inflammation. CD might be helpful in diagnosing and/or predicting treatment response in MDD from CD patients with comorbid depression we can identify the effects of anti-inflammatory drugs. Finally, the effects of psychotropic medication and psychotherapy on the course of CD should be further investigated, to assess if these interventions can modulate the illness in CD, next to treating the existing psychiatric condition. Conversely, further research should assess the effects of anti-inflammatory biological therapy such as anti-TNF-α on psychological symptoms and psychopathology in CD.

8.5 REFERENCES

1. Pallis AG, Mouzas IA, Vlachonikolis IG. The inflammatory bowel disease questionnaire: a review of its national validation studies. *Inflamm Bowel Dis*. 2004;10:261–269.
2. Irvine EJAN. Measuring quality of life in inflammatory bowel disease. In: Targan SR, Shanahan F, Karp LC, eds. *Inflammatory Bowel Disease: From Bench to Bedside*. 2nd Ed. Springer US; 2005:481–494.
3. Turnbull GK, Vallis TM. Quality of life in inflammatory bowel disease: the interaction of disease activity with psychosocial function. *Am J Gastroenterol*. 1995;90:1450–1454.
4. Sainsbury A, Heatley R V. Review article: psychosocial factors in the quality of life of patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2005;21:499–508.
5. Vidal A, Gomez-Gil E, Sans M, et al. Health-related quality of life in inflammatory bowel disease patients: the role of psychopathology and personality. *Inflamm Bowel Dis*. 2008;14:977–983.
6. Guthrie E, Jackson J, Shaffer J, et al. Psychological disorder and severity of inflammatory bowel disease predict health-related quality of life in ulcerative colitis and Crohn's disease. *Am J Gastroenterol*. 2002;97:1994–9.
7. Persoons P, Fischler B, Vandenberghe J, et al. Determinants of quality of life in Crohn's disease. In: *Gastroenterology*.Vol 122 (Suppl.); 2002:S1375: A216.
8. Zhang CK, Hewett J, Hemming J, et al. The Influence of Depression on Quality of Life in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2013;epub ahead of print:1–8.
9. Czuber-Dochan W, Ream E, Norton C. Review article: Description and management of fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2013;37:505–16.
10. Langenberg DR Van, Gibson PR. Systematic review: fatigue in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2010;32:131–143.
11. Beurskens AJ, Bültmann U, Kant I, et al. Fatigue among working people: validity of a questionnaire measure. *Occup Environ Med*. 2000;57:353–7.
12. Sostegni R, Daperno M, Scaglione N, et al. Review article: Crohn's disease: monitoring disease activity. *Aliment Pharmacol Ther*. 2003;17 Suppl 2:11–7.
13. Mikocka-Walus AA, Turnbull DA, Moulding NT, et al. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflamm Bowel Dis*. 2007;13:225–34.
14. Piche T, Ducrotte P, Sabate JM, et al. Impact of functional bowel symptoms on quality of life and fatigue in quiescent Crohn disease and irritable bowel syndrome. *Neurogastroenterol Motil*. 2010;22:626–e174.
15. Mardini HE, Kip KE, Wilson JW. Crohn's disease: a two-year prospective study of the association between psychological distress and disease activity. *Dig Dis Sci*. 2004;49:492–497.
16. Mittermaier C, Dejaco C, Waldhoer T, et al. Impact of depressive mood on relapse in patients with inflammatory bowel disease: a prospective 18-month follow-up study. *Psychosom Med*. 2004;66:79–84.
17. Bitton A, Dobkin PL, Edwardes MD, et al. Predicting relapse in Crohn's disease: a biopsychosocial model. *Gut*. 2008;57:1386–1392.
18. Camara RJ, Schoepfer AM, Pittet V, et al. Mood and nonmood components of perceived stress and exacerbation of Crohn's disease. *Inflamm Bowel Dis*. 2011;17:2358–2365.

19. Mackner LM, Clough-Paabo E, Pajer K, et al. Psychoneuroimmunologic factors in inflammatory bowel disease. *Inflamm Bowel Dis*. 2011;17:849–57.
20. Palazidou E. The neurobiology of depression. *Br Med Bull*. 2012;101:127–45.
21. Tracey KJ. The inflammatory reflex. *Nature*. 420:853–9.
22. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory bowel disease. *Gastroenterology*. 2013;144:36–49.
23. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry*. 2012;83:495–502.
24. Martino M, Rocchi G, Escelsior A, et al. Immunomodulation Mechanism of Antidepressants: Interactions between Serotonin/Norepinephrine Balance and Th1/Th2 Balance. *Curr Neuropharmacol*. 2012;10:97–123.
25. Dantzer R, O'Connor JC, Lawson MA, et al. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology*. 2011;36:426–36.
26. Maes M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011;35:664–75.
27. Raison CL, Rutherford RE, Woolwine BJ, et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA psychiatry*. 2013;70:31–41.
28. Baars JE, Nuij VJAA, Oldenburg B, et al. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis*. 2012;18:1634–40.
29. Gupta NK, Thaker AI, Kanuri N, et al. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis*. 2012;18:1214–20.
30. Maes M, Leonard BE, Myint AM, et al. The new "5-HT" hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to th. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011;35:702–21.
31. Stahl S. Chemical neurotransmission as the mediator of disease actions. In: *Essential psychopharmacology: neuroscientific basis and practical applications*. 2nd ed. Cambridge: Cambridge University Press; 2000:99–135.
32. Williams WA, Shoaf SE, Hommer D, et al. Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5-Hydroxyindoleacetic Acid in Normal Volunteers. *J Neurochem*. 2001;72:1641–1647.
33. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry*. 2001;178:399–405.
34. Russo S, Kema IP, Fokkema MR, et al. Tryptophan as a Link between Psychopathology and Somatic States. *Psychosom Med*. 2003;65:665–671.
35. Does AJ Van der. The effects of tryptophan depletion on mood and psychiatric symptoms. *J Affect Disord*. 2001;64:107–19.
36. Capuron L, Ravaud A, Neveu PJ, et al. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry*. 2002;7:468–73.
37. Capuron L, Neurauter G, Musselman DL, et al. Interferon-alpha–induced changes in tryptophan metabolism. *Biol Psychiatry*. 2003;54:906–914.
38. Capuron L, Dantzer R. Cytokines and depression: The need for a new paradigm. *Brain Behav Immun*. 2003;17:119–124.
39. Bonaccorso S, Marino V, Puzella A, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. *J Clin Psychopharmacol*. 2002;22:86–90.
40. Dantzer R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North Am*. 2009;29:247–64.
41. O'Connor JC, Lawson MA, André C, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*. 2009;14:511–22.
42. Raison CL, Dantzer R, Kelley KW, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry*. 2010;15:393–403.
43. Wichers MC, Koek GH, Robaeys G, et al. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol Psychiatry*. 2005;10:538–44.
44. Smith RS. The macrophage theory of depression. *Medical hypotheses*. 1991;35:298–306.

ABSTRACTS & SUMMARIES

Abstract of the research

Crohn's disease (CD) is a systemic, inflammatory bowel disease, characterized by a chronic and relapsing course and extraintestinal manifestations. The pathophysiology of CD is multifactorial and is determined by genetic susceptibility and environmental factors, which results in an upset immunohomeostasis, leading to an abnormal, exaggerated intestinal immune response. Ample evidence suggests that psychosocial factors also play a significant role in the pathophysiology of CD and it is clear that the association between psychosocial factors and CD goes beyond the mere psychological consequences of a severe illness. The interaction between CD and psychosocial factors should be considered as complex and multi-determined, with reciprocal influential processes, through which CD influence psychosocial factors (e.g. psychological functioning or symptoms, psychopathology or quality of life), and conversely, psychosocial factors may also affect several aspects of CD (e.g. expression symptoms, course of CD, response to treatment). Furthermore, progress in the field of psycho-neuro-immuno-endocrinology has provided a plausible, albeit incompletely understood framework of overlapping pathways for the proposed reciprocal modulation of CD and psychosocial factors. The basic hypothesis of this doctoral project was that the association between psychosocial factors and CD cannot be reduced to a simple, directional pathway of cause and effect, but should rather be approached as a complex, bidirectional association with mutually interacting systems at multiple levels. The research project aimed to contribute to the investigation of this association between psychosocial factors and CD. Table 1 summarizes the studies within the 3 different sections by presenting the subjects, the methodology, the primary endpoints and the results with the conclusions of each study.

The methodological studies of the ***first section*** present the validation of two questionnaires. The quick responsiveness of the Inflammatory Bowel Disease Questionnaire (IBDQ), a disease specific instrument measuring health related quality of life in CD, was demonstrated. Additionally, cutoff values for the IBDQ remission and partial response were determined. A comprehensive validation of the Checklist Individual Strength 20 items (CIS20R), a multidimensional fatigue questionnaire, was performed in a CD population. High prevalence rates of significant fatigue, determined on the basis of previously established cutoff values, and significant, chronic fatigue were present in CD patients.

