NUTRIENT EFFECTS IN INFLAMMATORY BOWEL DISEASE

NIKOLAOS KAMPERIDIS

THESIS SUBMITED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF MEDICINE MD(Res)

QUEEN MARY, UNIVERSITY OF LONDON

Statement of originality:

I, Nikolaos Kamperidis, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

Details of collaboration:

Data presented in chapter II were collected by Nikolaos Kamperidis and Dr Arati Rao, as stated at the relevant methods section. Dr Rao, being a paediatrician, has also collected growth data (which does not form part of this thesis); while the work presented in this thesis has focussed on duration of remission, and factors associated with it, as Dr Kamperidis is an adult gastroenterologist.

The intestinal epithelial cells that were isolated from patients and which I then used experimentally used for the work presented in chapter III were a kind donation of Dr Anna Vossenkamper and Dr Neil McCarthy, post-doctoral fellows of Queen Mary University.

The data presented in chapter IV have been collected by Nikolaos Kamperidis.

The data presented in chapter V have been collected by Nikolaos Kamperidis, James Goodhand, Mahmood Wahed and Neerav Joshi (clinical research fellows of Queen Mary University). This work has been supported by Dr James Oliver Lindsay and Professor David S Rampton. Data were analysed by Nikolaos Kamperidis and the relevant publication manuscript was prepared by Nikolaos Kamperidis and James Goodhand. This work is not presented in any other thesis

Publications:

1. Response to enteral nutrition predicts increased length of remission in children with Crohn's disease. Rao A, **Kamperidis N**, Koodun Y, Naik S, Croft N.M, Sanderson I.R. Gut, 2012; 61(Suppl:2): A239-A239. (Rao A and Kamperidis N, contributed equally and should be considered as joint first authors)
2. The phenotype and course of inflammatory bowel disease in UK patients of Bangladeshi descent. Goodhand JR, **Kamperidis N**, Joshi NM, Wahed M, Koodun Y, Cantor EJ, Croft NM, Langmead FL, Lindsay JO, Rampton DS. Alimentary Pharmacology Therapeutics, 2012 Apr;35(8):929-40. PMID: 22404452

Abstract

Background: Not only does IBD lead to nutritional deficiencies, but also nutrients influence its pathophysiology: exclusive enteral nutrition (EEN) is an effective primary treatment in Crohn's disease; and vitamin D (VitD) is involved in its pathogenesis and course.

Aims: We hypothesised that nutrients impact on the course of IBD. We therefore studied the effect of EEN i) on long term clinical course in children; ii) on CD58, a costimulatory molecule at the intestinal epithelial cell (IEC) lines, iii) adults with Crohn's disease. We examined the possible effect of serum vitamin D levels on the course of IBD and also the possible role of ethnicity in our paediatric and adult populations that were treated with EEN but also in our general adult population.

Results

Chapter II: 56 paediatric patients with Crohn's disesase were followed up for 5 years. 57% of patients achieved remission after 6 weeks of EEN. Achievement of clinical remission within 6 weeks of EEN was significantly associated with a longer time to relapse and to treatment escalation. VitD deficiency was common; and those patients who were deficient were significantly more likely to require corticosteroids and also needed thiopurines sooner.

Chapter III: CD58 was expressed in the IEC isolated from IBD patients and healthy controls. EN down-regulated the expression of CD58 on IEC lines.

Chapter IV: 22 adult patients with Crohn's disease with a mean age of 30.8 years were given EEN and followed up for a mean time of 1.9 years. 22.7% of patients went into

clinical remission and 77.3% experienced a clinical response. By the end of follow up 63.6% (14/22) of patients had clinically relapsed and 36.4% required surgery during their follow up. There was no difference between South Asian and Caucasian patients in the disease outcomes after administration of EEN.

Chapter V: Bangladeshis were more often vitamin D deficient than white Caucasian patients; however vitamin D status was not associated with the course of IBD. Bangladeshis developed perianal disease and required thiopurines earlier in their disease course. Bangladeshi patients with UC had more extensive disease.

Conclusions: EEN, when successful, improves the long term outcome of Crohn's disease in children, possibly in part, by down-regulating CD58 on the IEC. VitD deficiency may influence the clinical course of IBD; however our results were contradictory between children and adults and significantly limited by the assessment of the vitamin D level at a single time point.

Acknowledgements

I would like to thank my supervisor, Professor Ian Rutherford Sanderson for his guidance, supervision and financial support. Professor Sanderson has been a true inspiration to me, as he introduced me in the field of research during my MSc in gastroenterology in 2005 – 2006 and has been very enthusiastic in supporting me in the completion of the current work. He has always been a pleasure to work and collaborate with and a great example of an excellent supervisor. I am also grateful to Dr James Oliver Lindsay and Professor David Rampton for their clinical and research guidance throughout my work. I would like to thank Dr Nick Croft, Reader of Paediatric Gastroenterology, who is the director of the Digestive Diseases tissue bank. Samples taken for research during clinically-indicated procedures are taken with consent using the ethics committee approval P/01/23.

My gratitude extends to Professor Graham Foster who has funded me during my research. I am grateful to Dr James Goodhand and Dr Mahmood Wahed, Clinical research fellows for their active support and collaboration in completing my work. I would like to thank Anna Vossenkamper and Neil McCarthy, post-doctoral fellows for their help in providing me with intestinal epithelial cells that have been used for a part of my experimental work. I would like to say thank you to Nici Kingston and Abdul Monaf for their efficiency and support with administrative aspects of my work.

Finally I would like to thank my family for their encouragement throughout.

CONTENTS **PAGE**

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

5-ASAs	5 amino-salycilic- acid derivatives
AMP	Adenosine monophosphate
ATC	Artificial target cells
BMD	Bone mineral density
BMI	Body mass index
CD	Crohn's disease
CDAI	Crohn's disease activity index
CR	Complement receptor
CRP	C-reactive protein
CTLA	Cytotoxic T-lymphocyte-associated antigen
DC	Dendritic cell
DEXA	Dual energy X-ray absoptiometry
DSS	Dextran sodium sulphate
EN	Enteral nutrition
EEN	Exclusive enteral nutrition
ESR	Erythrocyte sedimentation rate
GVHD	Graft versus host disease
GWAS	Genome wide association study
Hb	Haemoglobin
HLA	Human lymphocyte antigen
HR	Hazard ratio

ICAM	Intercellular adhesion molecule
IEC	Intestinal epithelial cell
IBD	Inflammatory bowel disease
IFN	Interferon
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IkB	Inhibitor of kB
IL	Interleukin
IL1R2	Interleukin 1 receptor 2
IU	International units
JAK2	Janus Kinase 2
kDa	kilo Dalton
KO	Knock out
LPMC	Lamina propria mononuclear cells
LPS	Lipopolysacharide
MadCAM	Mucosal addressin cell adhesion molecule
MHC	Major histocompatibility complex
NFkB	Nuclear Factor kB
NOD	Nucleotide-binding oligomerization domain
NOGG	National osteoporosis guidelines group
OR	Odds ratio
PBMC	Peripheral blood mononuclear cell
PCDAI	Paediatric Crohn's disease activity index

PMA	Phorbol myristate acetate
SD	Standard deviation
SEM	Standard error of the mean
SLE	Systematic lupus erythematous
SNP	Single nucleotide polymorphism
TCR	T cell receptor
TGF	Transforming growth factor
Th	T helper cells
TLR	Toll like receptor
TNBS	Trinitrobenzene Sulfonic Acid
TNF	Tumor necrosis factor
Tregs	T regulatory cells
UC	Ulcerative colitis
UK	United Kingdom
USA	United states of America
VDR	Vitamin D receptor
VitD	Vitamin D
WHO	World health organization

CHAPTER I: INTRODUCTION

1.1 Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) represent the two main forms of inflammatory bowel disease. [1] UC was first described in the mid 19th century and CD, initially in 1913 by Kennedy Dalziel (Dalziel's disease) [2] and subsequently in 1932 as regional enteritis. [3] The incidence of IBD varies over different populations and has changed over time. In the past 50 years, the incidence of UC initially increased and then stabilized, while the incidence of CD continued to increase, mainly driven by CD in younger people. [4] The incidence and prevalence of IBD is highest in North America, Western Europe and UK (figure 1.1). [5] The prevalence of CD in North America is estimated between 26 and 198.5 cases per 100,000 of population, with an incidence rate of 3.1 to 14.6 new cases per 100,000 of population per year. 400 - 600,000 patients are affected. [6] In UK, the prevalence of CD has been reported about 157/100,000 with an incidence rate of 9.56 new cases per 100,000 population per year. [7] The prevalence of UC is 7.6 – 246/100,000 with an incidence rate of 1.2 – 20.3 new cases per 100,000 of population per year, with the wide range possibly implying different exposure to genetic and environmental risk factors. [3] The incidence of IBD is higher among countries with a western lifestyle; for example in Australia and New Zealand the reported incidence and prevalence rates of IBD are comparable to those of western Europe and North America. A recent study in Victoria in Australia, calculated and incidence rate of 29.3 new cases per 100,000 of population per year for the period 2007-2008, one of the highest reported in literature. [8] In Canterbury in New Zealand the estimated prevalence of IBD is about 332/100,000 , again one of the highest ever reported, with cases being more prevalent among Causians, of higher socioeconomic status living in a city. [9]

Similarly, in Europe, the incidence of IBD in Western European countries has been estimated to be twice that of the less wealthy Eastern European countries. [10] However, the diference in incidence between Eastern and Western European countries seems to decline: A recent Epicom study reports an incidence rate of 14/100,000 in Western Europe compared to 11.3/100,000 in Eastern Europe. [11] In fact, there is a worldwide trend towards an increase in the incidence and prevalence of IBD. It has been increasingly reported in countries where used to be rare, such as South Korea, China, India, Iran, North Africa, Thailand and French West Indies. [5] A systematic meta-analysis of the epidemiology of CD in China, reported that after 1980, it is increasingly prevalent in urban areas of the country, estimating a prevalence of 30.000-40,000 patients , which is probably an underestimate given the geographical and demographic complexity of China. [12] Similarly, a sharp rise in the incidence and prevalence of IBD has been reported in other Asian countries such as Japan, Hong Kong and South Korea. [4]

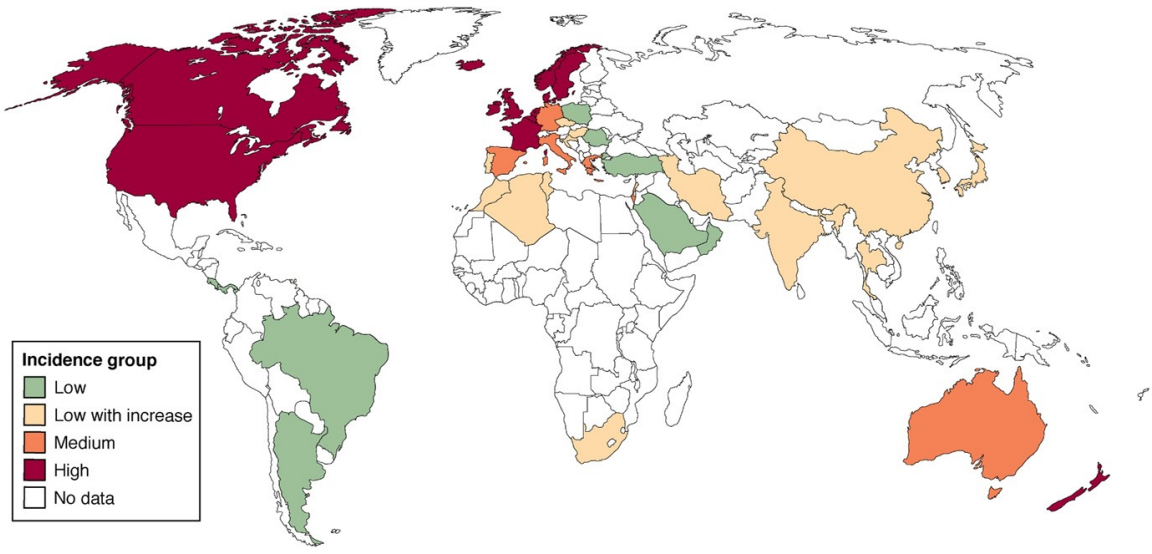


Figure 1.1. : The global map of IBD incidence. Taken from Cosnes et al, Gastroenterology 2011: 140: 1785 - 1794.