Table 1. Summary of the studies presented in the doctoral thesis

Section	Study	Chapter	Subjects	Methods	Primary endpoint	Results & Conclusions
I	1st	3	224 CD patients with active disease	Prospective questionnaire study	Responsiveness ratio IBDQ Cutoff value remission and response IBDQ	The change in disease activity correlated significantly with the change in HRQL over 4 weeks. The responsiveness ratios for the change of the IBDQ were high. A linear regression analysis based on a clinical remission of disease activity yielded a cutoff value of 168 points for the IBDQ. For the change in IBDQ, the respective cutoff values were 22 and 27 points for clinical improvement based on a change of disease activity of ≥ -70 and ≥ -100 points. The IBDQ is a responsive instrument which can reflect quick change in the quality of life of patients with CD.
	2nd	4	308 CD patients	Cross-sectional questionnaire study with prospective follow-up after intervention in 120 patients	Comprehensive validity CIS20R Prevalence of fatigue	Factor analysis confirmed the 4 CIS20R dimensions in CD patients. The internal consistency and reliability were excellent. Reproducibility and responsiveness of the CIS20R were demonstrated CD patients treated with infliximab treatment. Concurrent and convergent validity of the CIS20R were confirmed. Fatigue was worse in patients with active CD and within this group, more patients reported "significant fatigue" in contrast to patients with inactive CD. Significant, chronic (> 6 months) fatigue was present in 4 in 10 CD patients. The CIS20R is a valid responsive multidimensional questionnaire for the assessment of the severity and prevalence of fatigue in CD patients.
II	3rd	5	100 CD patients with active disease	Prospective, short-term and long-term follow-up study after intervention (infliximab)	Effect of MDD at treatment on short-term and long-term outcome	MDD is a risk factor for failure to achieve remission with infliximab and for earlier retreatment in patients with active luminal Crohn's disease. Assessment and management of major depressive disorder should be part of the clinical approach to patients with Crohn's disease
III	4th	7	39 CD patients with active disease 9 CD patients with stable disease	Prospective, controlled, intervention (infliximab treatment) study	Change in TRP after infliximab treatment Relationship between change in TRP and change in HRQL	TRP levels were significantly lower at baseline in CD patients with active disease and increased significantly after treatment with infliximab. Successful treatment with infliximab, with normalization of inflammatory parameters, resulted in a normalization of total TRP levels, whereas free TRP levels remained significantly lower. The change (i.e. improvement) in HRQL after treatment with infliximab was correlated with the change of total and free TRP levels. Furthermore, the improvement of HRQL as a result of treatment with infliximab was independently determined by the decrease in disease activity and by the change (increase) in free TRP levels. This could be interpreted as an indication of a role of the levels of TRP in CD patients' subjective well-being.
	5th	8	9 CD patients	ATD, randomized, double blind, controlled, cross-over	Difference in change in mood between control and depletion condition	Serum TRP concentrations decreased significantly after ATD, whereas the change in mood scores did not differ significantly between the ATD and the control condition. The results do not support the hypothesis of a serotonergic vulnerability due to inflammation-induced TRP reduction in quiescent CD, which can be elicited by an ATD paradigm. Inflammation-induced psychological symptoms are possibly not associated to the drastic decrease of TRP bioavailability, but to other mechanisms related to high levels of pro-inflammatory cytokines.

PHQ: Patient Health Questionnaire, MDD: major depressive disorder, CD: Crohn's Disease, IBDQ: Inflammatory Bowel Disease Questionnaire, ATD: acute tryptophan depletion, TRP: tryptophan, HRQL: health-related quality of life

In the ***second section***, the association between psychosocial factors and CD was approached from the **"psyche-somatic" point of view** (i.e. biopsychosocial variables as independent variables and CD-related variables as dependent variables). The prospective, intervention study in which biopsychosocial factors were investigated as possible determinants of the course of CD, assessed the influence of major depressive disorder (MDD) on the short- and long-term outcome of CD after treatment with infliximab. Biological and psychosocial parameters were measured in patients with active CD at the time of treatment with infliximab. They were re-evaluated 4 weeks after treatment and subsequently followed up until the moment they needed to be treated again due to relapse of CD activity. We found that patients with MDD at baseline had a lower chance to achieve short-term remission after treatment with infliximab, but MDD did not affect short-term clinical response. This short-term effect of MDD on the outcome of treatment seemed to be a reflection of the high prevalence of physical symptoms in depressed patients. Additionally, we found that the presence of MDD at the time of treatment predicted an earlier retreatment in these patients. These results argue for a modulatory role of MDD in the course of CD after treatment and support the notion of a possibly specific association between depression and CD. These findings illustrate the highly complex relationship between psychological factors in general – particularly MDD – on the one hand, and CD on the other hand.

We observed that the prevalence of MDD in this study, was significantly higher at baseline than after treatment with infliximab, because major depression was not diagnosed after treatment with infliximab in a subgroup of patients who did have MDD at baseline. The depressive disorder in these patients might have been "reactive" because their illness was more serious. An alternative interpretation should be considered, given the high inflammatory parameters in these patients before treatment. Thus, MDD in this subpopulation of CD patients was possibly associated with the severe inflammatory condition (i.e. induced by the high levels of pro-inflammatory cytokines), and these CD patients might have suffered from **inflammation-associated depression**, which responded favorably to infliximab.

The association between psychosocial factors and CD is approached from the "***somatic-psyche***" **point of view** in the ***third section***. The possible role of biological factors (i.e. inflammation), in the development of psychological symptoms in CD was explored, specifically, increased tryptophan catabolism by the inflammation-induced enzyme indoleamine 2,3 dioxygenase (IDO). We confirmed that total and free tryptophan concentrations were significantly decreased in CD patients with active inflammation. The tryptophan concentrations were also significantly and inversely correlated with the degree of inflammation. Both total and free tryptophan concentrations increased significantly after

treatment with infliximab, but only in the CD patients without elevated CRP after treatment. In patients with elevated CRP after infliximab treatment, the tryptophan concentrations did not change significantly. Furthermore, free tryptophan concentrations also remained significantly lower in CD patients with normalized CRP after infliximab in comparison with control CD patients. The evolution of tryptophan concentrations and the evolution of HRQL were significantly correlated and both disease activity and the change in free tryptophan concentration were significant determinants of the change in HRQL. From the finding that the change in tryptophan was associated with the change in HRQL and that the change in free tryptophan was a significant determinant of the change in HRQL, we hypothesized that the level of peripheral tryptophan might influence psychosocial factors (such as HRQL) in CD patients. In inflammation-associated depression, such a relationship between decreased tryptophan concentrations and depression has been observed. Tryptophan is the precursor of serotonin, which is a neurotransmitter in the central nervous system with a central role in the current theories on mood regulation. To investigate the response of mood in humans to a pharmacological challenge of the central serotonergic neurotransmission, the technique of acute tryptophan depletion (ATD) has been used extensively to reduce peripheral tryptophan levels. With the technique of ATD, we investigated if tryptophan depletion due to inflammation induced catabolism could be responsible for inflammation-associated psychological mood changes in CD patients. We assessed the effect of ATD on mood, fatigue and anxiety using a double blind, controlled, cross-over protocol. We found significant tryptophan depletion after ATD in comparison with the control condition. However, mood, fatigue and anxiety did not differ significantly in the ATD condition in comparison with the control condition. Possibly, the central serotonergic neurotransmission was not affected in this study population, since no significant inflammation was present in the participating CD patients. The results may also be interpreted as unsupportive of the hypothesized “serotonergic vulnerability” associated with the inflammation-induced depletion of tryptophan in CD patients, since ATD did induce adequate tryptophan depletion without any effect on the psychological measures, even though CD patients almost never achieve full remission. This interpretation is supported by recent evidence from animal and clinical human studies, which demonstrated that a decrease of peripheral tryptophan and the consequent change in central serotonin synthesis were insufficient to explain the complexity of the effects of inflammation and IDO activation on the development of psychological symptoms, thus rendering the hypothesis of a deficit of the serotonergic neurotransmission as a central mechanism for inflammation-associated depression untenable. This has led to a shift in the hypothesis from tryptophan depletion towards other possible mechanisms, such as the detrimental effects of some tryptophan catabolites as a result of IDO activation, and their role in the development of psychological symptoms and depression.

Wetenschappelijke samenvatting

De ziekte van Crohn (ZvC) is een systemische, inflammatoire darmziekte, gekenmerkt door een chronisch en terugkerend verloop, met extra-intestinale manifestaties. De pathofysiologie van de ZVC is multifactorieel en wordt bepaald door een genetische gevoeligheid en omgevingsfactoren, wat resulteert in een verstoorde immunohomeostasis, waardoor een abnormale, overdreven immuunrespons in de darm optreedt. Er zijn voldoende aanwijzingen dat psychosociale factoren een belangrijke rol spelen in de pathofysiologie van de ZvC. Het is duidelijk dat de associatie tussen psychosociale factoren en de ZVC niet louter het gevolg is van de ernst van de ziekte. De interactie tussen ZVC en psychosociale factoren moet worden beschouwd als complex, waarbij diverse processen elkaar beïnvloeden. Zo kan de ZvC een impact hebben op psychosociale factoren, en omgekeerd kunnen psychosociale factoren diverse aspecten van de ZVC beïnvloeden (bijv. expressie symptomen, verloop van de ziekte, respons op de behandeling). De vooruitgang op het gebied van de psycho-neuro-immuno-endocrinologie kan een aannemelijk, zij het onvolledig begrepen, kader bieden voor de hypothetische, wederzijdse modulatie van ZVC en psychosociale factoren. De fundamentele hypothese van dit doctoraatsonderzoek was dat de associatie tussen psychosociale factoren en de ZvC niet kan worden teruggebracht tot een eenvoudig, directioneel pad van oorzaak en gevolg, maar veeleer moet worden benaderd als een complexe, reciproque associatie van diverse systemen die op meerdere niveaus met elkaar in wisselwerking treden. Het project had tot doel een bijdrage te leveren aan het onderzoek van deze complexe relatie tussen psychosociale factoren en ZvC. Tabel 1 geeft een overzicht van de studies in de 3 secties van dit doctoraatsproject.

De methodologische studies van de eerste sectie presenteren de validatie van twee vragenlijsten. De snelle responsiviteit van de “Inflammatory Bowel Disease Questionnaire” (IBDQ), een ziekte-specifiek instrument voor gezondheid gerelateerde kwaliteit van leven bij ZvC werd aangetoond. Daarnaast werden cutoff waarden voor remissie en gedeeltelijke respons op de IBDQ bepaald. Een uitgebreide validatie van de Checklist Individuele Sterkte 20 items (CIS20R), een multidimensionale vermoeidheid vragenlijst, werd uitgevoerd in een ZvC populatie. Er was een hoge prevalentie van significante vermoeidheid en belangrijke chronische vermoeidheid aanwezig bij ZvC patiënten.

Tabel 1. Samenvatting van de studies opgenomen in de doctoraatsthesis

Sectie	Studie	Hfdstk	Proefpersonen	Methode	Primair doel	Resultaten en Conclusies
I	1st	3	224 ZvC patiënten met actieve ziekte	Prospectieve vragenlijststudie	Responsiviteit IBDQ Cutoff waarde remissie en respons IBDQ	De verandering in ziekteactiviteit correleerde significant met de verandering in HRQL na 4 weken. De responsiviteit ratio's voor de verandering van de IBDQ waren hoog. Een lineaire regressie-analyse op basis van een klinische remissie van de ziekteactiviteit leverde een cutoff van 168 punten voor de IBDQ op. De verandering in IBDQ de respectievelijke cutoff waarden waren 22 en 27 punten voor klinische verbetering gebaseerd op een verandering van ziekteactiviteit van ≥ -70 en -100 ≥ punten. De IBDQ is een responsief instrument dat een snelle verandering in de kwaliteit van leven van patiënten met CD kunnen reflecteren.
	2nd	4	308 ZvC patiënten	Cross-sectionele vragenlijst studie met prospectieve opvolging na interventie bij 120 patiënten	Validiteit van de CIS20R Prevalentie van vermoeidheid	Factor analyse bevestigde de 4 CIS20R dimensies bij ZvC patiënten. De interne consistentie en betrouwbaarheid waren uitstekend. Reproduceerbaarheid en responsiviteit van de CIS20R werden aangetoond bij ZvC patiënten behandeld met infliximab. Concurrente en convergente validiteit van de CIS20R werden bevestigd. Vermoeidheid was slechter bij patiënten met actieve ZvC en binnen deze groep meldden meer patiënten "significante vermoeidheid" in tegenstelling tot patiënten met inactieve ZvC. Significante, chronische (>6 maanden) vermoeidheid was aanwezig bij 4 op de 10 ZvC patiënten. De CIS20R is een valide, responsieve multidimensionale vragenlijst voor de beoordeling van de ernst en prevalentie van vermoeidheid bij ZvC patiënten.
II	3rd	5	100 ZvC patiënten met actieve ziekte	Prospectieve, korte en lange termijn opvolgstudie na interventie (infliximab)	Effect van MDD at treatment on short-term and long-term outcome	MDD is een risicofactor om geen remissie te bereiken na behandeling met infliximab en voor vroegtijdige herbehandeling bij patiënten met actieve, luminale ziekte van Crohn. De inschatting en behandeling van een depressieve stoornis zou deel moeten uitmaken van de klinische benadering bij patiënten met de ziekte van Crohn.
III	4th	7	39 ZvC patiënten met actieve ziekte 9 ZvC patiënten met stabiele ziekte	Prospectieve, gecontroleerde interventie (infliximab behandeling) studie	Verandering in TRP na infliximab behandeling Verband tussen verandering in TRP en verandering in HRQL	TRP niveaus waren significant lager bij aanvang bij ZvC patiënten met actieve ziekte en verhoogden significant na behandeling met infliximab. Succesvolle behandeling met infliximab, met normalisatie van inflammatoire parameters, resulteerde in een normalisatie van de totale TRP niveaus, terwijl de vrije TRP significant lager bleven. De verandering (d.w.z. verbetering) in HRQL na behandeling met infliximab was gecorreleerd met de verandering van totale en vrije TRP niveaus. Bovendien werd de verbetering van de HRQL als gevolg van behandeling met infliximab onafhankelijk bepaald door de afname van ziekteactiviteit en door de verandering (toename) van de vrije TRP niveaus. Dit zou kunnen geïnterpreteerd worden als een indicatie van een rol van TRP niveaus in het subjectieve welbevinden bij ZvC.
	5th	8	9 CD patiënten	ATD, gerandomiseerde, dubbelblinde, gecontroleerde, cross-over studie	Verschil in verandering in stemming tussen controle en depletie conditie	Serum TRP concentraties namen aanzienlijk af na ATD, terwijl de verandering in de stemming scores niet significant verschilden tussen de ATD en de controle conditie. De resultaten verwierpen de hypothese van een serotonerge kwetsbaarheid als gevolg van ontsteking-geïnduceerde TRP vermindering van de latente CD, die kan worden uitgelokt door een ATD paradigma. Ontsteking geïnduceerde psychische symptomen zijn mogelijk niet gekoppeld aan de drastische afname van de biologische beschikbaarheid van TRP, maar andere mechanismen in verband met hoge pro-inflammatoire cytokines spelen mogelijk een rol.

PHQ: Patient Health Questionnaire, MDD: depressieve stoornis, CD: Crohn's Disease, IBDQ: Inflammatory Bowel Disease Questionnaire, ATD: acute tryptofaan depletie, TRP: tryptofaan, HRQL: gezondheidsgerelateerde kwaliteit van leven

In het tweede deel, werd de associatie tussen psychosociale factoren en de ZvC benaderd vanuit het **“Psyche-Somatisch" standpunt** (i.e. biopsychosociale variabelen als onafhankelijke variabelen en de ZvC-gerelateerde variabelen als afhankelijke variabelen). De prospectieve interventiestudie waarbij biopsychosociale factoren als mogelijke determinanten van het verloop van de ZvC werden onderzocht, ging de invloed na van een depressieve stoornis (MDD) op het korte-en lange termijn verloop van de ZvC na een behandeling met infliximab. Biologische en psychosociale parameters werden gemeten bij patiënten met actieve CD op het moment van behandeling met infliximab. Zij werden gerevalueerd 4 weken na behandeling en vervolgens opgevolgd tot wanneer een nieuwe behandeling nodig was voor herval van ziekte activiteit. We vonden dat patiënten met MDD een lagere kans hadden op korte-termijn remissie na behandeling met infliximab, maar MDD had geen invloed op de korte-termijn klinische respons. Dit korte-termijn effect van MDD op de resultaten van de behandeling leek een weerspiegeling van de hoge prevalentie van lichamelijke klachten bij depressieve patiënten. Daarnaast was de aanwezigheid van MDD op het moment van de behandeling met infliximab voorspellend voor een eerdere herbehandeling bij deze patiënten. Deze resultaten kunnen wijzen op een modulerende rol van MDD in het verloop van ZvC en ondersteunen een mogelijk specifiek verband tussen depressie en de ZvC. Het verschillende effect van MDD op korte en lange termijn illustreert de complexiteit van de interactie tussen psychosociale factoren en de ZvC. Opmerkelijk was ook dat de prevalentie van depressie bij de eerste meting significant hoger was dan na behandeling met infliximab, omdat de depressieve stoornis werd niet meer werd vastgesteld in een subgroep van patiënten. De MDD in deze subgroep was mogelijk "reactief" omdat hun ziekte was ernstiger was en verdween door de verbetering van de lichamelijke problematiek. Gezien de hoge inflammatoire parameters bij deze subgroep voor de behandeling met infliximab, is een alternatieve interpretatie ook mogelijk: de MDD in deze subpopulatie van ZvC patiënten was mogelijk het gevolg van de ontsteking (i.e. geïnduceerd door de hoge niveaus van pro-inflammatoire cytokines), zodat deze ZvC patiënten leden aan **een inflammatie-geassocieerde depressie**, die gunstig reageerde op infliximab.

In de derde sectie werd de associatie tussen psychosociale factoren en de ZvC benaderd vanuit het ***'Somatisch-psyche*" standpunt**. De mogelijke rol van biologische factoren (i.e. inflammatie) in de ontwikkeling van psychische klachten bij de ZvC werd onderzocht, meer bepaald de rol van het verhoogde tryptofaan (TRP) katabolisme door het inflammatie-geïnduceerde enzym, indoleamine 2,3 dioxygenase (IDO). We bevestigden dat de totale en vrije TRP concentraties significant verminderd ware bij ZvC patiënten met actieve ontsteking. De TRP concentraties waren ook significant en omgekeerd gecorreleerd met de mate van inflammatie. Zowel totale als vrije TRP concentraties waren significant gestegen na

behandeling met infliximab, maar alleen bij de ZvC patiënten met genormaliseerde CRP concentraties na behandeling. Bij patiënten bij wie de CRP gestegen bleef na infliximab behandeling, veranderden de TRP concentraties niet significant. Verder bleven de vrije TRP concentraties ook significant lager bij behandelde ZvC patiënten met genormaliseerde CRP, in vergelijking met de controlegroep. De evolutie van de TRP concentraties en de ontwikkeling van HRQL waren significant gecorreleerd en zowel de ziekteactiviteit en de verandering in vrije TRP concentratie waren significante determinanten van de verandering in kwaliteit van leven. Uit de bevinding dat de verandering van TRP niveaus geassocieerd was met de verandering van kwaliteit van leven en dat de verandering van vrije TRP niveaus een belangrijke determinant was voor de verandering van HRQL, veronderstelden we dat de perifere TRP niveaus mogelijk psychosociale factoren (waaronder HRQL) zou kunnen beïnvloeden bij patiënten met ZvC. In het geval van "*inflammatie-geassocieerde depressie*", werd dergelijke relatie tussen verlaagde TRP concentraties en depressie waargenomen. TRP is de precursor van serotonine (5-HT), een neurotransmitter in het centrale zenuwstelsel die een centrale rol in de huidige theorieën over stemmingsregulatie. Met de acute TRP depletie (ATD) kan een reductie van de perifere TRP beschikbaarheid bereikt worden en kan de centrale 5-HT neurotransmissie farmacologisch uitgedaagd worden, waarmee onder andere de stemming bij de mens onderzocht kan worden. We onderzochten met de techniek van ATD of inflammatie-geïnduceerd TRP katabolisme mogelijk aan de basis lag van stemmingsveranderingen bij ZvC patiënten. Het effect van ATD op stemming, vermoeidheid en angst werd nagegaan met een dubbelblind, gecontroleerd cross-over protocol. We vonden significante TRP depletie na ATD in vergelijking met de controleconditie. Stemming, vermoeidheid en angst verschilden niet tussen de ATD conditie en de controleconditie. De centrale 5-HT neurotransmissie werd mogelijk niet getroffen, aangezien er geen significante ontsteking aanwezig bij de deelnemende patiënten. Anderzijds ondersteunen de resultaten de uitgangshypothese in verband met "5-HT kwetsbaarheid" door ontsteking-geïnduceerde afbraak van TRP bij ZvC patiënten mogelijk ook niet, gezien ATD wel degelijk TRP depletie uitlokte zonder effect op de psychologische parameters, ook al is er bij ZvC bijna nooit volledige remissie aanwezig. Deze interpretatie wordt ondersteund door recent bewijsmateriaal dat aantoont dat gedaalde perifere TRP niveaus en de daaruit voortvloeiende gewijzigde serotoninesynthese, onvoldoende verklaring biedt voor de complexe effecten van inflammatie en IDO activatie op de ontwikkeling van psychische klachten. Hierdoor blijkt de hypothese van een deficiënte 5-HT neurotransmissie als centraal mechanisme voor inflammatie-geassocieerde depressie onhoudbaar, waardoor andere mogelijke mechanismen naar voor worden geschoven, zoals de nadelige effecten van sommige TRP katabolieten als gevolg van IDO activatie en hun rol in de ontwikkeling van psychische symptomen en depressie.