Crohn's disease is usually firstly diagnosed among adult patients aged 20-30 years and UC among patients aged 30-40 years. In the paediatric population, which represents about 7-20% of IBD patients, [5] IBD is diagnosed at 11.9 years on average [13] and the incidence of UC has remained stable over the years, while that of Crohn's disease has been steadily increasing. [5] In Scotland the incidence of paediatric Crohn's disease has increased from 2.86/100,000 per year in 1990 – 1995 to 4.75/100,000 per year in 2003 – 2008. [14] In California, the annual incidence of Crohn's disease increased between 1996 and 2006 from 2.2 to 4.3 and that of UC from 1.8 to 4.9 new cases per 100,000 population. [15] In France the reported incidence of paediatric Crohn's disease increased between 1990 and 2007 by 71% from 6.5 to 11.1 new cases every year per 100,000 persons. [16] Similarly, in Finland the mean annual incidence of IBD among the paediatric population almost doubled, increasing from 3.9 in 1987 to 7.0/100,000 in 2003. [17]

The rising trend in the incidence of IBD in populations of uniform and relatively steady genetic background over the years, and the increasing incidence in populations where IBD has been traditionally rare, is likely to reflect the influence of environmental factors rather than fundamental genetic alterations. This is strongly supported by studies in immigrant populations. In a population with traditionally high rates of abdominal tuberculosis and low incidence of IBD, the East London Bagladeshi community, IBD rates have been reported to rise over the course of time. [18] In a recent study from Spain, emigration was an independent risk factor for IBD, and emigration to European industrialized countries would be more strongly associated with a diagnosis of IBD compared to emigration to Asia or Latin America. [19]

1.2 The natural history of IBD

1.2.1. The natural history of Crohn's disease

Crohn's disease is characterized by transmural inflammation that can affect any point of the gastrointestinal tract from mouth to anus and can lead to strictures, fibrosis or penetrative complications. The primary lesion of Crohn's disease, in the macroscopic / endoscopic level is the apthous ulcer and the evolution of this lesion is best described by studies of the post-operative recurrence of Crohn's disease. A landmark study, following up 89 patients who underwent ileal resection for isolated ileal Crohn's disease, proved that endoscopic lesions were present in 73% of patients 1 year after surgery and 85% of patients 3 years after surgery with a clinical recurrence rate of 20% and 34% respectively. [20] Similar results were obtained by others, proving that although endoscopic recurrence occurs within a year in the majority of patients, clinical symptoms take about 3 years to recur. [21]

Crohn's disease patients are best described by the Montreal classification according to their age of diagnosis, the disease location, the disease behaviour, and the presence of upper GI and perianal disease. [22] Recently this has been modified to improve the description and classification of paediatric Crohn's disease according to the Paris modification. [23] However as most of the work presented in this thesis was undertaken prior to the Paris modification, Crohn's disease will be classified according to the Montreal Classification.

The disease location remains relatively stable over time, but still about 16% of patients will progress to a more extensive phenotype in the 10 years after diagnosis. 20% of

patients with predominantly ileal disease and 17% of patients with colonic disease had extension of location involvement with median time to extension 5.5 and 7.5 years respectively. 4.8% of patients with ileocolonic disease presented upper GI involvement in a median of 8.3 years. Perianal fistulas occur in about 43% of patients. [24] The median time from diagnosis to first perianal fistula presentation is about 4.8 years. Although the majority of patients with perianal fistulation will have only one fistulizing episode, 33% of the will have a second or a third episode during the course of their disease. [25]

Crohn's disease behaviour, changes and progresses during the course of time, as most patients will develop intestinal complications (figure 1.2). In a retrospective study from Minessota including 306 patients diagnosed with Crohn's between 1970 and 2004, with a median follow up of 8.6 years, 81.4% of patients had inflammatory disease without stricturing or penetrative complications at baseline. The cumulative incidence of a stricturing or penetrative complication was 4.1% at 1 year, 18.5% at 5 years, 24.7% at 10 years, 39.5% at 20 years and 43.9% at 30 years. 81.6% of patients with a progression in behavior of their disease would require surgery within the 6 months after the identification of the complication. Ileal involvement and 5-ASA utilization at diagnosis were associated with higher risk of complications in the follow up period. [26] Another study including 297 patients with Crohn's disease confirmed the dynamics of behavioral evolution: 29.5% of patients initially diagnosed with inflammatory disease developed stricturing or penetration at 5 years after the diagnosis, 43% developed complications between 5 and 10 years from diagnosis and 33.3% between 20 and 25 years from diagnosis, proving that the change in behaviour can occur even decades after the diagnosis. The proportion of patients changing from an inflammatory to a stricturing

behavior was 27.1% with a median time to change 5.5 years and the proportion of change from an inflammatory to a penetrative phenotype was 29.4% with a median time to change of 6 years. New strictures or penetrative complications developed in 33% of patients after surgery. [27]

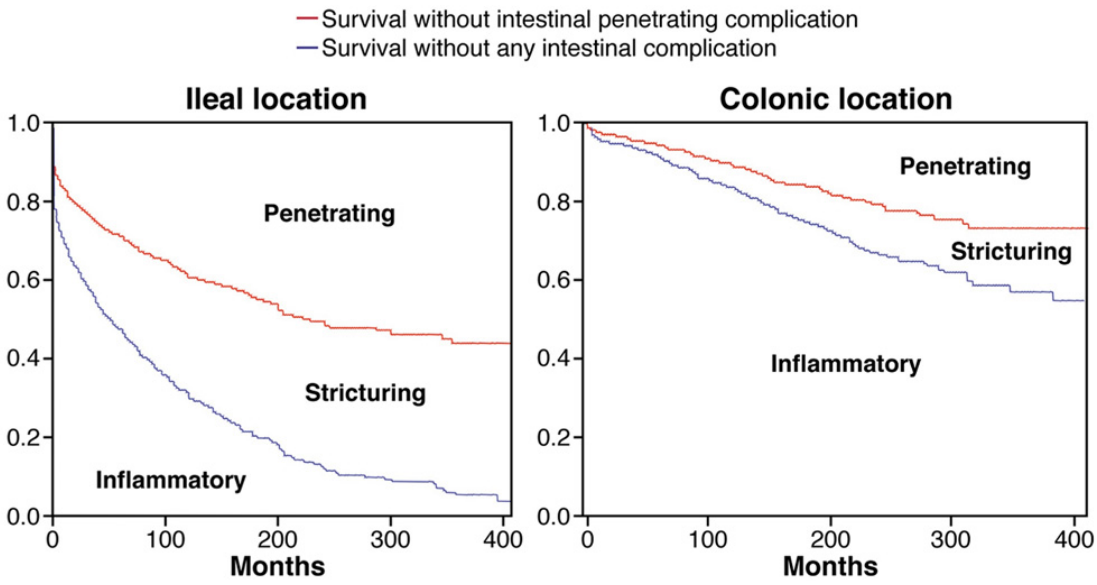


Figure 1.2.: The natural history of Crohn's disease phenotype. Taken from Cosnes et al, Gastroenterology 2011: 140: 1785 – 1794. Almost all Crohn's disease patients with ileal involvement developed stricturing or penetrating complications during their disease course. Only 40-50% of patients diagnosed with colonic Crohn's disease developed stricturing or penetrating complications during their disease course.

The natural history of Crohn's disease is more aggressive among patients being diagnosed during their childhood: A landmark study, presenting the follow up of 276 patients with CD diagnosed during their childhood and 596 adult onset CD patients in Scotland, showed that childhood onset CD is associated with panenteric involvement. Furthermore, disease extent progressed over time more rapidly among these patients with more than 30% of them presenting expansion of their disease location in 2 years. The rate of stricturing and penetrating complications over time was similar between adult and childhood onset Crohn's disease. Although the time to surgery was longer among paediatric onset CD patients, this might reflect the more extensive disease phenotype in this group, which makes surgery a less attractive option compared to medical treatement. (Figure 1.3) [28]

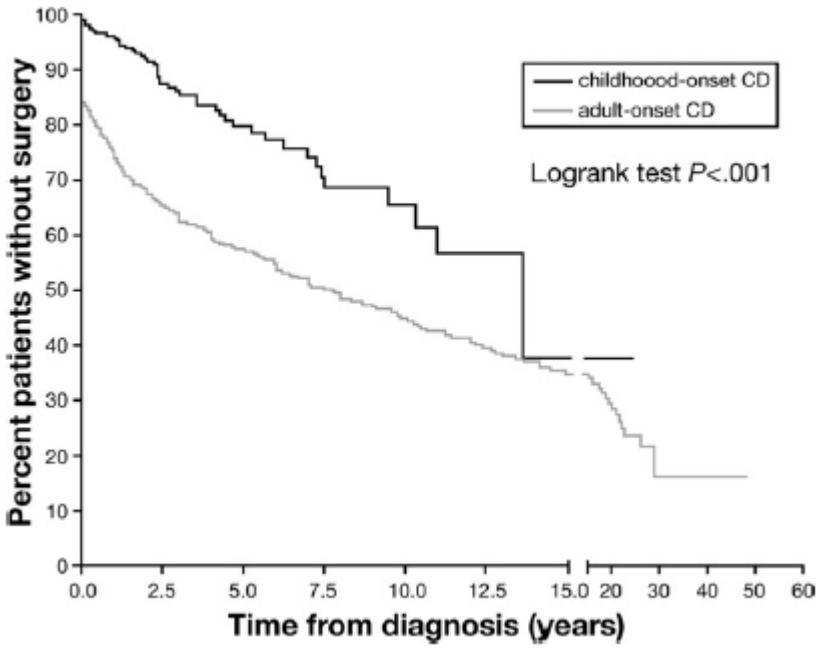


Figure 1.3.: Time to surgery in childhood and adult onset CD. (Van Limbergen et al, Gastroenterology, 2008. 135(4): p. 1114-22 [28]) Patients with childhood onset CD took longer to require surgery compared to patients with adult onset Crohn's disease.

Crohn's disease runs in a periodic remission – exacerbation pattern for most patients. However, there have been identified patients with very benign disease course and a minority of 10-15% of patients who will have chronic active and progressive disease. The relapse rate in the first 3 years after the diagnosis correlates with the relapse rates of the 5 subsequent years, perhaps underlining the importance of early aggressive management and achievement of remission [29] in clinical and endoscopic level. The achievement of mucosal healing 1 year post diagnosis is associated with reduced requirement for corticosteroid treatment and reduced need for surgery in the 5 years post initial follow up. [30] The patterns of disease course for Crohn's disease have been very elegantly elucidated by a Norwegian prospective follow up study of 237 new Crohn's disease patients followed up for 10 years. 90% of patients had at least one episode of relapse and 38% of them required surgery especially those diagnosed before the age of 40, those with ileal involvement or complicated behavior phenotype at diagnosis. Overall, 4 patterns were identified: i) patients with decrease in symptomatic severity over follow up, ii) patients with increase in the severity of symptoms over follow up, iii) patients with continuous symptoms of active disease, iv) patients with chronic relapsing bowel symptoms during the course of disease. [31] These patterns are graphically shown in figure 1.4.

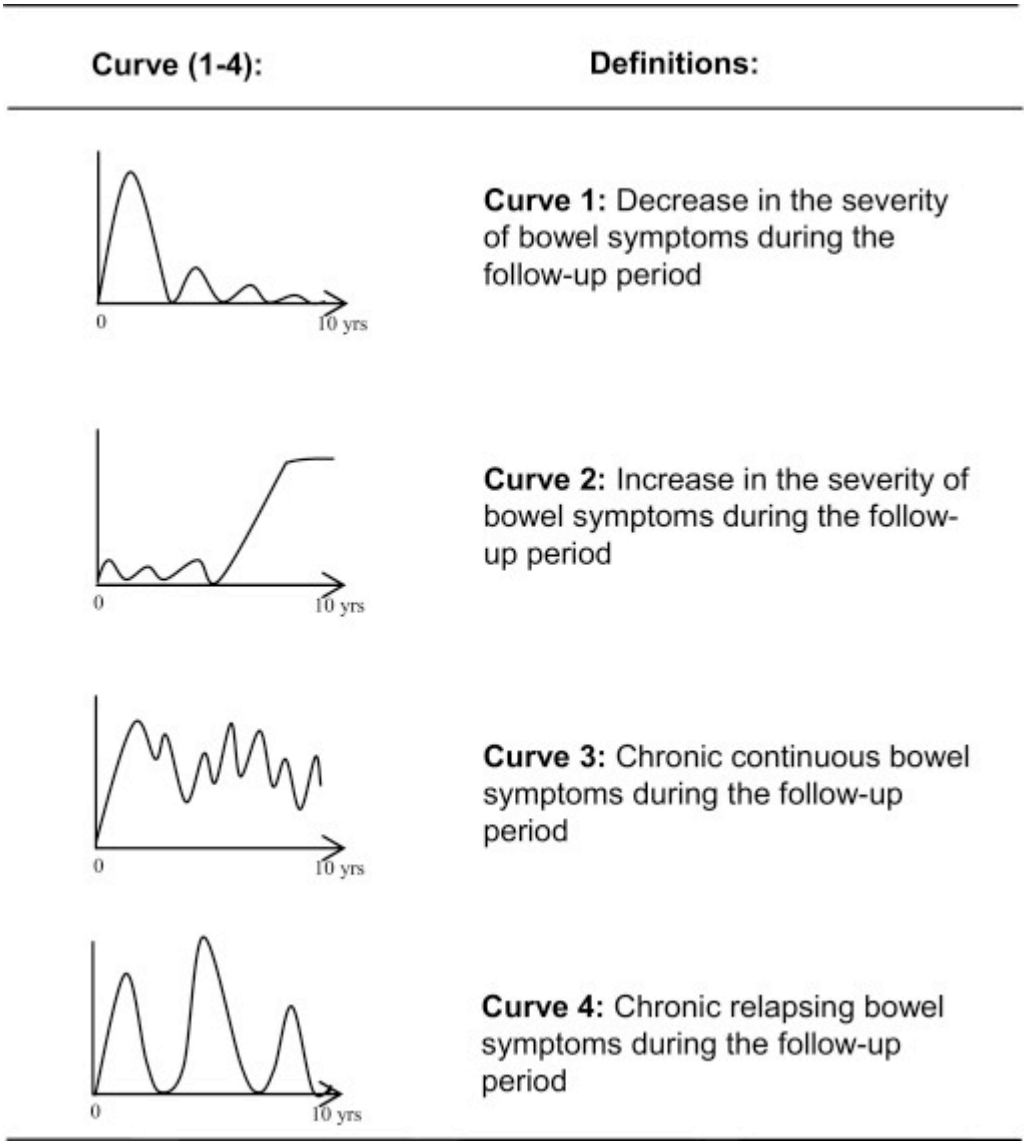


Figure 1.4.: Four predefined curves reflecting different patterns of CD activity from diagnosis to 10-year follow-up. Taken from Solberg et al, Clin Gastroenterol Hepatol, 2007. 5(12): p. 1430-8 [31]

Chronic active inflammation may lead to treatment failure or intestinal complications that can not be managed solely by medical treatment. 5-15% of patients require surgery within the first 3 months of diagnosis and about 3.5% of patients per year require surgery for the management of strictures, perforation and active inflammation. In this cohort with a follow up of 25 years, a third of patients were operated on at least once, with patients having at least ileal disease and absence of rectal involvement being at greatest risk. The cumulative probability for surgery was not affected by the increasing use of immunosuppresants. [32] Similar results were obtained by others. [31, 33]

Recurrent need for surgery can lead to short bowel syndrome and intestinal failure. Young age at first surgery and the presence of stricturing or penetrative disease have been shown to be independently associated with intestinal failure [34] pointing the need to maximize the benefits of the available treatments early in the disease course.