Popular summary

Crohn's disease is a chronic, inflammatory disease of the intestine with a variable and unpredictable disease course. Crohn's disease can manifest in the entire digestive system and inflamed bowel segments alternate with healthy segments. Extra-intestinal symptoms, such as arthritis, skin lesions, eye injuries, etc, are common. Although the exact cause is still unknown, we know that genetic vulnerability, along with environmental factors play an important role in the etiology of Crohn's disease. This disturbs the balance of intestinal immune system, which overreacts and causes inflammation.

Parallel to other chronic diseases, there is a link between Crohn's disease and emotional problems and this serious illness affects the functioning and well-being of all patients. Yet, previous research suggested a more complex relationship between Crohn's disease and psychological and social factors. The starting point for the research proposed in this thesis (hypothesis) is that the relationship between psychosocial factors and Crohn's disease cannot be reduced to a simple "cause and effect" question. Crohn's disease and psychosocial factors exert a mutual influence, at different levels and through various systems that communicate with each other. The required communication between the different levels, namely the bowels and brains, is called the brain-gut-axis. This axis is comprised of different systems (including the gastro-intestinal nervous system, the autonomic nervous system, hormonal (endocrine) system, the immune system) that interact with each other. With the 3 parts of the research in this thesis, we want to contribute to a better understanding of the functioning of this complex system.

The first part of the thesis is preparatory work and consists of 2 studies on respectively, the responsiveness of a Crohn's disease-specific "quality of life" questionnaire and the validity of a fatigue questionnaire. Because these questionnaires are used for the study of Crohn's patients in this thesis, it is important to examine their properties within this population. The study on fatigue also shows that this complaint very frequently occurs in patients with Crohn's disease.

The second part of the thesis looks at the influence of psychosocial factors on the course of Crohn's disease. The objective of the study from this point of view, is to determine if the presence of a depressive disorder at the time of the treatment of a serious flare of Crohn's disease with infliximab, has any influence on the short-term outcome of this treatment or on the course of the disease. In patients with a depressive disorder at the time of the treatment, the disease activity score is less frequently indicative of remission of the disease. The presence of a depressive disorder probably does not affect the short term response to the treatment. Patients with major depressive disorder at the time of their treatment seem to report more symptoms and have a higher disease activity score. This makes it harder to achieve the required score for "clinical remission", while the medication still seems to be as effective, since their disease activity decreases equally as in patients who are not depressed. Long-term, patients with a depressive disorder at the time of treatment with infliximab, will need a new treatment for relapse of disease activity sooner. The depressive disorder is an independent prognostic factor and seems to exert an influence on the course of Crohn's disease.

A final noteworthy finding in this study was the large number of patients with Crohn's disease at the start of the study who had a depressive disorder, but not after treatment with infliximab. It is possible that the inflammatory factors released during a flare of Crohn's disease play a role in the emergence of this depressive disorder. In this case, this is called a so-called an "inflammation-associated depressive disorder." It is still unclear if such a depressive disorder occurs in Crohn's disease. Infliximab interferes with the process of immune activation and stops the production of inflammatory factors. It is possible that the depressive disorder in a number of Crohn's patients can improve through this process, by interfering with the pathophysiology of inflammation-associated depressive disorder, and that this is the explanation for the relatively large decrease in the number of depressed patients following treatment with infliximab in this group.

The third part of the thesis investigates if biological mechanisms in Crohn’s disease play a role in the development of psychological problems. The two studies in this section examine the possible link between a decreased availability of tryptophan in the body due to the inflammation in Crohn's disease, and psychosocial factors. Tryptophan is an essential, large, neutral amino acid which is necessary for the production of serotonin. Serotonin is a messenger substance (neurotransmitter) that occupies a central place in the biological theories on the regulation of mood, behavior and cognitive functioning. In anxiety and depression, a disturbed serotonin neurotransmission is present. By oral administration of a mixture of large, neutral amino acids without tryptophan, a rapid decrease of the tryptophan availability can be achieved. We call this rapid or acute tryptophan depletion. In vulnerable

subjects, acute tryptophan depletion leads to a change in the production of serotonin and a temporary change in mood or depressive symptoms. The availability of tryptophan is also reduced by inflammatory processes. The first part of this study confirms this and shows that the tryptophan availability is much lower in patients with Crohn's disease before treatment with infliximab than afterwards. Moreover, the tryptophan availability is also lower in patients who still have elevated inflammatory parameters. There appears to be a correlation between the change in tryptophan availability and the change in quality of life, possibly indicating an effect of tryptophan availability on psychosocial factors. In inflammation-associated depression, a correlation with decreased tryptophan availability was also demonstrated in other populations.

The second study shows that rapid tryptophan depletion in patients with Crohn's disease does lead to a reduced availability of tryptophan in the blood, but has no effect on mood, anxiety or fatigue. This argues against a possible vulnerability due to the decreased tryptophan availability as a result of inflammation as a direct mechanism for the development of psychological problems in patients with Crohn's disease. This interpretation of the results is supported by the findings of recent research showing that a reduced tryptophan availability insufficient explanation for the effects of inflammation on mood, but that the increased degradation of tryptophan and their products could play a role in the development of the psychological symptoms of Crohn's disease.

In summary we can say that the research in this thesis demonstrates that there is probably a complex mutual influence between the biological factors of Crohn's disease and psychosocial factors, where different systems at different levels play a role.

Vulgariserende samenvatting

De ziekte van Crohn is een chronische, ontstekingsziekte van de darm met een onvoorspelbaar en wisselend ziekteverloop. De ziekte van Crohn kan zich over het verloop van het hele spijsverteringsstelsel manifesteren en ontstoken darmsegmenten zijn afgewisseld met gezonde darmsegmenten. Er kunnen zich ook ziekteverschijnselen buiten de darm voordoen, zoals gewrichtsontsteking, huidletsels, oogletsels, etc. Hoewel de precieze oorzaak nog onbekend is, weten we dat genetische kwetsbaarheid, samen met omgevingsfactoren een belangrijke rol spelen bij het ontstaan van de ziekte van Crohn. Hierdoor wordt het evenwicht in de darm verstoord en treedt een overdreven reactie van het immuunstelsel op, waarbij ontstekingsstoffen worden vrijgezet.

Zoals bij vele andere chronische ziekten, bestaat er een verband tussen de ziekte van Crohn en een verstoring van het emotionele leven. Het spreekt voor zich dat deze ernstige aandoening het functioneren en het welzijn van de patiënten sterk aantast. Eerder uitgevoerd onderzoek leek toch ook te wijzen in de richting van een complexer verband tussen de ziekte van Crohn en psychologische en sociale factoren. De uitgangspositie voor het onderzoek dat wordt voorgesteld in deze thesis (hypothese) is dat het verband tussen psychosociale factoren en de ziekte van Crohn niet herleid kan worden tot een eenvoudige "*oorzaak-en-gevolg*" vraag. De ziekte van Crohn en psychosociale factoren oefenen een wederzijdse invloed uit, op verschillende niveaus en via diverse systemen die met elkaar communiceren. De vereiste communicatie tussen de verschillende niveaus, namelijk de darmen en de hersenen, wordt de hersen-maag-darm-as (*brain-gut-axis*) genoemd. Deze as bestaat op zijn beurt uit verschillende systemen (onder andere het maag-darm-zenuwstelsel, het autonome zenuwstelsel, het hormonale (endocriene) stelsel, het immuunsysteem) die met elkaar in wisselwerking treden. Met de 3 onderdelen van het onderzoek in deze thesis willen we bijdragen aan een beter inzicht in de werking van dit complexe systeem.

Het eerste onderdeel van de thesis is voorbereidend werk en bestaat uit 2 studies die respectievelijk de responsiviteit van een ziekte van Crohn-specifieke "kwaliteit van leven" vragenlijst en de validiteit van een vermoeidheidsvragenlijst nagaan. Omdat deze vragenlijsten gebruikt worden voor het onderzoek bij Crohn patiënten in deze thesis, is het

belangrijk om hun eigenschappen binnen deze populatie na te gaan. Uit het onderzoek over vermoeidheid blijkt ook dat deze klacht heel frequent voorkomt bij patiënten met de ziekte van Crohn.

In het tweede onderdeel van de thesis wordt de invloed van psychosociale factoren op het verloop van de ziekte van Crohn nagegaan. Het doel van de studie die deze invalshoek onderzoekt, is om na te gaan of de aanwezigheid van een depressieve stoornis op het moment waarop een patiënt met de ziekte van Crohn behandeld met infliximab wordt voor een ernstige opstoot, een invloed heeft op het korte termijn effect van de behandeling of op het verloop van de ziekte nadien. Patiënten bij wie een depressieve stoornis aanwezig was op het moment van de behandeling, hebben na de behandeling minder vaak een ziekteactiviteitscore die wijst op remissie van de ziekte. De aanwezigheid van een depressieve stoornis beïnvloedt waarschijnlijk niet in welke mate een patiënt beantwoordt aan de behandeling op korte termijn. Patiënten met een depressieve stoornis op het moment van hun behandeling lijken meer klachten te hebben en hebben daardoor op dat moment een hogere ziekteactiviteitscore, waardoor ze moeilijker de vereiste score behalen om te voldoen aan “klinische remissie”, terwijl de medicatie nog wel even efficiënt lijkt te werken, aangezien hun ziekteactiviteit wel even veel afneemt als bij patiënten die niet depressief zijn.
Op lange termijn, hebben patiënten die een depressieve stoornis hebben op het moment van de eerste behandeling met infliximab wel sneller opnieuw een nieuwe behandeling nodig voor een herval van ziekteactiviteit. De depressieve stoornis is hiervoor een onafhankelijk voorspellende factor en lijkt dus een invloed uit te oefenen op het verloop van de ziekte van Crohn.
Een laatste opmerkelijke bevinding in deze studie was het grote aantal patiënten met ziekte van Crohn die bij het begin van de studie wel een depressieve stoornis hadden, maar niet meer na de behandeling met infliximab. Het is mogelijk dat de ontstekingsfactoren die vrijkomen bij een opstoot van de ziekte van Crohn mee aan de basis liggen van het ontstaan van de depressieve stoornis. In dat geval spreken we van een zogenaamde “ontstekings-geassocieerde depressieve stoornis”. Het is nog onduidelijk of een dergelijke depressieve stoornis voorkomt bij de ziekte van Crohn. Infliximab grijpt in op het proces van immuunactivatie, onder andere door het stopzetten van de productie van ontstekingsfactoren. Dit zou kunnen ingrijpen op het ontstaan van de “ontstekings-geassocieerde depressieve stoornis”. Het is mogelijk dat daardoor ook de depressieve stoornis bij een aantal Crohn-patiënten kan verbeteren en dat dit de verklaring is voor de relatief grote afname van het aantal depressieve patiënten na behandeling met infliximab in deze groep.

In het derde deel van de thesis wordt nagegaan of biologische mechanismen bij de ziekte Crohn een rol spelen bij het ontstaan van psychologische problemen. De twee studies van dit onderdeel onderzoeken het mogelijke verband tussen een gedaalde beschikbaarheid van TRP in het lichaam als gevolg van de ontstekingsprocessen bij de ziekte van Crohn, en psychosociale factoren. TRP is een essentieel, groot, neutraal aminozuur dat noodzakelijk is voor de aanmaak van serotonine. Serotonine is een boodschapperstof die een centrale plaats bekleedt in de biologische theorieën over de regeling van stemming, gedrag en cognitief functioneren. Bij angst en depressie is een verstoorde serotoninehuishouding aanwezig. Door middel van orale toediening van een mengsel van grote, neutrale aminozuren zonder TRP, kan een snelle daling van de TRP beschikbaarheid bereikt worden. Dit noemen we *snelle TRP depletie*. Bij kwetsbare personen leidt snelle TRP depletie tot een verandering in de productie van serotonine en tot een tijdelijke verandering van de stemming of zelfs depressieve symptomen. De beschikbaarheid van TRP wordt ook verminderd door ontstekingsprocessen. De eerste studie van dit onderdeel bevestigt dit en toont aan dat het TRPgehalte veel lager is bij patiënten met de ziekte van Crohn voordat ze behandeld worden met infliximab dan nadien. Bovendien blijft het TRP gehalte ook lager bij patiënten die nog steeds verhoogde ontstekingsparameters hebben. Er blijkt ook een verband te bestaan tussen de verandering in TRP gehalte en de verandering van de kwaliteit van leven, wat mogelijk wijst op een invloed van TRP op psychosociale factoren. Bij depressie die aan ontsteking geassocieerd is, werd in andere populaties ook een verband aangetoond met een verminderde TRP beschikbaarheid. De tweede studie toont aan dat een snelle TRP depletie bij patiënten met ziekte van Crohn wel leidt tot een verminderde beschikbaarheid van TRP in het bloed, maar geen effect heeft op stemming, angst of vermoeidheid. Dit pleit mogelijk tegen een kwetsbaarheid door een verminderde TRP beschikbaarheid als gevolg van ontsteking als rechtstreeks mechanisme voor het ontstaan van psychologische problemen bij patiënten met de ziekte van Crohn. Deze interpretatie van de resultaten wordt gesteund vanuit de bevindingen in recent onderzoek dat aantoont dat een verminderde TRP beschikbaarheid onvoldoende verklaring biedt voor de effecten van ontsteking op stemming, maar dat de verhoogde afbraak van TRP en de producten hiervan wel een rol zouden kunnen spelen in het ontstaan van psychologische symptomen bij de ziekte van Crohn.

Samenvattend kunnen we stellen dat het onderzoek in deze thesis aantoont dat er waarschijnlijk een complexe, wederzijdse invloed bestaat tussen de biologische factoren van de ziekte van Crohn en psychosociale factoren, waarbij diverse systemen op verschillende niveaus een rol spelen.

ADDENDUM

QUESTIONNAIRES

CROHN'S DISEASE ACTIVITY INDEX (CDAI)

Crohn's Disease Activity Index (CDAI)
Range 0 – 600
< 150 – Inactive Disease
>450 – Critically Ill

Ziekte Activiteit		Totaal	x Factor	Subtotaal
Totaal aantal vloeibare of zeer zachte stoelgangen gedurende de voorgaande 7 dagen			**x 2**	
Abdominale pijn / krampen (totaal voor de laatste 7 dagen): *0=geen 1=mild 2=matig 3=ernstig*			**x 5**	
Algemeen welzijn (totaal voor de laatste 7 dagen): *0=algemeen goed 1=een beetje minder goed 2=slecht 3=heel slecht 4=verschrikkelijk*			**x 7**	
Patiënt lijdt momenteel aan				
A. arthritis / arthralgie			**x 20**	
B. Iritis / Uveitis			**x 20**	
C. Erythema Nodusum/Pyoderma Gangrenosum/Afteuze Stomatitis			**x 20**	
D. Anale fissuur, fistel of abces			**x 20**	
E. Andere fistels			**x 20**	
F. Koorts > 37,8°C			**x 20**	
Medicamenteuze behandeling voor diarree (bv. Immodium, opiaten) *0=geen 1=ja*			**x 30**	
Abdominale massa: *0=geen 1=mogelijk 5=zeker*			**x 10**	
Hematocriet	Exact			
Mannen (47 - Exact)			**x 6**	
Vrouwen (42 - Exact)				
Gewicht				
Eigenlijke gewicht in kg				
Standaard gewicht (zie tabel) in kg				
Berekende gewicht			**x 1**	
TOTAAL				

PATIENT HEALTH QUESTIONNAIRE – 9 ITEMS (PHQ-9)

Vragen over stemming

Hoe vaak heeft u in de voorbije 2 weken last gehad van één van de volgende problemen?

	Helemaal Niet	Verschillende dagen	Meer dan de helft van de dagen	Bijna elke dag
a. Weinig interesse of plezier in uw gewone activiteiten	O	O	O	O
b. Zich neerslachtig, depressief, hopeloos voelen	O	O	O	O
c. Moeilijk inslapen, moeilijk doorslapen of te veel slapen	O	O	O	O
d. Zich moe voelen of gebrek aan energie hebben	O	O	O	O
e. Weinig eetlust of overmatig eten	O	O	O	O
f. Een slecht gevoel hebben over uzelf -of het gevoel hebben dat u een mislukkeling bent -of het gevoel dat u zichzelf of uw familie heeft teleurgesteld	O	O	O	O
g. Problemen om u te concentreren, bv. om de krant te lezen of om T.V. te kijken	O	O	O	O
h. Zo traag bewegen of zo langzaam spreken dat andere mensen dit zouden kunnen gemerkt hebben. Of integendeel, zo zenuwachtig of rusteloos zijn dat u veel meer rondliep	O	O	O	O
i. De gedachte dat u beter dood zou zijn of de gedachte uzelf op een bepaalde manier pijn te doen	O	O	O	O

INFLAMMATORY BOWEL DISEASE QUESTIONNAIRE

De volgende vragen zijn opgenomen om een idee te krijgen hoe u zich gedurende de afgelopen twee weken heeft gevoeld. Er wordt gevraagd naar klachten die kunnen behoren bij uw darmziekte, maar ook zijn vragen opgenomen over algemeen welbevinden en stemming.
Telkens het meest toepasselijke antwoord aankruisen.

1. **Hoe vaak heeft u in de afgelopen twee weken ontlasting gehad in vergelijking met een rustige fase van uw darmziekte?**
   1 ☐ ontlasting vaker dan ooit
   2 ☐ extreem vaak
   3 ☐ erg vaak
   4 ☐ matige verhoging in vergelijking met rustige fase
   5 ☐ enige verhoging in vergelijking met rustige fase
   6 ☐ lichte verhoging in vergelijking met rustige fase
   7 ☐ geen verhoging in vergelijking met rustige fase

2. **Hoe vaak was moeheid of het gevoel "uitgeblust te zijn" de afgelopen twee weken voor u een probleem?**
   1 ☐ de hele tijd
   2 ☐ grootste deel van de tijd
   3 ☐ een groot deel van de tijd
   4 ☐ een matig deel van de tijd
   5 ☐ een klein deel van de tijd
   6 ☐ bijna geen deel van de tijd
   7 ☐ helemaal niet

3. **Hoe vaak was u de afgelopen twee weken gefrustreerd, ongeduldig of rusteloos?**
   1 ☐ de hele tijd
   2 ☐ grootste deel van de tijd
   3 ☐ een groot deel van de tijd
   4 ☐ een matig deel van de tijd
   5 ☐ een klein deel van de tijd
   6 ☐ bijna geen deel van de tijd
   7 ☐ helemaal niet

4. **Hoe vaak heeft u de afgelopen twee weken school moeten missen of uw werk niet kunnen doen als gevolg van uw darmklachten?**
   1 ☐ de hele tijd
   2 ☐ grootste deel van de tijd
   3 ☐ een groot deel van de tijd
   4 ☐ een matig deel van de tijd
   5 ☐ een klein deel van de tijd
   6 ☐ bijna geen deel van de tijd
   7 ☐ helemaal niet

5. **Hoe vaak heeft u de afgelopen twee weken dunne ontlasting gehad?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

6. **Hoeveel energie had u de afgelopen twee weken?**

1 ☐ totaal geen energie
2 ☐ heel erg weinig energie
3 ☐ weinig energie
4 ☐ enige energie
5 ☐ matige hoeveelheid energie
6 ☐ veel energie
7 ☐ zeer veel energie

7. **Hoe vaak bent u in de afgelopen twee weken ongerust geweest over een eventueel noodzakelijke operatie voor uw darmziekte?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