1.2.2 The natural history of ulcerative colitis

Ulcerative colitis involves the rectum and spreads in a retrograde fashion to the rest of the colon, however for some patients there may be rectal sparing and/or backwash ileitis. In a prospective evaluation of the disease extension involving 408 patients, at diagnosis 32% had proctitis, 33.5% had left sided disease and 34.5% had extensive disease. [35] In follow up colonoscopic investigation (median time 14 months), there was disease expansion in 14% of patients, extent regression in 22% and normal findings in 30% of patients. 22% of patients with initially diagnosed proctitis, 28% of patients with initially diagnosed left sided colitis or rectosigmoiditis presented expansion of the distribution of active inflammation in their follow up colonoscopy. [35]

The clinical course of ulcerative colitis is characterized by periods of remission and periods of symptomatic exacerbation. In a study from Denmark, 23% of the 1161 patients diagnosed with UC in Copenhagen between 1962 and 1987 had one episode of active disease during the observation period (median 3 years) and remained inactive thereafter, and 77% of patients presented a continuous relapsing course after the initial episode. [36] However the cumulative probability of a relapse free course decreased significantly over the course of time, reaching 18.4% after 5 years and 10% after 25 years. After the first 2 years from diagnosis about 40% of patients will be in remission every year and this percentage increases to 50% at 20 years after diagnosis. [36] This improvement might be due to the disease becoming more inactive over the course of time, or simply reflect the number of patients who underwent "curative" colectomy (20% within the first 10 years and 30% within 25 years). Excluding patients who underwent a colectomy, a minority of 5-10% presented chronic active inflammation and 40% of patients presented intermittent disease activity. [36]

The course of UC can be more aggressive in children; In a study including 99 patients with childhood onset UC and 701 patients with adult onset UC, more children than adults had extensive disease at diagnosis (82% vs 48%) and this reflected on the time to colectomy which was significantly shortened among the childhood onset UC patients. (figure 1.5) [28] A retrospective longitudinal cohort study study shows that 28% of children with UC need to be hospitalized with acute severe colitis, 53% of them manage to respond to intravenous corticosteroids but 42% required colectomy at the same hospitalization. [37]

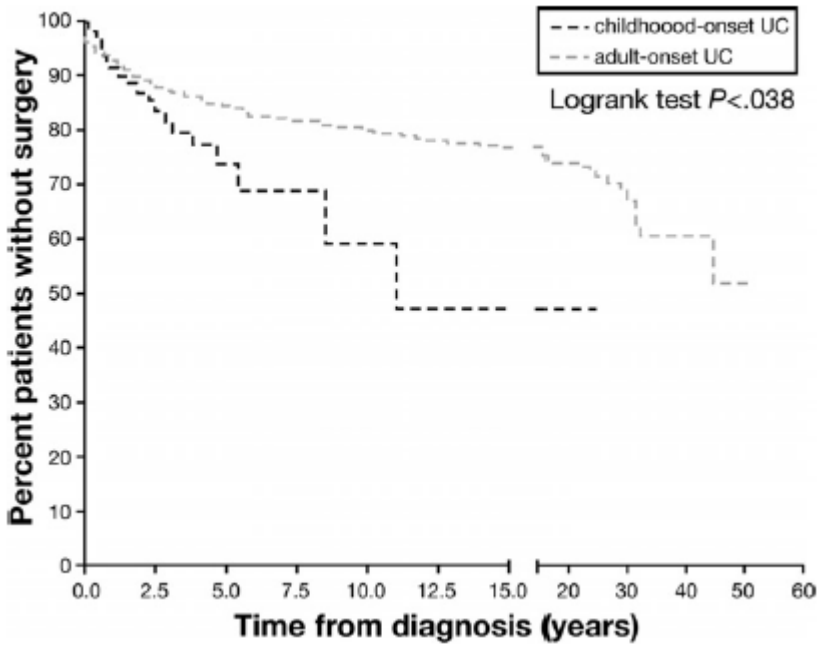


Figure 1.5.: Time from diagnosis to surgery in childhood and adult onset UC. Taken from Van Limbergen et al, Gastroenterology, 2008. 135(4): p. 1114-22. [28]

Patients diagnosed with UC during their childhood required sooner and more often surgical treatment.

The rate of mortality for both patients with Crohn's and UC is not different to that of the general population, [5] however the impact of IBD on patient's quality of life can be detrimental.

1.2.3 Paediatric and adult onset Inflammatory Bowel Disease

It has been suggested that paediatric onset IBD may represent a different form of the disease and that it may also have a distinct aetiology. [38]. Given the increased prevalence of family history among those who are diagnosed with IBD early in life, the impact of environmental modifiers could be less prominent; and therefore IBD in this group may represent a more "pure" genetically derived form of disease with different phenotypic characteristics and natural history. [39] Two big studies have attempted to provide data on the natural history of IBD in the paediatric population and to compare it with the adults: one from Scotland [28] and one from France. [38] Both studies agree, that childhood onset Crohn's disease is associated with more extensive, pan-enteric phenotype and less commonly isolated ileal or colonic disease. [28, 38] 50-70% of children had ileocolonic disease on diagnosis according to these two studies with 27% of children presenting with maximum disease extent according to their Montreal Classification (L3 + L4). [28, 38] The incidence of pan-entreric involvement was significantly higher among patients with childhood onset compared to those with adult onset Crohn's disease, among whom the incidence of this phenotype on diagnosis is only 3.2%.

Disease progression was frequent among paediatric onset Crohn's disease patients: 4 years after diagnosis the incidence of stricturing and penetrative disease increased from 4.4% to 12.9% and 11.4% respectively. [28] 60-90% of patients with paediatric onset CD required treatment with immunomodulators by the end of follow up. [28, 38] Surgery was more often required among patients with adult onset, compared to paediatric onset

Crohn's disease (53% vs 17% respectively) and the time to first resection was 7.8 and 13.7 years respectively. [28]

Among patients with paediatric onset UC, 75% had extensive (E3) disease at diagnosis according to their Montreal Classification and from the remaining 25%, almost half patients had disease progression during follow up. [28] 10 years after diagnosis 40% of patients with childhood onset UC had undergone a colectomy compared to 20% of adult onset UC patients. [28]

Within the paediatric population, a distinct phenotype has been described as well: those with very early onset disease (before their 8th birthday). [38] These children develop IBD with more frequent colonic involvement and less frequent ileal or panenteric involvement. [38, 40] Also, Crohn's disease limited to the mouth or perianal area was more common in this sub-group of patients (13%) compared to patients diagnosed during their childhood, but after their 8th birthday (2.7%). [28]

The above described phenotypic differences between paediatric and adult onset IBD have led to the hypothesis that the two phenotypes may stem from different genetic backgrounds. Recently, a GWAS study, including 3,426 patients and 11,963 controls from Europe and North America, identified five genomic regions that are associated with early onset IBD, one of which is relevant to the regulation of IL-27. [41] This was also confirmed at tissue level, were IL-27 mRNA was expressed at significantly lower levels in early onset CD and UC mucosa compared to normal controls. [41] A recent study using high density genotyping to evaluate genetic differences between paediatric and adult onset IBD suggested that the allelic architecture of common susceptibility variants for paediatric onset IBD is similar to the one of adult onset IBD. [42] However, this study

did not focus on very early onset IBD. This is important because, while it is likely that paediatric and adult IBD are similar within their disease types (Crohn's disease and ulcerative colitis), it has been known for over 25 years that IBD presenting in infancy is a distinct condition. [40]

Recenly, the composition of faecal microbiota among IBD patients has been the focus of a number or studies attempting to explore the pathophysiology and course of IBD. [43] Among adult patients with Crohn's disease, the levels of Faecalibacterium Prausnitzii which has been shown to produce an anti-inflammatory 15 KDa protein [44] have been proved to be reduced in the intestinal mucosa. [45] Adult patients who undergo surgery are more likely to develop post surgical de novo inflammation if the proportion of Faecalibacterium Prausnitzii in the microbiota of the resected mucosa is low. [45] Interestingly, a Scottish study of the intestinal microbiota including 22 children with newly diagnosed IBD who were treatment naive and 12 controls observed an increase in Faecalibacterium Prausnitzii among children with Crohn's disease compared to controls, challenging the outcomes of studies in adults and possibly suggesting that the microbiota of paediatric onset Crohn's disease differs to the one of adult onset Crohn's disease. [43]

The above findings suggest that childhood onset IBD may have different phenotypic characteristics and natural history and distinct genetic background. However, this notion is not universally accepted, except in very early onset disease, which was always thought to be a distinct entity from Crohn's disease and UC of adulthood. [40]

1.3. Impact of IBD on the quality of life

The symptomatology, hospitalizations, treatment and outpatient visits related to a diagnosis of IBD have a significant impact on patients' quality of life. The study that developed the health related quality of life questionnaire, which is now widely used in clinical and research practice, included 150 patients and identified that IBD patients experience a moderate degree of functional impairment, mostly in the social and psychological rather than physical level. [46] Concerns about surgery, fatigue and body image issues were associated with social and psychological dysfunction, which are more prevalent among Crohn's disease rather than UC patients. [46] In a recent study comparing quality of life between IBD and IBS patients, the psychological distress had a more significant impact on quality of life than the degree of gastrointestinal symptoms, and both were more severe in IBD than IBS patients. [47] Factors associated with poorer quality of life included the disease course, as defined by number of disease relapses, anxiety, level of social support and female gender. [48] Similar results were obtained in studies of the paediatric population; similar to the adult studies disease activity was associated with poorer quality of life scores. [49] Although a diagnosis of IBD has not been shown to be associated with poorer education and professional function – although absenteeism has been reported - [50] adolescents with IBD had significant impairment in their physical and psychosocial functioning. [51]

The detrimental impact of IBD on patients' lives is largely reversed by the successful management of IBD. Initiation on thiopurines on the correct clinical grounds was associated with a significant and long lasting positive effect on quality of life scores among IBD patients. [52] Similarly induction of clinical remission with infliximab

restored the quality of life of the majority of patients. [53] Achievement of clinical and endoscopic remission by any medical means has been associated with restoration of quality of life in IBD patients. [54] Therefore, successful treatment enhanced quality of life on validated assessment. Furtermore, the comprehension and elucidation of the pathophysiological mechanisms the lead to IBD is very important in order to optimize our current therapeutic tools and develop new treatments for the management of IBD.