8. **Hoe vaak heeft u de afgelopen twee weken een afspraak moeten uitstellen of afzeggen in verband met uw darmziekte?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

9. **Hoe vaak heeft u de afgelopen twee weken last gehad van buikkrampen?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

10. **Hoe vaak heeft u zich de afgelopen twee weken niet lekker gevoeld ("algemeen onwel zijn")?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

11. **Hoe vaak heeft u zich de afgelopen twee weken zorgen gemaakt om niet op tijd bij een toilet te kunnen komen?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

12. **Hoeveel moeite heeft het u de afgelopen twee weken gekost, door uw darmproblemen, om tijd te besteden aan ontspanning of sport?**

1 ☐ zeer veel moeite, ontspanning en sport waren niet mogelijk
2 ☐ veel moeite
3 ☐ best wel moeite
4 ☐ enige moeite
5 ☐ weinig moeite
6 ☐ praktisch geen moeite
7 ☐ helemaal geen moeite, geen beperking van sport/ ontspanning door darmproblemen

13. **Hoe vaak heeft u de afgelopen twee weken last gehad van buikpijn?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

14. **Hoe vaak heeft u gedurende de afgelopen twee weken problemen gehad een goede nachtrust te krijgen of had u last van 's nachts wakker worden?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

15. Hoe vaak voelde u zich de afgelopen twee weken depressief of teneergeslagen?

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

16. **Hoe vaak heeft u de afgelopen twee weken gelegenheden vermeden waarvan u niet zeker wist dat er een toilet in de buurt was?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

17. **Hoe vaak heeft u gedurende de afgelopen twee weken last gehad van veel winderigheid?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

18. **Hoe vaak heeft u zich de afgelopen twee weken zorgen gemaakt over het behouden of verkrijgen van het gewicht dat u nastreeft?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

19. **Veel patiënten met chronische darmziekte maken zich vaak zorgen of hebben angsten die te maken hebben met hun ziekte. Onder andere zorgen om kanker te krijgen, zich nooit beter te voelen en angsten om weer veel last van hun darmziekte te krijgen. Hoe vaak heeft u dergelijke zorgen gehad gedurende de afgelopen twee weken?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

20. **Hoe vaak heeft u gedurende de afgelopen twee weken last gehad van een opgeblazen gevoel?**

1 ☐ de hele tijd

2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

21. **Hoe vaak heeft u zich gedurende de afgelopen twee weken relaxed en ontspannen gevoeld?**
1 ☐ nooit
2 ☐ een klein deel van de tijd
3 ☐ enig deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ grootste deel van de tijd
6 ☐ bijna de hele tijd
7 ☐ de hele tijd

22. **Hoe vaak heeft u de afgelopen twee weken last gehad van bloedverlies bij de ontlasting?**
1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

23. **Hoe vaak heeft u zich gedurende de afgelopen twee weken opgelaten gevoeld door uw darmprobleem?**
1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

24. **Hoe vaak heeft u de afgelopen twee weken het gevoel gehad dat u naar de toilet moest gaan waarbij dan echter geen ontlasting kwam?**
1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

25. **Hoe vaak heeft u zich de afgelopen twee weken verdrietig of van streek gevoeld?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

26. **Hoe groot was het probleem voor u gedurende de afgelopen twee weken dat per ongeluk wat ontlasting werd verloren?**

1 ☐ een ernstig probleem
2 ☐ een belangrijk probleem
3 ☐ een matig probleem
4 ☐ enige last
5 ☐ weinig last
6 ☐ bijna geen last
7 ☐ geen last

27. **Hoe vaak heeft u zich de afgelopen twee weken kwaad gemaakt vanwege uw darmziekte?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

28. **In welke mate heeft uw darmprobleem gedurende de afgelopen twee weken ertoe bijgedragen dat er een beperking was in uw sexuele leven?**

1 ☐ geen sexuele activiteit door darmproblemen
2 ☐ zeer grote beperking door darmproblemen
3 ☐ grote beperking door darmproblemen
4 ☐ matige beperking door darmproblemen
5 ☐ enige beperking door darmproblemen
6 ☐ nauwelijks beperking door darmproblemen
7 ☐ geen beperking door darmproblemen

29. **Hoe vaak heeft u zich gedurende de afgelopen twee weken misselijk gevoeld?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

30. **Hoe vaak was u gedurende de afgelopen twee weken geïrriteerd?**

1 ☐ de hele tijd
2 ☐ grootste deel van de tijd
3 ☐ een groot deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ een klein deel van de tijd
6 ☐ bijna geen deel van de tijd
7 ☐ helemaal niet

31. **Hoe vaak had u gedurende de afgelopen twee weken het gevoel niet door uw omgeving begrepen te worden?**

1 ☐ de hele tijd
2 ☐ bijna de hele tijd
3 ☐ grootste deel van de tijd
4 ☐ een matig deel van de tijd
5 ☐ enig deel van de tijd
6 ☐ een klein deel van de tijd
7 ☐ nooit

32. **Hoe tevreden, gelukkig of blij was u gedurende de afgelopen twee weken met uzelf?**

1 ☐ zeer ontevreden, grootste deel van de tijd ongelukkig
2 ☐ over het algemeen ontevreden, ongelukkig
3 ☐ iets ontevreden, ongelukkig
4 ☐ over het algemeen tevreden, blij
5 ☐ tevreden, meeste deel van de tijd gelukkig
6 ☐ zeer tevreden, meeste deel van de tijd gelukkig
7 ☐ extreem tevreden, kon niet gelukkiger of blijer zijn

HARTELIJK DANK VOOR UW MEDEWERKING

CHECKLIST INDIVIDUAL STRENGTH 20 ITEMS (CIS20R) – DUTCH VERSION

CIS 20R

(Copyright J. Vercoulen et al. (Katholieke Universiteit Nijmegen)

Instructies:

Op de volgende pagina's staan 20 uitspraken. Met behulp van deze uitspraken willen wij een indruk krijgen van hoe u zich de **laatste twee weken** heeft gevoeld.

Voorbeelden:

Er staat bijvoorbeeld de uitspraak "Ik voel me moe"

*Wanneer u vindt dat het **helemaal klopt** dat u zich de laatste 2 weken moe heeft gevoeld, plaatst u een kruisje in het linker hokje op de volgende manier:*

Ja, dat klopt ☒☐☐☐☐☐☐ nee, dat klopt niet

Wanneer u vindt dat uw antwoord tussen **"ja dat klopt" en "nee, dat klopt niet"** in zit, zet dan een kruisje in het hokje dat het meest overeenstemt met uw gevoel. Bijvoorbeeld, als u zich wel wat moe heeft gevoeld, maar niet zo heel erg moe, kunt u het kruisje in een van de volgende hokjes zetten die in de buurt staan van de antwoordmogelijkheden "ja dat klopt". Dus bijvoorbeeld als volgt:

Ja, dat klopt ☐☐☒☐☐☐☐ nee, dat klopt niet

Sla aub geen uitspraken over en plaats telkens één kruisje bij iedere uitspraak

1.	Ik voel me fit	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
2.	Ik zit vol activiteit	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
3.	Nadenken kost me moeite	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
4.	Lichamelijk voel ik me uitgeput	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
5.	Ik heb zin om allerlei leuke dingen te gaan doen	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
6.	Ik voel me moe	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
7.	Ik vind dat ik veel doe op 1 dag	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
8.	Als ik ergens mee bezig ben, kan ik er mijn gedachten goed bijhouden	Ja, dat klopt	☐☐☐☐☐☐☐ ee, dat klopt niet
9.	Ik voel me slap	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
10.	Ik vind dat ik weinig doe op één dag	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
11.	Ik kan me goed concentreren	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
12.	Ik voel me uitgerust	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
13.	Het kost me moeite ergens mijn aandacht bij te houden	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
14.	Lichamelijk voel ik me in een slechte conditie	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
15.	Ik zit vol plannen	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
16.	Ik ben gauw moe	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
17.	Er komt weinig uit mijn handen (d.w.z. 'Ik presteer weinig')	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
18.	De zin om dingen te ondernemen ontbreekt mij	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
19.	Mijn gedachten dwalen gemakkelijk af	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet
20.	Lichamelijk voel ik me in een uitstekende conditie	Ja, dat klopt	☐☐☐☐☐☐☐ nee, dat klopt niet

Professional Career

Philippe Persoons (°1974) graduated as a Medical Doctor magna cum laude at the Katholieke Universiteit Leuven in 1999. He started his residency in Psychiatry at the University Hospitals of the KU Leuven in July 1999 and graduated as a specialist in general adult psychiatry in July 2006. From July 1999 until September 2000, he was part-time scientific collaborator of the scientific project "*Pilootproject voor een geïntegreerde Liaisonpsychiatrie in de Algemene Geneeskunde*" of the Department of Health and Social Affairs, Belgium. From October 2000 until September 2004, he was a research fellow of the Fund for Scientific Research – Flanders, Belgium. He was enrolled as a PhD student at the Doctoral School of Biomedical Sciences of the KU Leuven during his PhD training. Additional training comprised of the post-graduate course of specialization in Cognitive Behavioral Therapy at the Faculty of Psychology of the KU Leuven (to be completed) and he obtained the European Certificate in Mood and Anxiety Disorders, an international postgraduate program, endorsed by the universities of Maastricht, Bristol and Tel Aviv.

From July 2006 until March 2007, he was senior resident of the Department of Psychiatry at the University Hospital of the KU Leuven and worked at the campus University Psychiatry Center (UPC) Lubbeek, further specializing as an Old Age psychiatrist. Since April 2007, he is working as an Old Age psychiatrist (adjunct-kliniekhoofd) and supervisor at the University Psychiatric Center (UPC) of the KU Leuven, campus Sint-Jozef Kortenberg. From 2007 until November 2012, he continued to work as an Old Age psychiatrist at the campus UPC Lubbeek, until the transfer of the patients to the campus Sint-Jozef. From 2007 until the end of 2008, he worked as a psychiatrist at the community mental health care center CGG (Centrum Geestelijke Gezondheidszorg) Vlaams-Brabant Oost vzw, Aarschot branch.