1.4. The pathogenesis of IBD

The pathophysiology of IBD is largely unknown, however several aspects of it have been described. The clinical description of familial tendencies in IBD has led to the exploration of significant genetic aspects of its pathogenesis. A German study recruiting 189 twin pairs in which at least one member had IBD confirmed a 35% and 16% concordance rate for Crohn's disease and ulcerative colitis respectively in monozygotic pairs. [55] In dizigotic pairs, the concordance rate fell to 3% and 2% for Crohn's disease and UC respectively. [55] In a British study of 250 twin pairs, the concordance rate was 11% for any IBD. [56] In both UC and Crohn's disease monozygoric concordant twins, there was good concordance for the Montreal classification of the disease and the need for medical treatments. This suggests that disease phenotype and course are to a certain extent genetically regulated. [56] In a study of the concordance rate following up 421 patients with UC and 197 with Crohn's disease for 10 years described a 26 and 9 fold risk for Crohn's disease among siblings and parents respectively. The same study confirmed a 8.6 and 1.5 fold risk for UC among siblings and parents respectively. [57]

The role of genetics in the pathogenesis of IBD has been further supported by genome wide association studies (GWAS); In a recent meta- analysis of 15 GWAS studies, 163 genetic regions were identified to be related to the risk of IBD. [58] Of these 163 regions, 110 were common for Crohn's disease and UC, while from the remaining 53 loci, 30 were associated with Crohn's disease and 23 with UC. The loci implicated imply an interaction between the immune system and microbes both at the intestinal mucosal level and in the gut lumen [58] Another meta analysis of 6 GWAS including a total of 6333 cases and 15056 controls confirmed the identification of 71 loci associated with Crohn's disease, most of them being associated with loci relevant to the inate immune response. [59] One of the best described mutations that is associated with risk of developing Crohn's disease and with the risk of more aggressive disease phenotype is NOD2. NOD2 is involved with the recognition of muramyl dipeptide, a component of bacterial peptidoglycan and leads to NF-kB activation, priming of pro-inflammatory cytokine transcription and T cell activation. It is located on chromosome 16 and although more than 30 single nucleotide polymorphisms have been identified in association with Crohn's disease, 3 of these are most commonly involved. [60]

Similarly, in ulcerative colitis, a meta-analysis of 6 GWAS including 6687 cases and 19718 controls describes 99 loci associated with the pathogenesis of the disease. Again these were in genomic regions associated with the regulation of the inate immune response and T cell activation, such as IL1R2, IL8 and JAK2.

The genomically described affected pathways mostly lead to immunological dysfunction and inappropriate T cell activation. Luminal antigen sensing starts at the intestinal epithelium, where this interaction occurs through cell surface or intracellular structures

called pattern recognition molecules, such as Toll like receptors (TLR) and NODs, [61] and it is now widely accepted that IBD represents an inappropriate and dysregulated immunological response to luminal antigens.

A key event in the pahogenesis of IBD is the breakdown of the intestinal epithelial barrier, which gives direct access for the luminal antigens to the mucosal immune cells and leads to T cell activation through innate and adaptive immune processes. [1] Epithelial tight junctions, a key component of the epithelial barrier are less resistant and more permeable among Crohn's disease patients. [61] Apart from a leaky epithelium, the expression of TLRs is different in IBD patients compared to controls; TLR4, which is stimulated by lipopolysacharide, is minimally expressed in colonic and small bowel biopsies of healthy controls but significantly upregulated among IBD patients. [62] TLR3 which is constitutively expressed in normal mucosa is significantly down-regulated in Crohn's disease patients. [62] TLR5, which is expressed on the basolateral surface of the colonic epithelium, becomes accessible to luminal antigens after the primary epithelial injury and its activation through bacterial flagellin leads to IL-6 and IL-8 production. [63] These data clearly suggest a defect in the innate immune processes of the mucosal immune system that lead to NF-kB activation and attraction of effector T cells (figure 1.6).

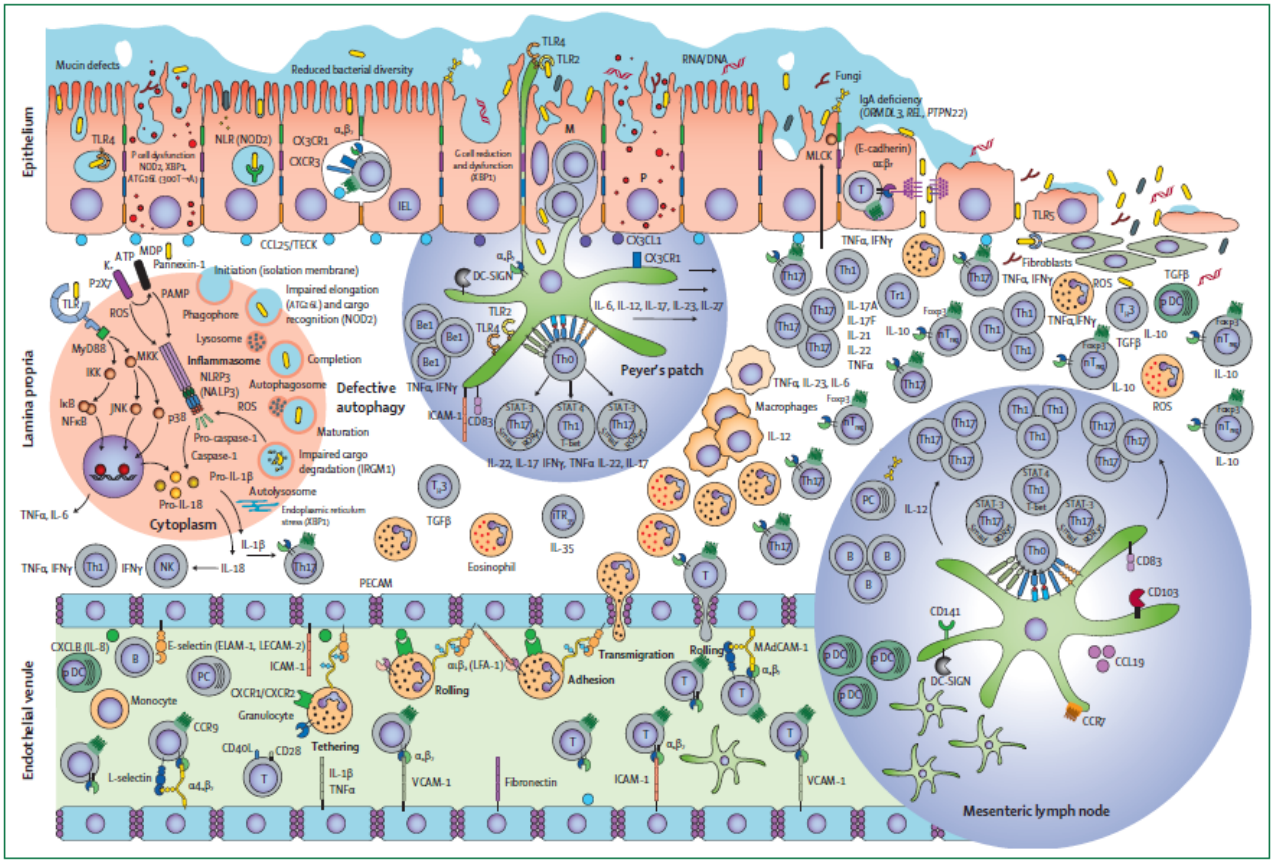


Figure 1.6. : The mucosal immune system in the pathogenesis of Crohn's disease

Taken from Baumgart et al, Lancet 2012, 380: 1593 [1]

In the lamina propria, the dendritic cells, a subset of antigen presenting cells that express TLRs and NODs control the immunological response as they distinguish between commensals and pathogens. Dendritic cells present antigens to T cells and can lead to T cell activation, however the same cells educate the immune system at the very early stages of development on the tolerance towards self antigens (figure 1.6). [64] IBD patients have been shown to have significantly reduced numbers of immature and possibly tolerogenic dendritic cells during periods of flare up. [65] They also have high numbers of plasmocytoid dendritic cells both in the lamina propria and the mesenteric

lymph nodes, express higher levels of co-stimulatory molecules (CD86 and CD40), secrete higher concentrations of pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α than in controls [66] and express higher levels of TLR-2 and TLR-4. [67] Hence IBD patients have a distinct phenotype of dendritic cells populating their peripheral blood and mucosal immune system.

This particular phenotype of innate immunity in IBD shifts the adaptive immune response towards a pro-inflammatory behaviour. Migration of naïve T cells to the secondary lymphoid tissues occurs through the high endothelial venules. The rolling and homing of the lymphocytes is promoted by the L selectin and α4β7 integrin on the surface of T cells with MAdCAM on the high endothelial venules. When naïve CD4 T cells enter the mesenteric lymph nodes and Peyer patches they interact with antigens presented through MHC II molecules on the surface of dendritic cells. Binding of the T cell receptor to the MHC II – antigen complex leads to T cell activation and eventually proliferation, enhancement of gut homing adhesion molecules and differentiation towards a colitogenic phenotype. [68] Following this initial priming event, T cells re-enter the systemic circulation and subsequently home to the gut, where they encounter their specific antigen presented on a variety of antigen presenting cells (macrophages, B cells, dendritic cells). This leads to a more vigorous response of activated T cells with increased production of IL-17, IFN-γ, TNF-α and IL-2. IL-2 enhances the proliferation of T helper cells. Following T cell activation, IFN-γ, TNF-α and IL-17 further enhance endothelial cell adhesion molecules expression and macrophage derived proinflammatory cytokines. This results in an uncontrolled secretion of Th1, Th17 and macrophage derived proinflammatory mediators and chronic active inflammation. [68] IBD is characterized

by an imbalance in the lamina propria level between pro-inflammatory cytotoxic T cells such as Th1 and Th17 and the natural peacekeepers, the regulatory T cells that suppress inflammation through the production of IL-10, TGF-β and IL-35 (figure 1.7). [1] It has been long established that Crohn's disease is characterized by Th1 cell activation and production of IL-12, IFN-γ and TNF-α. UC on the other hand is associated with Th2 response and increased production of IL-4, IL-5 and IL-13 and this has been supported by studies in animal models of IBD. [69]

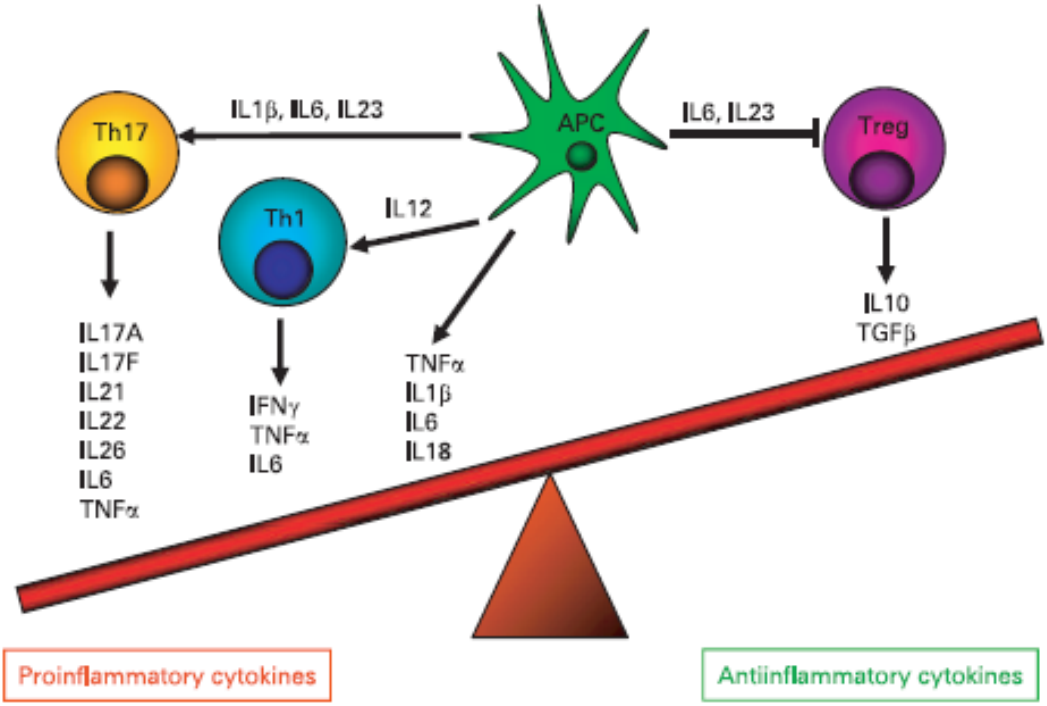


Figure 1.7: The imbalance of pro- and anti-inflammatory cytokines in Crohn's disease. Taken from Brand S, Gut, 2009, 58(8): p. 1152-67. [70].