Philippe Persoons is a laureate of the travel and research award from the Crohn- en Colitisvereniging vzw (CCV), Belgium (2003) and of the Eli-Lilly – Vlaamse Vereniging voor Zenuwartsen Award (2005). His unit Sint-Lutgardis, specialized in Behavioral and Psychiatric Symptoms in Dementia, at the UPC KUL, campus Sint-Jozef Kortenberg, will be supported by the "Hulpfonds Prins Alexander van België", from 2013.

He is a member of the Vlaamse Vereniging Psychiatrie, the BCNBP, the International Psychogeriatric Association. He was an active member of the Vlaamse Vereniging Assistenten Psychiatrie (2000-2005), the CoM-Ment group (Communication in Mental health, 2006-2008) and member of the Medical Advisory Board of the CCV vzw, Belgium.

He teaches psychopharmacotherapy in Old Age Psychiatry in the postgraduate interuniversity program for psychiatry trainees (2011-present), the part Old Age Psychiatry in the Selected Topics Mental Health Care II of the Master in Nursing and Midwifery (MSc.) program (coordinated by Stephan Claes, MD, PhD and Pascal Sienaert, MD, PhD) and the part Old Age Psychiatry in the course Rehabilitation of Psychopathological Disease of the bachelor of Kinesiology and Rehabilitation Science (coordinated by Michel Probst, PhD and Marc De Hert, MD, PhD).

PUBLICATIONS IN PEER REVIEWED JOURNALS

Fischler B, Tack J, De Gucht V, Shkedy ZI, **Persoons P**, Broekaert D, Molenberghs G, Janssens, J. Heterogeneity of symptom pattern, psychosocial factors, and pathophysiological mechanisms in severe functional dyspepsia. Gastroenterology 2003;124(4):903-10

Blockmans D, **Persoons P**, Van Houdenhove B, Lejeune M, Bobbaers H. Combination therapy with hydrocortisone and fludrocortisone does not improve symptoms in chronic fatigue syndrome: a randomized, placebo-controlled, double-blind, crossover study. Am J Med 2003;114(9):736-41.

Persoons P, Luyckx K, Desloovere C, Vandenberghe J, Fischler B. Anxiety and mood disorders in otorhinolaryngology outpatients presenting with dizziness: Validation of the self-administered PRIME-MD Patient Health Questionnaire and epidemiology. Gen Hosp Psychiatry 2003;25(5):316-323.

Vandenberghe J, Dupont P, Fischler B, Bormans P, **Persoons P**, Janssens J, Tack J.. Regional brain activation during proximal stomach distention in humans: a positron emission tomography study. Gastroenterology 2005; 128 (3): 564-573.

Vandenberghe J, Vos R, **Persoons P**, Demyttenaere K, Janssens J, Tack J. Dyspeptic patients with visceral hypersensitivity: sensitisation of pain specific or multimodal pathways? Gut 2005; 54 (7): 914-919.

Persoons P, Vermeire S, Demyttenaere K, Fischler B, Vandenberghe J, Van Oudenhove L, Pierik M, Hlavaty T, Van Assche G, Noman M, Rutgeerts P. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. Aliment Pharamcol Ther 2005; 22(2): 101-110.

Blockmans B, **Persoons P**, Vanhoudenhove B, Bobbaers H. A Randomised Double-blind, Placebo-controlled Cross-over Study with Methylphenidate in Sixty Patients with Chronic Fatigue Syndrome. Am J Med, 2006; 119 (167): e23-e30.

Hlavaty T, **Persoons P**, Vermeire S, Ferrante M, Pierik M, Van Assche G, Rutgeerts P. Evaluation of short-term responsiveness and cutoff values of inflammatory bowel disease questionnaire in Crohn's disease. Inflamm Bowel Dis 2006; 12(3):199-204.

Van Oudenhove L, Vandenberghe J, Geeraerts B, Vos R, **Persoons P**, Demyttenaere K, Fischler B, Tack J. Relationship between anxiety and gastric sensorimotor function in functional dyspepsia. Psychosom Med. 2007 Jun;69(5):455-63.

Van Oudenhove L, Vandenberghe J, Geeraerts B, Vos R, **Persoons P**, Fischler B, Demyttenaere K, Tack J. Determinants of symptoms in functional dyspepsia: gastric sensorimotor function, psychosocial factors or somatisation? Gut. 2008 Dec;57(12):1666-73.

Dockx K, **Persoons P.** De vertraagde slaapfase: een ongekend en vaak miskend type van circadiaanse slaapstoornis. Tijdschrift voor Geneeskunde. 2010; 66: 1034-1037.

Van Assche L, Luyten P, Bruffaerts R, **Persoons P**, van de Ven L, Vandenbulcke M. Attachment in old age: Theoretical assumptions, empirical findings and implications for clinical practice. Clin Psychol Rev. 2012 Oct;33:67-81.

Archie A, **Persoons P**, Vandenbulcke M. Clozapine en levodopa als behandeling van persisterende visuele hallucinaties en motorische symptomen bij dementie met Lewy-lichaampjes. Tijdschr Psychiatr. 2013; 55(4): 287-291.

Hulstaert A, **Persoons P**. The Management of anxiety symptoms in patients with vascular dementia: a case report about treatment with pregabalin. Acta Psychiatrica Belgica. Accepted for publication 2013

Persoons P, Vandenberghe J, Demyttenaere K, Rutgeerts P. Fatigue in Crohn's Disease: validity of the Checklist of Individual Strength and prevalence. Submitted

ABSTRACTS OF ORAL OR POSTER PRESENTATIONS AT INTERNATIONAL SCIENTIFIC MEETINGS WITH PEER-REVIEW

Fischler B, **Persoons P.** Luyckx K. PRIME-MD PHQ in the specialist medical setting: validation for anxiety and mood disorders. J Psychosom Res 2000; 48: A1018. *Poster Presentation at the American Psychiatric Association Annual Meeting 2000, Chicago, USA*

Fischler B, **Persoons P.** Luyckx K. Epidemiology of axis-I disorders in medical patients. J Psychosom Res 2000; 48: A1035. *Poster Presentation at the American Psychiatric Association Annual Meeting, Chicago 2000*

Persoons P. Fischler, B, Luyckx K, Van Oudenhove L, Igodt P. Recognition of mental disorders in a gastroenterology unit. J Psychosom Res 2000; 48: A1019. *Poster Presentation at the American Psychiatric Association Annual Meeting 2000, Chicago, USA*

Fischler B, De Gucht V, **Persoons P.** Broekaert D, Janssens J, Tack J. Differential Psychopathological Associations of Symptom Clusters in Functional Dyspepsia. J Psychosom Res 2000; 48: A1068. *Poster Presentation at the American Psychiatric Association Annual Meeting 2000, Chicago, USA*

Fischler B, Vandenberghe J, **Persoons P,** De Gucht V, Broekaert D, Luyckx K, Tack J. Evidence-Based Subtypes in Functional Dyspepsia with Confirmatory Factor Analysis: Psychosocial and Physiological Correlates. Gastroenterology 2001; 120(7) Suppl; 268: A-51. *Oral Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2001, Atlanta, USA*

Persoons P, Luyckx K, Fischler B. Psychiatric Diagnoses in Gastroenterolgy: Validation of a Self-Report Instrument (PRIME-MD PHQ), Epidemiology and Recognition. Gastroenterology 2001; 120(7) Suppl; 615: A-114. *Oral Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2001, Atlanta, USA*

Fischler B**, Persoons P,** Luyckx K, Vander Mijnsbrugge D. Validation and Assessment of Practical Use of a Self-Rating Scale for Axis-I Disorders (PRIME-MD TODAY) in General Hospitals. *ECNP Congress, 2001, Istanbul, Turkey.*

Persoons P, Fischler B, Noman M, Vandenberghe J, Rutgeerts P. Determinants of Psychosocial Distress in Crohn's Disease. Gastroenterology 2002; 122(7) Suppl; W1322: A-609. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2002, San Francisco, USA*

Persoons P, Fischler B, Vandenberghe J, Rutgeerts P. Determinants of Quality of Life in Crohn's Disease. Gastroenterology 2002; 122(7) Suppl; S1375: A-216. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting, 2002, San Francisco, USA*

Persoons P, Fischler B, Vandenberghe J, Rutgeerts P. Fatigue in Inflammatory Bowel Disease: Determinants and Association with Quality of Life. Gastroenterology 2002 122(7) Suppl; W1329: A-610. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2002, San Francisco, USA*

Catthoor K, De Hert M, **Persoons P,** Peuskens J. Stigma in Family Members of Patients with Psychotic Disorder. *American Psychiatric Association's Annual Meeting, 2002, Philadelphia, USA.*

Persoons P, Vandenberghe J, Demyttenaere K, Bonnewyn A, Rutgeerts P. The Severity of Somatic Complaints in Crohn's Disease: the Role of Psychological Factors. Gastroenterology 2003; 124 (7) Suppl; S1425. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2003, Orlando, USA*

Persoons P, Vandenberghe J, Demyttenaere K, Bonnewyn A, Rutgeerts P. Severity and Determinants of Fatigue in Crohn's Disease. Gastroenterology 2003; 124 (7) Suppl; S1404. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2003, Orlando, USA*

Vandenberghe J, Dupont P, **Persoons P,** Demyttenaere K, Tack J. Regional Cerebral Blood Flow During Gastric Balloon Distention in Functional Dyspepsia. Gastroenterology 2004; 124 (7) Suppl; 194. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2004, New Orleans, USA*

Persoons P, Demyttenaere K, Vandenberghe J, Van Oudenhove L, Rutgeerts P. Fatigue in Crohn's Disease patients treated with infliximab: a prospective study. Gastroenterology 2004; 126 (7) Suppl; *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2004, New Orleans, USA*

Persoons P, Demyttenaere K, Fischler B, Vandenberghe J, Van Oudenhove J, Rutgeerts P. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease after treatment with anti-TNF-alfa (infliximab): a prospective study. Psychosom Med 2005, 67 (1) Suppl; A-76: A1082. *Poster Presentation at the American Psychosomatic Society's annual meeting, 2005, Vancouver, Canada*