Proinflammatory cytokines secreted by antigen presenting cells (APC) after they come in contact with antigens, promote the differentiation of pro-inflammatory T cell lineages (Th17 and Th1). The proinflammatory cytokines released by pro-inflammatory T cells, outweight the effect of anti-inflammatory cytokines released by regulatory T cells (Treg). [70]

1.5 CD58 (LFA – 3)

1.5.1 T cell activation

Because of the importance described above of the antigen presenting cells, it is clear that T cell activation is a central event in the pathogenesis of IBD. The main stimulus for T cell activation is the interaction between the T cell receptor (CD3) with the antigen which is held by the MHC complex on the surface of antigen presenting cells.[71] As the affinity of the T cell receptor for the antigens is low, a number of co-stimulatory molecules are involved in the activation process. [71] T cell receptor stimulation results in either clonal expansion or anergy, depending on whether co-stimulatory molecules are involved in the process (figure 1.8). [72] There are a number of molecules that may provide a co-stimulatory signal for T cell activation such as B7.1 (CD80), B7.2 (CD86), and CD58. The best characterized co-stimulatory molecules are those of the B7 family. They are members of the immunoglobulin gene superfamily and have 25% amino-acid identity. Their cytoplasmic domains are remarkably different with that of B7.2 being considerably longer and containing potential phosphorylation sites. The genes coding for B7.1 and B7.2 are located at 3q13.3-3q21. [73] B7.1 and B7.2 are expressed on the antigen presenting cells and ligate to CD28 or CTLA-4 on the surface of T cells. B7-CD28 co-stimulation in the presence of TCR signaling results in upregulation of IL-2 receptor, cytokine transcription and T cell proliferation. In contrast B7-CTLA4 co-stimulation down regulates T cell responses, [72] and CTLA-4 has a greater affinity for B7.1 and B7.2 than CD28. [74]

The role of B7 molecules on antigen presenting cells such as dendric cells, leucocytes and macrophages in the pathogenesis of IBD is vital. In patients with active IBD the number

of CD86 positive dendritic cells in the peripheral blood is significantly higher compared to healthy controls. [65] In DSS colitis it has been proven that the number of B7 positive leukocytes increase 20 fold compared to normal mice by day 7 of colitis. [75] Similar results were obtained from human studies. In both UC and Crohn's patients the population of B7.1 and B7.2 positive macrophages is markedly increased compared to controls in colonic mucosa biopsies. [76] Similar results, confirming the importance of co-stimulatory molecules have been reported by others. [77]

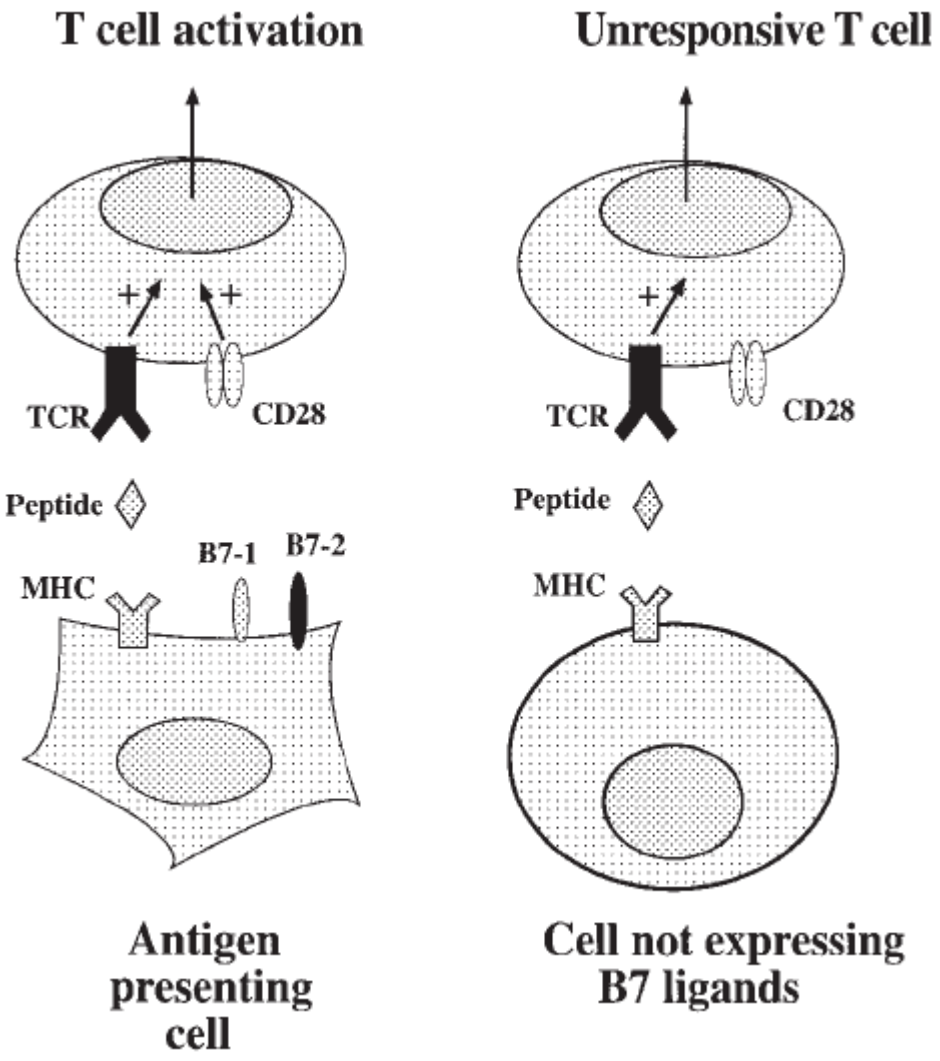


Figure 1.8: The two signal model of T cell activation. Taken from Harris et al, [72]

These data led to the exploration of therapeutic strategies blocking the B7-CD28 interaction. Abatacept, a recombinant fusion protein comprising a fragment of the Fc domain of human IgG1 and the extra-cellular domain of human CTLA-4, has proven clinical efficacy in the treatment of auto-immune disorders as rheumatoid arthritis and juvenile arthritis. [74] In animal studies using immunocompromised mice injected with CD4+CD45RBhigh T cells in order to induce colitis, abatacept treatment resulted in body weight preservation at 1 week, as opposed to untreated or cyclosporine treated mice that lost about 20% of their body weight by that time. Similarly, colonic length was preserved in the abatacept treated mice compared to controls. These clinical observations were in line with the understanding of the underlying mucosal immunology; Abatacept treatment significantly prevented the secretion of TNF-α, IFN-γ, IL-1 and IL-4 from the lamina propria lymphocytes in this model of IBD. [78] The application of these encouraging results was attempted in human IBD patients with moderate, or moderate to severe Crohn's disease and UC. 451 Crohn's disease and 490 UC patients were recruited and treated with 3 different doses of abatacept or placebo. In contrast to the results in studies using mice, abatacept did not prove effective in inducing clinical remission or achieving a clinical response in either Crohn's disease or UC. [74]

Although the results of this trial in IBD patients contrast to the results of the experiments in mouse models of IBD discussed above, they do not necessarily refute the targeting of co-stimulatory molecules as a treatment strategy for IBD. In fact, in CD28 deficient mice, T cells activate and proliferate with certain stimulants and these mice mount a normal cytotoxic T cell response in vivo when infected with the lymphocytic choriomeningitis virus. [79] These facts imply that in the complex immunological milieu of IBD, including

perhaps other co-stimulatory molecules should be taken into account in attempting to control mucosal inflammation. Therefore a part of this thesis will analyse a possible role for CD58-CD2 interactions.

1.5.2 CD2 – CD58

CD58 on antigen presenting cells is the ligand for CD2 on T cells (figure 1.9): CD2 is a 50kD glycoprotein expressed in all peripheral T cells. It was initially described as the sheep red cell receptor. [80] It consists of an extra-cellular domain made up of 185 aminoacids, a transmembrane domain of 24 aminoacids and an intra-cellular one of 117 aminoacids. [81] The extra-cellular domain binds to its ligands, the transmembrane is used to anchor the receptor to the cell and the intracellular presumably for signal transduction and interaction with intra cellular structures. [82, 83] Initially, CD2 was perceived as an adhesion molecule, as experiments showed that blocking it with antibodies results in a significant reduction in the T cell binding (rosette formation) ability; [84] however subsequent experiments showed that CD2 stimulation caused T cell proliferation, IL–2 secretion and IL–2 receptor expansion. [85] Blocking CD2 caused inhibition of T cell proliferation when this was attempted through a variety of pathways. [82]

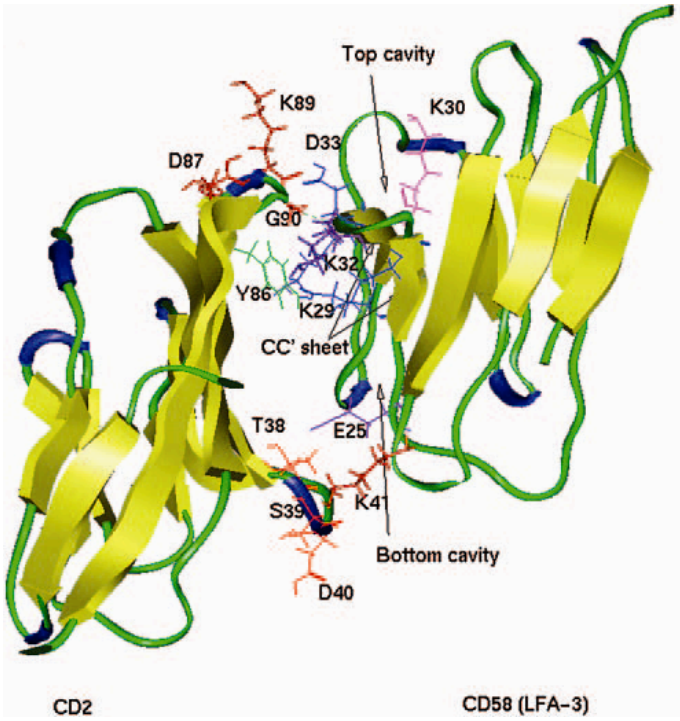


Figure 1.9: The crystal structure of CD58 – CD2 complex. (Taken from Jining et al, European Journal of Biochemistry. 2004; 271: 2873 – 2886 [86])

Competition experiments with antibodies against several epitopes, demonstrated that CD58 (LFA – 3) [but not other adhesion molecules such as LFA – 1, complement receptor – 1 (CR1) and HLA – A, B] is the specific natural ligand to CD2. [85] This was reconfirmed with experiments using artificial target cells (ATC) reconstituted with purified CD58 and Jurkat T cells. Antibodies against CD58 and CD – 2, [but not against LFA – 1 or HLA] blocked the ATC – T cell conjugate formation. [87] The role of this interaction in T cell activation was initially studied by incorporating CD58 to murine L cells; CD58 positive, but not negative L cells promoted T cell proliferation in the presence anti – CD3 antibodies or low concentrations of PMA. The results could not be reproduced in the presence of an anti – CD5 antibody or medium suggesting an

indispensable role for CD58 in the T cells activation process. [88] The presence of pro-inflammatory cytokines as IL-1 and IL-6 did not abrogate the need for CD2 – CD58 mediation for T cell activation. [89] The same interaction in a different experimental setting using murine T cell hybridomas expressing the CD2 cDNA and liposomes expressing CD58 and HLA – DR, promoted IL-2 production by T cells. This was inhibited by blocking CD2 or CD58. [90] Moreover CD2 – CD58 interaction increased T cell intracellular cAMP, implying a role for the initiation of intracellular biochemical changes or a functional regulatory role. [91]

This interaction is a dynamic process; CD3 stimulation up-regulates CD2 expression through an intracellular inside-out signaling pathway, while the avidity of CD2 to CD58 is regulated by a number of biochemical molecules such as tyrosine kinases, protein kinase C and cAMP. [92]

CD58 is a 40 – 70 kDa glycosylated protein made up of six distinct epitopes and is expressed in haematopoetic and non haematopoetic cells. [93] It is encoded by a 65 kbp gene that has six exons and is located at the human chromosome locus 1p13. [94]

It is expressed on a mumber of immune molecules that play a central role in the pathogenesis of IBD, such as the dendritic cells. In an allogeneic mixed lymphocyte reaction model using blood dendritic cells as the antigen presenting cells, antibodies against CD58 blocked T cell activation. [95] In another model of T cell activation using dendritic cells and T cells, it was shown that when dendritic cells overexpressed a triad of co-stimulatory molecules (CD58, B7-1, ICAM-1) they had increased antigen presenting potency and could achieve increased T cell proliferation which produced 60% more IL-2 and 66% more IFN-γ. [96]

1.5.3 The expression of CD58 in the GI tract

CD58 was initially shown to be expressed in intestinal epithelial cells. It was not affected by cytokine stimulation (TNF-a, IL-1, IL-6, IFN – gamma). [97] Its expression was highly polarized and restricted to the basolateral membrane and its inhibition blocked the stimulation of CD4 T cell proliferation. [98] The functional role of CD58 on intestinal epithelial cells (IECs) has not been extensively studied, but in vitro experiments using co-cultures of HT – 29 cells and intraepithelial lymphocytes obtained by the jejunum of otherwise healthy patients undergoing gastric bypass showed a synergistic increase in the production of IL – 8, which was cell contact dependent and abrogated by the addition of antibodies blocking CD58. [99] CD58 is also expressed in the lamina propria mononuclear cells. In LPMC cultures blocking CD2 or CD58 resulted in decreased cell proliferation. When exposed to APCs, CD2 or CD58 again caused inhibition of proliferation and IFN-gamma production. [100]

1.5.4 CD58 in the gastrointestinal diseases.

Very limited data are available concerning the role of CD58 in inflammatory bowel disease. A soluble form of CD58 that can block the CD2 – CD58 interaction was decreased in the serum of UC and Crohn's disease patients; this reduction was correlated to inflammatory markers and clinical disease activity. [101] When compared to healthy controls, the mucosal expression of CD58 in the ileum of Crohn's disease patients was reduced and this reduction was reversed after treatment with infliximab. [102]

In mice the molecule CD48 is the ligand to CD2, hence acts as the CD58 analogue. When wt naïve CD4 T cells are transferred into wt or CD48 -/- mice, they result in clinical and

histological features of colitis. [103] This does not occur when CD48 -/- CD4 T cells are transferred to CD48 knock out mice. Additionally, treating mice with established colitis with anti-CD48 antibodies decreased the clinical activity of the colonic inflammation. (figure 1.10) These results suggest a critical role for CD48 – CD2 interaction in the pathogenesis of colonic inflammation. [103]

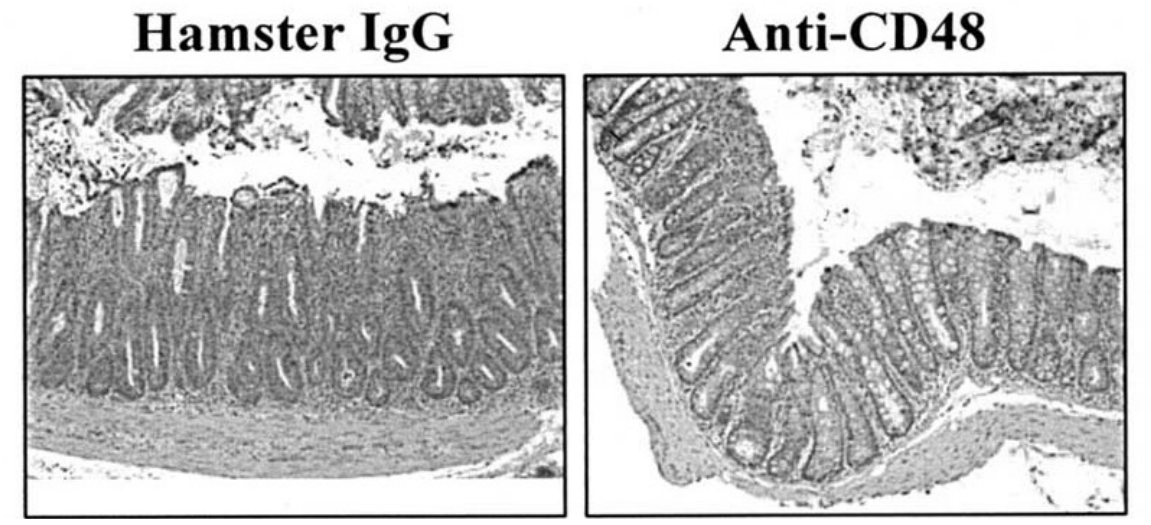


Figure 1.10: CD48 (the murine analogue of CD58) as a target for the treatment of intestinal inflammation. Treating mice with anti-CD48 (the murine analogue of CD58) antibodies improves established colitis and prevents development of colonic inflammation in animal models of IBD. Taken from Abadia – Molina et al. Gastroenterology; 2006; 130: 424 – 434. [103]

The role of CD58 has been studied in colorectal cancer patients. It was shown that about 50% of colorectal cancer patients had reduced expression of CD58, without this having an impact on the natural history of cancer. [104-106] The role of CD58/CD48 in the

development of colorectal cancer was also studied in mice; When a recombinant vaccinia virus containing CD48 was inoculated in colonic cancer cells these did not grow into a tumour when injected to immunocopmetent mice. [107]

The importance of CD58 in the development of chronic active inflammation was examined in hepatocytes and PBMCs of hepatitis B patients where the levels of CD58 correlated with HBV DNA levels and biochemical disease severity [108, 109] Similar results were obtained in a small study with hepatitis C patients. [110] The expression of CD58 was reduced after treatment with interferon – alpha among the responder hepatitis B and C patients. [111]

1.5.5 CD58 in chronic inflammation outside the GI tract

CD58 has been studied in a number of immune mediated disorders outside the GI tract.

Rheumatoid arthritis

CD58 expression is increased in the synovial fluid lymphocytes of patients diagnosed with rheumatoid arthritis (RA) compared to its expression in the PBMCs of RA patients or healthy controls. [112] CD58 has been shown to play a fundamental role in the pathogenesis of rheumatoid arthritis rather than just being a part of the disregulated immune response involved, as the SNP rs11586238 that is located near the CD2 – CD58 region is strongly associated with the disease in a large GWAS study. [113] In a mouse model of arthritis (collagen induced arthritis), a CD2 derived peptide that can bind to CD58, when administered intravenously suppressed the clinical and histopathological activity of arthritis and this effect was not present in the placebo treated group. [114]

Multiple sclerosis

Activated autoreactive T cells play a central role in the pathogenesis of multiple sclerosis and a SNP within the first intron of the CD58 gene has been identified in a GWAS of multiple sclerosis as a significant genetic protective factor for susceptibility to the disease. RNA studies confirmed that this allele is associated with an increase in RNA expression of CD58. [115] However, T cells in multiple sclerosis patients express higher levels of CD2. [116]

Psoriasis

Psoriasis is characterized by skin CD4 and CD8 memory T cell infiltration. CD58 is not expressed in normal skin, but is expressed in the keratinocytes, the blood vessels and the mononuclear cells of psoriasic lesions. The molecular expression on the mononuclear cells is downregulated after treatment with ciclosporine. [117] Additionally, in cultures of mixed epidermal T cells from psoriasic lesional biopsies, cell proliferation was significantly inhibited by the action of anti-CD2 and anti-CD58 mAbs. [118] Psoriasis was the first systemic immune mediated disorder where the CD2 – CD58 interaction has been investigated as a potential therapeutic target; Alefacept is a recombinant protein (CD58 IGG1 fusion protein) that binds to the CD2 on lymphocytes in order to block the CD2 – CD58 interaction (figure 1.11). [119] In vitro it has been shown to inhibit T cell responses. [120] In a placebo controlled trial including 229 patients with psoriasis (59 on placebo and 170 in 3 different doses of alefacept administered once weekly intravenously for 12 weeks) it showed increased efficacy in terms of psoriasis area and severity index. Clinical remission that was sustained for at least 10 weeks after the end of treatment and

was associated with a reduction in the number of peripheral CD4 cells and CD4+CD45RO+ T cells. [119] The efficacy of the drug was reconfirmed in a larger phase III trial. [121]

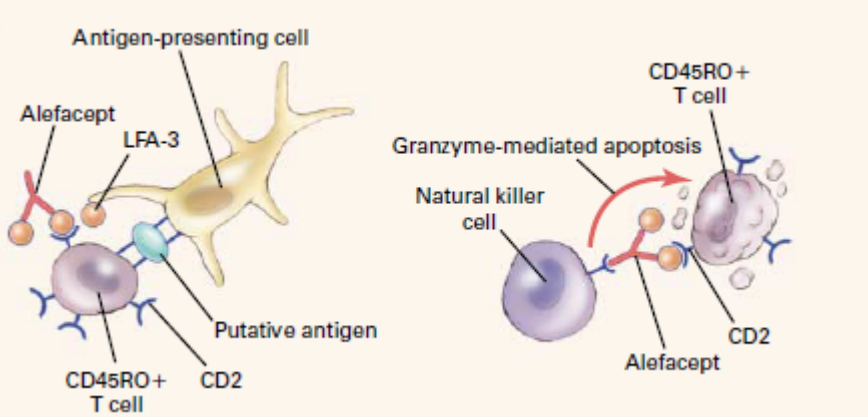


Figure 1.11: Blocking CD2 – CD58 interaction as a way to immunomodulation. Reproduced with permission from Ellis et al, NEJM 2001; 345 (4): 286 [119]

Graft versus host disease (GVHD)

Apart from its use in dermatology, there is some evidence that alefacept can be an efficient treatment option in more challenging immunological cases as graft versus host disease which is a rare potentially lethal T cell mediated condiation that occurs post transplantation. [122] 3 small studies involving a total of 22 patient who developed steroid refractory GVHD after bone marrow transplantation, show an efficacy of about 50% for alefacept. [123-125] A recent case report suggests durable effectiveness for GVHD post liver transplantation [122]

These results show that blocking the CD2 – CD58 interaction can be a treatment option for the successful management of refractory T cell mediated disorders. In this thesis we will examine if this interaction maybe important in the immune modulation of IBD.

1.6 Environmental factors that influence IBD

The previous chapter elucidated important aspect of the immuno-pathogenesis of IBD. This is largely defined by genetics, but it is also widely recognized that environmental factors play a significant role in concert with genetic susceptibility.

1.6.1 Smoking

Smoking has been reported as one of the most consistent risk factors for the pathogenesis of Crohn's disease in western countries, associated with a two-fold risk. [4] Tobacco exposure can influence the phenotype and disease course; In a study comparing 81 never smokers with 92 smokers with Crohn's disease, heavy smoking was associated with more small bowel involvement, more aggressive penetrative and perianal disease and higher risk for surgery. Smokers were 14% more likely to have an operation within 10 years from diagnosis, while the risk of re-operation was significantly higher among heavy smokers (OR 1.79 [95% CI 1.12, 2.55] p=0.015). [126] This is consistent with another study proving a significantly higher risk of post operative recurrence among smokers (OR 2.96 [95% CI 1.5, 5.6]), [127] while post operative smoking cessation remains an important therapeutic intervention reducing the risk of recurrence and re-operation. [128] Interestingly, the impact of smoking in ulcerative colitis is protective (OR 0.58). The cause of the different impact of tobacco use in Crohn's and UC is largely unknown; The impact of smoking on faecal microbiota and the immunological function have been quoted as possible biological mechanisms. [4]

1.6.2 Appendicectomy

Appendicectomy has been extensively studied as an environmental factor influencing the pathogenesis of IBD. A number of studies associate appendicectomy with a reduced risk of developing ulcerative colitis; A landmark study of 212,963 patients and the same number of controls showed that appendicectomy for acute appendicitis or mesenteric lymphadenitis reduces the risk of UC occurrence by 27% and 52% respectively. This association was not proved when appendicectomy was done for non specific abdominal pain. [129] This was further confirmed in a meta-analysis of 13 case control study, showing that appendicectomy reduces the risk of UC by 62-65%. [130] Further studies investigated the role of appendicectomy on the course of UC: In a case control study 6.5% of UC patients and 16.3% of the controls had undergone appendicectomy; Patients having undergone appendicectomy had less extensive disease and milder disease course with fewer relapses, less requirements for immunosuppressive therapy [131] and a lower risk of colectomy. [132]

1.6.3 Antibiotics

As the intestinal microbiota plays an important role in the pathogenesis of IBD, the use of antibiotics has been investigated as a possible factor in the pathogenesis of IBD. In a recent nationwide prospective study from Denmark, following up 577,627 children, antibiotic users had an almost 2-fold risk to develop IBD (OR 1.84) and every course of antibiotics prescribed increased the risk of IBD by 12%. Antibiotic users were 3.41 times more likely to develop Crohn's disease and children having been prescribed at least 7 courses of antibiotics were 7.32 times more likely to develop Crohn's disease. No significant association was shown between antibiotics and ulcerative colitis. [133] Another case control study from Canada, proved that individuals having been prescribed antibiotics were 50% more likely to develop Crohn's disease or UC within the next 2-5 years. [134] Similarly a large scale study using data from general practices in UK involving more than 1 million children and more than 6 million person years of follow up, confirmed that exposure to anti-anaerobic antibiotics in childhood is linked with an almost 2 fold risk of developing IBD by the age of 19. The risk was higher among those who were prescribed antibiotics earlier in life: Exposure before the first year was related with a 5.5 fold risk, exposure before the 5^{th} year was related with a 2.6 fold risk and before 15 years with a 1.5 fold risk. [135] Of course this trend might be explained by the possibility that children prescribed antibiotics after the 15^{th} year of age would have shorter follow up period than those exposed earlier as patients were followed up to their 19^{th} year of age.

1.6.4 Diet

Diet has been investigated extensively as a possible factor related to the development of IBD. Most studies have focused on particular dietary patterns or nutrients. Increased sugar intake has been reported as a possible risk factor for the development of Crohn's disease in questionnaire based studies. [136, 137] In a prospective study of 67,581 female healthy individuals in France, high intake of animal protein was associated with a 3 fold risk of developing IBD. [138] A similar multi-ethnic European study following up more than 200,000 healthy individuals suggested that increased intake of linoleic acid, an n-6 polyunsaturated fatty acid that is present in red meat, cooking oils and margarines is associated with developing UC. Patients at the highest quartile of intake had a 2.5 fold risk of developing UC. [139] This constitutes an epidemiological rather than a direct aetiological relationship in all studies, however the authors of the last study suggest that linoleic acid, through its incorporation in the colonic cell membrane might cause the release of proinflammatory molecules, such as prostagladines and leukotrienes leading to the aggregation of immune cells. [139] Similarly a study from Japan concluded that there is a strong correlation between animal fat and n-6 polyunsaturated fatty acids with a new diagnosis of Crohn's disease and [140] the administration of n-3 polyunsaturated fatty acids had a therapeutic benefit in TNBS induced colitis in mice, as opposed to n-6 polyunsaturated fatty acids. [141] A recent longitudinal study assessing the risk of IBD in 170,776 women followed up for 26 years, concluded that higher dietary intake of fiber is associated with significantly associated with reduced risk of developing Crohn's disease and especially of ileal or ileocolonic distribution but did not impact on the risk of developing UC. [142] In practice it is difficult to interpret these data given that perhaps

patients with undiagnosed small bowel Crohn's disease would avoid eating large amounts of fiber to avoid symptoms of diarrhea and abdominal pain.