Van Oudenhove L, Vandenberghe J, Geeraerts B, **Persoons P,** Demyttenaere K, Tack J, Gregory L, Aziz Q. Influencde of anxious emotional context on gastric sensorimotor function in men. Psychosom Med 2005, 67 (1) Suppl; A-77: A1085. *Poster Presentation at the American Psychosomatic Society's annual meeting, 2005, Vancouver, Canada*

Hlavaty T, **Persoons P,** Vermeire S, Ferrante M, Pierik M, Henckaerts L, Joossens M, Van Assche G, Rutgeerts. IBDq Score Is a Sensitive Instrument To Assess Short-Term Changes in Disease Related Quality Of Life in Patients With Luminal Crohn's Disease. Gastroenterology 2005. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2005, Chicago, USA*

Persoons P, Vermeire S, Joossens M, Van Oudenhove L, Fischler B, Demyttenaere K, Rutgeerts P. The Impact Of Major Depressive Disorder on the Short-Term and Long-Term Outcome Of Crohn's Disease Treatment With Infliximab: a Prospective Study. Gastroenterology 2005. *Poster Presentation at the Digestive Disease Week, Annual American Gastroenterological Association meeting 2005, Chicago, USA*

Van Oudenhove L, Vandenberghe J, **Persoons P,** Demyttenaere K, Geeraerts B, Vos R, Tack J. Determinants of symptoms in functional dyspepsia. Psychosom Med 2006, 68(1) Suppl; A-38: A1300. *Oral Presentation at the American Psychosomatic Society Annual Meeting 2006, Denver, USA.*

Persoons P, Van Oudenhove L, Demyttenaere K, Vermeire S, Servaes S, Rutgeerts P. Crohn's disease and psychosocial problems: exploring a possible biological link with inflammation. Psychosom Med. 2007, 69(1) Suppl A-11: 1278. *Oral Presentation at the American Psychosomatic Society Annual Meeting 2007, Budapest, Hungary.*

PUBLICATIONS IN NON-PEER REVIEWED JOURNALS

Persoons P, Vandenberghe, J. De posttraumatische stress-stoornis bij volwassenen. Deel 1: Ontstaan en diagnose. Neuron 2003; 8: 108 – 112.

Persoons P, Vandenberghe, J. De posttraumatische stress-stoornis bij volwassenen. Deel 2: Behandeling. Neuron 2003; 8:170 – 172.

Persoons, P. Vermoeidheid en Inflammatoire Darmziekten. Crohniek 2003; 85: 12-22.

Persoons, P. Slaaphygiëne en Inflammatoire Darmziekten. Crohniek 2004; 88.

Persoons, P. Het mysterieuze geschenk van Hypnos: de normale slaap. Crohniek 2004; 89.

Persoons P, Vandenberghe J, Van Oudenhove L. Psychopathologie bij Ziekte van Crohn: Is Tryptofaan een Zwakke Schakel? Patient Care België 2004; Speciale Editie april: 13-20 en Patiënt Care Nederland 2004

Persoons P. Psychopathologie bij ziekte van Crohn: Is Tryptofaan een zwakke schakel? Crohniek 2005;103.

Persoons P. Lichamelijke klachten zonder duidelijke verklaring bij chronische darmziekten: wederzijdse beïnvloeding tussen het lichaam en het brein. Crohniek 2010; 114.

Persoons P. Vermoeidheid Crohniek 2012

BOOK CHAPTERS

Persoons P. Psychosociale aspecten van chronische darmziekten. In D'Haens G, Rutgeerts P, editors. 101 vragen over langdurige darmziekten. 1e ed. Tielt: Lannoo; 2005; Vragen 93-96.

Persoons P. Praktische farmacotherapie. In van der Mast R, Heeren T, Kat M, Stek M, Vandenbulcke M, Verhey F, editors. Handboek Ouderenpsychiatrie. 1^{e} ed. Utrecht; 2010: 245-275.

INTERNAL REPORTS

Tussentijds verslag en eindverslag 1999-2000: "*Pilootproject voor een geïntegreerde Liaisonpsychiatrie in Algemene Geneeskunde*" van het Ministerie van Volksgezondheid en Sociale Zaken. Projectleider: Prof. Benjamin Fischler, MD, PhD

Dankwoord

Dit proefschrift wordt respectvol voorgesteld aan Prof. Dr. M. Waer en Prof. Dr. M. Casteels, respectievelijk Rector en vice-Rector van de KU Leuven, en aan Prof. Dr. J. Goffin, Decaan van de Faculteit Geneeskunde. Ik dank de Universiteit en de Faculteit Geneeskunde voor de mogelijkheid om dit onderzoek uit te voeren en ook de verantwoordelijken van de UZ Leuven en de UPC KUL om dit proces te faciliteren. Ook wil ik het Fonds voor Wetenschappelijk Onderzoek-Vlaanderen danken voor het mandaat van aspirant-onderzoeker dat ze mij gedurende 4 jaar verleenden.

I wish to express my gratitude to all the members of the jury: Prof. Dr. G. Moser, Prof. Dr. S. Claes, Prof. Prof. Dr. D. De Ridder, Prof. Dr. F. Dobbels, Prof. Dr. G. Lemmens and Prof. Dr. P. Pelckmans for the time they devoted to the critical reading of this manuscript and for their constructive comments and suggestions which helped improve the quality of this thesis.

Ik dank mijn promotor, Prof. Dr. K. Demyttenaere die altijd even enthousiast bleef wanneer ik weer eens aankondigde dat ik zou verder werken aan mijn thesis. Bedankt Koen om in mij te blijven geloven, ook al heb ik je geduld stevig op de proef gesteld.

Ik dank mijn co-promotor, Prof. Dr. P. Rutgeerts voor de steun en begeleiding die ik doorheen de jaren heb mogen ervaren. Ook wil ik Prof. Rutgeerts bedanken voor de unieke kans die hij mij geboden heeft door een psychiater toe te laten onderzoek te doen bij patiënten met Inflammatoire Darmziekten. Ik dank u oprecht voor alle mogelijkheden die ik kreeg en voor de unieke samenwerking over de grenzen van de disciplines heen.

Ik dank mijn co-promotor, Prof. Dr. Joris Vandenberghe die nog laat formeel in het project is gestapt, maar die vanaf het begin van heel nabij betrokken is geweest, als collega, labogenoot, coauteur, vriend en vooral, te volgen en onnavolgbaar voorbeeld. Zonder jou, Joris, was dit mij nooit gelukt. Ik sta bij jou in het krijt en *we hebben toch veel meegemaakt…*

Ik dank Prof. Dr. B. Fischler die destijds als promotor met mij de eerste stappen zette in de wetenschap en in dit project. Ik neem mee wat is geweest.
Ik dank Prof. Dr. Em. P. Igodt die me de mogelijkheid gaf om een doctoraat te beginnen toen hij diensthoofd Psychiatrie was en die mij bleef aansporen om af te maken waar ik mee begonnen was, toen hij als emeritus actief aanwezig bleef.

Ik dank de patiënten en de medewerkers van de Crohn en Colitisvereniging VZW voor hun bereidwillige medewerking aan de studies en voor hun interesse voor de voortgang van het onderzoek. Ik hoop dat ik met mijn bijdrage iets kan teruggeven voor al hun inspanningen

Ik dank alle medewerkers van de dienst Gastro-enterologie, Prof. Dr. S. Vermeire, Prof. Dr. G. Van Assche, Dr. I. Arijs, Dr. M. Noman, Isolde, Karen, het team van de infuuseenheid en het team van E443. Jullie hulp bij het verzamelen van de nodige gegevens en de creatieve inbreng was onmisbaar. Ik dank Dr. T. Hlavaty voor de vruchtbare samenwerking aan het artikel dat hoofdstuk 3 werd van deze thesis. Ik dank ook de heer R. Servaes voor zijn analyses die cruciaal waren voor de verwerking en interpretatie van de gegevens die inzicht gaven in de rol van tryptofaan bij Crohn patiënten.

Ik dank mijn labogenoten Paul, Koen, Lukas, Anke, Els en iedereen met wie ik vele uren doorbracht. Ook wil ik mijn diensthoofd en huidige collega's, Prof. Dr. M. Vandenbulcke, Dr. F. Bouckaert en Dr. D. Broekaert, samen met de respectievelijk huidige en vorige hoofdgeneesheer van het UC Kortenberg, Prof. Dr. M. De Hert en Prof. Dr. J. Peuskens bedanken voor de faciliteiten en tijd die ik kreeg om mijn thesis af te werken. Mijn dank gaat ook uit naar Marc, Hilde, Frie en alle medewerkers van de dienst Ouderenpsychiatrie die mij gesteund hebben tijdens de afwerking van mijn thesis. Ook Els, die weet waarom, wil ik hier expliciet bedanken. Ik wil ook iedereen bedanken die hier niet expliciet vermeld wordt maar die in de loop der jaren heeft bijgedragen aan het tot stand komen dit werk.

Ik dank ook mijn moeder die al die jaren is blijven vragen hoe het met mijn thesis was en die nooit op het juiste moment belde. Dank je mama om op jouw eigen manier in mij te geloven en nooit af te geven, klein maar dapper als je bent. Ik dank mijn vader om er te zijn op de juiste momenten. Ik weet je te vinden wanneer ik je nodig heb. Ook op Stéphanie, mijn zus, en de hele warme familie rond mij kon ik rekenen.

Liene en Mattijs, het boek van papa is af. Jullie hebben lang moeten wachten. Bedankt voor jullie geduld. Wat ze ook beweren, ik wilde eigenlijk niets missen en zou niets meer willen missen, want "*Childhood is short and maturity is forever"! (Bill Watterson)*

Dank je liefste An, om met mij naar dit moment toe te leven. Wat jij voor mij gedaan hebt is te veel om op te noemen, wat jij voor mij betekent is onbeschrijflijk. Voor jou en mij is het nu tijd om ons leven samen verder te zetten, langs het ingeslagen pad…

We took the one less traveled by,

And that might make all the difference

Twee maal zeven woorden naar Robert Frost

“It's the questions we can't answer that teach us the most.

They teach us how to think.

If you give a man an answer, all he gains is a little fact.

But give him a question and he'll look for his own answers.”

From The Wise Man’s Fear by Patrick Rothfuss