In one of the few studies of the impact of dietary components in IBD investigating the underlying pathophysiology as well, it was proven that the administration of fructo-oligosaccharides in patients with active Crohn's disease achieved a significant reduction in the IL-6 positive and a significant increase in the IL-10 positive dendritic cells in the lamina propria; [143] However, within the 4 week period of the intervention, this biological turnover failed to be translated in a clinical benefit. [143]

In summary, diet seems to be another risk factor for IBD, at least epidemiologically. However more pathophysiological studies are required.

1.6.5 The epigenetics of IBD

In the previous sections of this thesis a number of genetic and environmental factors that influence the pathogenesis and course of IBD have been analysed. The genetic factors account for a fraction of the disease variance (13.6% for CD and 7.5% for UC). [144] However the evidence for the environmental factors and their impact on the disease variance has been mostly observational and the large variability in patients' duration, intensity and frequency of exposure to different environmental factors make the study of these even more difficult to conduct and interpret. [144]

The evolution of epigenetics has led to a new pathway of thinking when considering the impact of environemental factors in disease pathogenesis and outcome. Epigenetics can be defined as mitotically inherited changes in gene function that do not involve a change in DNA sequence like SNPs, but DNA methylation, histone modification, RNA

interference and positioning of nucleosomes. [144] The role of epigenetics in the pathogenesis of IBD has been studied to a certain extent mostly by identifying epigenetic changes around genes identified in previous GWAS studies but has not in a way specific to environmental factors.

However, the epigenetic changes mediated by different environmental factors that have been epidemiologically linked to IBD have been studied by other disciplines of medicine and on the general population. For example, the impact of smoking on the epigenome has mainly been studied in the context of Chronic Obstructive Airway disease: DNA methylation has been shown to be relevant to ever-smoking status, the age of smoking initiation and CRP levels [145] and smokers' macrophages have been shown to have downregulation of many miRNAs, [146] implying a possible link between the epigenetic changes related to smoking to the regulation of the inflammatory process.

Dietary components during pregnancy (folic acid, poly-unsaturated fatty acids) have been shown to cause epigenetic changes relevant to the transcription of NF-kB, influencing the immune function [147] and the complex relationship of nutrients and the intestinal microbiota with the intestinal epigenetic programming is the target interest of an up and coming research discipline, the nutrigenomics. [148] Both gut microbiota and nutrients are increasingly considered as epigenetic regulators of the intestinal gene expression. [149, 150] The bacteria that reside the gut lumen, through the secretion of low molecular weight molecules (eg buyrate) can activate or inhibit the epigenomic regulation by affecting the activity of relevant enzymes. [150] Studies on Vitamins A, D and fatty acids have shown that they can directly activate nuclear receptors and induce gene transcription. [151]

1.6.5.1 Epigenetic changes specific to IBD mechanism according to mechanism involved:

1.6.5.1.1 DNA methylation

An epigenome wide association study using an Illumina Methylation assay and including 16 paediatric patients with crohn's disease, 21 adult patients with CD and 19 controls, identified significant differences in DNA methylation between patients and controls in 50 sites. [152] These included sites of the genome that are implicated with the immune system processes, the immune response and defense response to bacteria. [152] Another study assessing DNA methylation in B cells from 18 IBD patients (9 UC and 9 CD) and 18 non-IBD siblings, identified 11 CpG sites with IBD, 14 with CD specific and 24 with UC specific associated methylation. [153] Again, genes implicated with differential methylation included genes relevant to the immune response such as IL-6, IL-10, IL-12B, PPAR-γ and MUC1. [153] The epigenetic changes in IBD have been also studied specifically on intestinal tissue: A study of 20 monozygotic twin pairs that were disconcordant for UC using colonic biopsies taken during colonoscopy 61 sites of differential DNA methylation were identified between cases and controls, the majority of which is implicated in the immune process. These loci were subsequently validated in an independent cohort. [154] Another study using rectal biopsies from UC, CD patients and healthy controls showed differential methylation of several genes that are involved in epithelial cells tight junction, antigen processing, B and T cell receptor signaling and cell adhesion. [155]

1.6.5.1.2 Histone modification

The role of histone modification has not been extensively studied in IBD and most of the evidence about it is indirect. Histones can undergo a range of modifications including the acetylation, phosphorilation, methylation and ubiquitination of their N-terminal aminoacid tails. [144] These processes are regulated by enzymes such as the histone acetyl-transferase (HAC) and the histone deacetylase (HDAC). HDAC inhibitors, such as sodium butyrate and valproate have been shown to have anti-inflammatory properties [144] and oral administration of HDAC inhibitors in animal models of colitis resulted in clinical and histological improvement with a corresponding shift of tissue cytokine profile towards a more anti-inflammatory phenotype. [156] TLR-4 regulates mucosal tolerance via mechanisms that could be at least partially regulated by histone acetylation and DNA methylation; Experiments undertaken in HT-29 cells showed a link between the responsiveness to LPS and the degree of histone deacetylation of the 5' region of the TLR-4 gene. [157]

1.6.5.1.3 RNA interference

Micro-RNAs (miRNA) are single stranded non coding RNAs that regulate gene expression by inhibiting mRNA translation or causing mRNA degradation. [144] Studies in animals have shown that miRNAs play a role in gut homeostasis and studies in patients with IBD have shown a differential expression of miRNA that are involved in the apoptotic and inflammatory process, in inflamed tissue compared to controls. [144]

1.7 IBD and nutrition

1.7.1 Nutritional aspects of IBD

The role of diet as a risk factor for the development of ulcerative colitis was first described during the 1960s when a questionnaire based study of cases vs controls showed that established breast feeding in infancy was less frequent among UC patients than healthy controls. [158] Around the same time, malnutrition in Crohn's disease patients, and its implications due to poor intake, malabsorption, short gut, fistulation and micronutrient loss through the GI tract were increasingly identified. [159, 160] Now it is recognized that malnutrition affects 30-80% of Crohn's disease patients. [161] In an anthropometric study taking into account muscle and subcutaneous fat, malnutrition was confirmed among IBD patients. 20% of patients are below the 90% of their ideal body weight. Malnutrition was linked with extensive small bowel Crohn's disease and post surgical recurrence of Crohn's disease. [162] Mid arm circumference was identified as a useful malnutrition screening tool for these patients. [163] Malnutrition would also be an issue among Crohn's disease patients in remission or with mildly active disease with about 50% of them having a substandard body weight and fat stores despite adequate caloric intake. [164]

1.7.2 Mechanisms of malnutrition

1.7.2.1 Decreased oral intake:

The symptoms of diarrhea, abdominal pain and nausea that are linked with food intake result in reduced oral intake from IBD patients. [165] In a prospective study assessing the nutritional status and food intake in paediatric patients with Crohn's disease, the weight, height and BMI of patients with active Crohn's disease is significantly lower that that of patients with inactive Crohn's disease. The total caloric intake was significantly lower than controls in both children with active (29% less) and inactive (6% less) Crohn's disease compared to healthy controls. [166] In a cross - sectional questionnaire based study in Canada, adult patients with active Crohn's disease, ingested similar amount of calories and micronutrients as patients with inactive Crohn's disease. Despite this similarity both groups ingested substandard quantities of vitamins and minerals. Patients with penetrative or obstructive Crohn's disease were excluded from this study and it would be risky to extrapolate the equality in intake for the general Crohn's disease population. [167] Energy intake, appetite and pleasure from eating are lower among patients with colonic Crohn's disease presenting with weight loss in another study. [161]

1.7.2.2 Loss of nutrients:

Loss of nutrient energy is another factor in the pathogenesis of malnutrition among IBD patients. Loss of nutrients is more prevalent in active Crohn's disease and among patients that have undergone bowel resections or have stomas. [168] In a recent study of adult patients with Crohn's disease in remission, measuring BMI, food intake and faecal gross energy, despite the fact that patients with a BMI < 18.5 had no difference in the caloric

intake compared to patients with BMI > 18.5, energy malabsoprtion was significantly higher among the patients with the lower BMI. [169] Additionally it is documented that energy requirements are higher among IBD patients; Although there is increased resting energy expenditure among patients with active disease the total energy expenditure remains stable despite the possibly lower levels of physical activity. [170] This adds to the nutritional imbalance already caused by reduced oral intake.

1.7.2.3. Short bowel syndrome:

50% of Crohn's disease patients will require surgery witin 10 years from diagnosis and the disease will recur to as many as 45-55% of them. [1] Hence, Crohn's disease is the most common cause of short bowel syndrome. Patients with a short bowel are divided in two categories: Those with an ileostomy and those with a jejuno – colonic anastomosis; the former group needs at least 100cm of jejunum and the latter at least 50cm of jejunum left in order to avoid parenteral supplementation. [171] The main mechanism of malabsorption in these patients is the loss of intestinal surface; in addition to this, patients with an end – jejunostomy have a significantly higher gastric output than patients whose colon is preserved and in continuity with the remaining jejunum. [172] Patients who develop short bowel syndrome will require regular electrolyte supplementation [173] or home total parenteral nutrition with a negative impact on their quality of life. [174]

1.7.3 Micronutrient deficiency:

1.7.3.1 Iron deficiency:

About 48% of Crohn's disease patients and 20% of UC patients are anemic at diagnosis, making anaemia the commonest extra-intestinal manifestation of IBD. [175] In a study undertaken by the candidate but not a part of this thesis as iron does not have an immunomodulatory role in IBD, 70% of the children, 42% of the adolescents and 40% of the adults were anaemic and aneamia was accounted for by iron deficiency in 88%, 83% and 55% respectively. [176] Anaemia has been linked with IBD to the point of haemoglobin levels being part of the Crohn's disease activity index. [177] In up to 90% of cases, the cause of anaemia is iron deficiency, [178] and the pathophysiology of iron deficiency is multifactorial.

The dietary intake of iron in IBD patients is low: In a study of 23 male and 24 female Crohn's disease patients, 50% of the female patients had inadequate iron intake. [179] In a multicenter questionnaire based study of 91 Crohn's disease patients and 91 controls in London, the mean dietary intake was significantly lower among cases by 2.3 mg/day compared to controls. This was mostly accounted for by lower intake of non-haem iron which is contained in fiber rich dietary products which could possibly cause symptom deterioratrion. [180] In UC patients iron intake is significantly lower in periods of disease relapse. [181] The impact of low dietary intake is further accentuated by the loss of iron rich erythrocytes from intestinal ulceration and bleeding. [177]

In active inflammation the absorption of iron from enterocytes is impaired. [182] This is mainly mediated by hepcidin.

The role of hepcidin: Hepcidin is a peptide secreted by the hepatocytes that was initially shown to have anti-microbial properties, [183] and its role in iron homeostasis in human has been initially studied among patients with hereditary hemochromatosis where decreased expression of the gene coding for hepcidin compared to controls was shown. [184] Its synthesis is dependent on the amount of dietary iron entering the blood and the amount of iron in iron stores. Hepcidin regulates the absorption of iron (figure 1.12). Blocking the expression of hepcidin in mice resulted in increased iron absorption and iron overload in most tissues, while transgenic mice overexpressing hepcidin-1 transplacental and intestinal iron transport was blocked causing death at birth unless iron was administered parenterally. [185] Hepcidin expression is linked to inflammation. Treatment of murine hepatocytes with IL-6 induced the mRNA expression of hepcidin. [186] Hepcidin mRNA was not induced during inflammation (terpentine abscess induction) in IL-6 KO mice as compared to WT mice, proving that IL-6, a central cytokine in the pathogenesis of IBD is an indispensable mediator of its synthesis. Similarly, iron levels decreased in WT but not IL-6 KO mice. In the same study, infusion of IL-6 to healthy human individuals caused remarkable increase in hepcidin levels and decreased serum iron and transferrin saturation levels by 34% and 33% respectively. [185] Moreover, in experiments using Caco-2 cells grown in transwell inserts, exposure to hepcidin decreased the iron transport from Caco-2 cells proving a direct relationship between hepcidin and iron malabsorption. [187] Serum levels of hepcidin are higher in UC and Crohn's disease patients compared to controls making hepcidin a key factor of iron malabsorption among them. [188] This in turn has implications in oral iron replacement therapy administration. In a study of 19 Crohn's disease patients, ferrous

sulphate absorption was significantly lower among patients with active disease and inversely correlated with IL-6 and CRP levels. [182] However, this is in contradiction with our series of patients where oral iron, when administered, led to an increase in corresponding Hb levels. [176] Effective treatment of iron deficiency is very important as if left untreated leads to chronic fatigue, physical and psychological dysfunction, compromising the quality of life of affected patients. [177]

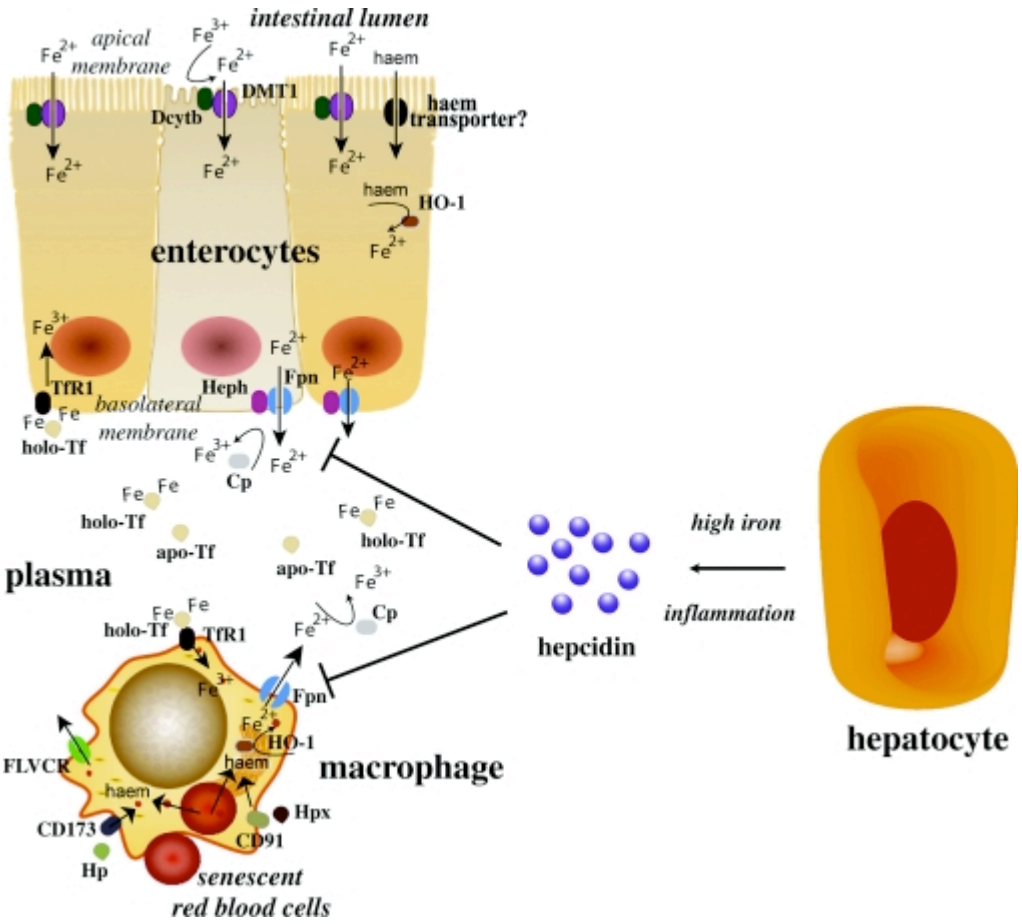


Figure 1.12 : Iron metabolism and the role of hepcidin

This figure was originally published in The Biochemical Journal, Wang et al, 2011 Mar 15; 434(3): 365–381 [189]

1.7.3.2 Trace elements:

Trace elements deficiencies have been widely described in inflammatory bowel disease and especially in Crohn's disease. A large number of studies examine trace elements and as these are not central to the current thesis a brief summary follows

1.7.3.2.1 Magnesium:

Magnesium deficiency in Crohn's disease was initially described in 4 patients in1970. [190] In later study of Crohn's patients requiring parenteral nutrition 88% (15/17) were deficient in magnesium and 12% of them were symptomatic. [191]

1.7.3.2.2 Zinc:

In a study comparing zinc levels between paediatric IBD patients, normal controls and controls of short stature and reduced weight (primordial short status and anorexia nervosa respectively) hypozincaemia was more prevalent among IBD patients compared to healthy controls. The mean serum zinc level was significantly lower among the IBD patients in comparison to the healthy controls and the controls for weight and height. In a univariate analysis, zinc levels were lower among those with moderate or severely active IBD and those with growth retardation, but not significantly different between Crohn's and ulcerative colitis patients. The poor response to oral zinc supplementation among IBD patients with moderately or severely active IBD provides evidence for a decreased absorption of zinc among IBD patients with active disease. [192]

1.7.3.3 Vitamins:

Vitamin deficiencies are common in IBD. A full description of all vitamin deficiencies is beyond the scope of the present work. In this chapter a brief description of vitamin deficiencies and their role in IBD will follow, including of more extensive details on the role of vitamin D, whose importance has been increasingly appreciated in recent years.

In a prospective study involving children with IBD, 14.4% of the patients were Vitamin A deficient and 6.2% were vitamin E deficient. Hypovitaminosis was not observed in the control population. In Crohn's disease vitamin A and E deficiency could be predicted by disease activity either in the form of PCDAI or ESR. [193] Similar results were obtained in a study of vitamin A levels in adults. [194] In another study, serum levels of vitamin A would normalize after induction of remission without supplementation and hypovitaminosis A was linked with decreased serum levels of retinol binding protein rather than malabsorption. [195] Vitamin A supplementation has been tried as a means of remission induction in patients with Crohn's disease, but without a favorable outcome. [196] More recently in an animal model study, all trans retinoic acid administered in mice with TNBS colitis resulted in decreased inflammation, an increase in anti-inflammatory cytokines (IL-4 and IL-10) and a decrease in pro-inflammatory cytokines (IFN-gamma, TNF-alpha and IL-12). [197]

Vitamin C levels are lower among patients with IBD compared to healthy controls irrespective of their disease activity status. [198] The mucosal levels of ascorbic acid are also decreased in active Crohn's disease and ulcerative colitis [199] but a small pilot study in eleven patients failed to show any benefit for ascorbic acid. [200]

1.8 Vitamin D:

Vitamin D exists in mainly 2 forms: Vitamin D3 (cholecalciferol) which is mainly synthetised through exposure to sunlight, and vitamin D2 (ergocalciferol) which is obtained from plant sources. [201] Vitamin D is synthetised when UVB radiation photolyses 7-dihydroxycholesterol at the skin to pre-vitamin D3 which is then processed into vitamin D3, which is converted in the liver to 25(OH)vitamin D3 (the main circulating form) before it undergoes the final hydroxylation in the kidneys to form 1,25(OH)vitamin D, the main biologically active form of vitamin D. [202]

Severe vitamin D deficiency leading to osteomalacia was reported for the first time over 30 years ago in a prospective study of a cohort of 25 patients who had undergone a small bowel resection; 36% of them suffered from osteomalacia. [203] In a larger study of 82 patients with Crohn's disease, 65% of them had low vitamin D levels with the lowest levels observed among those who had undergone ileal resections. [204] In a study of 504 IBD patients (403 CD and 101 UC), the prevalence of vitamin D deficiency was almost 50% and among Crohn's disease patients, vitamin D deficiency was associated with increased disease activity and poorer quality of life scores. [205] Factors apart from surgical resection and malabsorption that contribute to this are active inflammation, reduced oral intake and reduced sunlight exposure. [201]

In addition to its well known impact on calcium metabolism, vitamin D has immunoregulatory properties; Vitamin D can suppress the proliferation of B and T cells as well as the mRNA production of cytokines as IL-2 and IFN – gamma and the production of IL-12 from monocytes and B cells. [206] Experiments using leukocytes from peripheral blood, proved that preincubation with Vitamin D inhibited the expression

of HLA-DR even when this was attempted to be induced by IFN – gamma hence impairing their antigen presenting properties. [207] Addition of Vitamin D to LPS stimulated dendritic cells inhibits their maturation by inhibiting the induction of co-stimulatory molecules as CD40, CD86 and CD80 and induce the apoptosis of mature dendritic cells. [208] Vitamin D could reduce the expression of TLR2 and TLR4 among the monocytes of healthy individuals as well as individuals suffering from immune mediated disorders where the baseline TLR expression is higher. [202] This was accompanied by reduced translocation of NF-kB to the nucleus in Vitamin D treated monocytes. [209] The effect of Vitamin D to NF-kB activity is achieved by significantly increasing the mRNA levels and the half – life of IkB-alpha (Inhibitor of kB alpha) and decreasing its phosphorylation. [210] This is further supported by a study on mice embryonic fibroblasts showing that deletion of the vitamin D receptor gene resulted in a reduction of IkB-alpha protein levels by 40% [211] The vitamin D receptor (VDR) is expressed by monocytes and activated B and T cells. [212]

Dendritic cells pre-incubated with Vitamin D attempting to stimulate CD4+ T cells shift their differentiation towards a Foxp3 positive phenotype that is known for its immunosuppressive and anti-inflammatory properties. [213] Foxp3 positive Tregs that are treated with vitamin D express higher levels of Foxp3 protein and are significantly more potent at suppression than the not treated ones even when a fixed number of Tregs interacts with an increased number of CD4+CD25- T cells. [214] The impact of Vitamin D on Foxp3 expression is mediated by direct binding of the VDR in the intronic (non-coding) region of the Foxp3 gene. [215] The regulatory effect of Vitamin D on Foxp3 expression is further supported by a study on 15 healthy volunteers whose Foxp3 levels

in the peripheral blood Tregs would be higher during the summer months when Vitamin D levels increase. [216]

The immunoregulatory properties of vitamin D are summarized in figure 1.13.

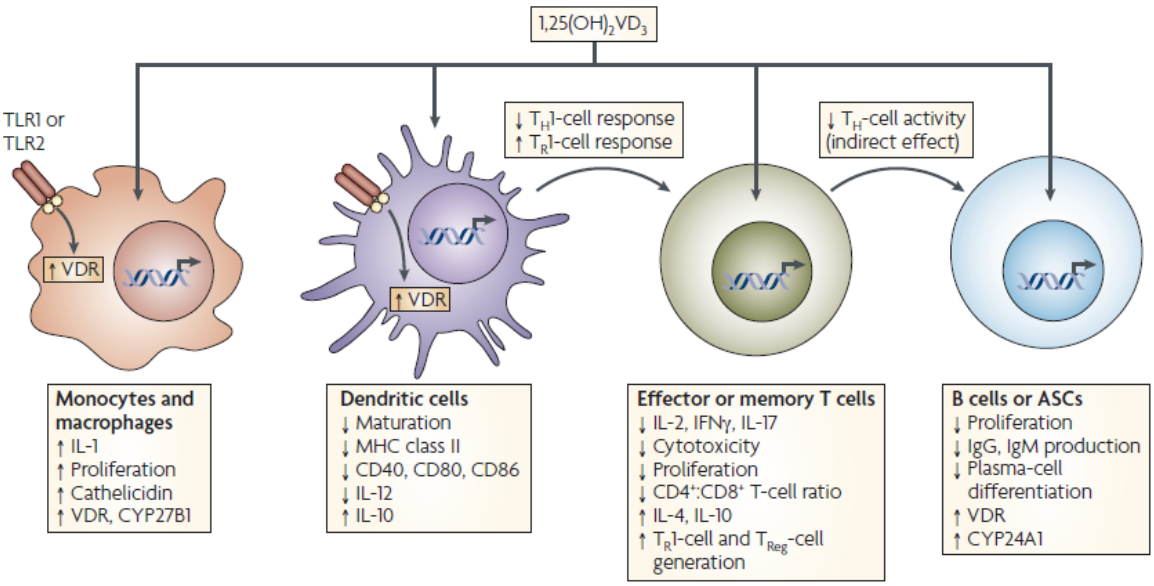


Figure 1.13: The immune regulatory properties of vitamin D.

Taken from Mora et al. Nature Reviews Immunology, 2008. 8: 685 – 698. [217]

TH17 cells have been shown to play a significant role in the mediation of inflammation in IBD. In an in vitro system of antigen presentation and T cell differentiation, the presence of vitamin D leads to an inhibition of TH17 differentiation and reduced IL-17 mRNA levels. This effect is mediated through the vitamin D receptor but was not observed in VDR -/- T cells and is independent of IL-10. [218] It is mediated through a reduction in RORγ and IL23 mRNA levels. [218]

Additionally, the inhibitory role of vitamin D on the TH17 population has been shown in animal models and patients with immune mediated disorders. The number of TH17 cells in the spleen and the CNS of mice injected with myelin oligodendrocyte glycoprotein in a model of experimental autoimmune encephalomyelitis were significantly less among mice treated with oral vitamin D and the vitamin D treated mice had reduced disease activity scores (and very close to zero) during the 18 day follow up. [219] In peripheral blood T cells of treatment naïve patients with rheumatoid arthritis (RA), vitamin D suppressed the levels of IL-17 and IFN-gamma and increased the levels of IL-4. [220] In another RA study, vitamin D reduced the levels of IL17 in synovial biopsy cultures and this was enhanced by TNF blockage, but not achieved by blocking TNF in the absence of vitamin D. [221] In a small study of patients with undifferentiated connective tissue disease who were deficient in vitamin D, vitamin D supplementation decreased the number of TH17 and Th1 cells. [222] Simirarly, in patients with SLE and hypovitaminosis D, vitamin D supplementation and restoration to normal levels results in decreased numbers of TH17 cells. [223]

Vitamin D might play a role in the pathogenesis and treatment of IBD. In an animal model of IBD (DSS colitis) administration of a vitamin D analogue could attenuate the clinical and histological disease activity of the chemically induced colitis. [224]

1.8.1 The role of Vitamin D in the pathogenesis of IBD

1.8.1.1 Epidimiological observation

Evidence from epidemiological data suggests that the incidence and prevalence of IBD is higher in countries, where exposure to sunlight is low, such as Northern Europe, America, Australia and New Zealand in comparison to sunnier countries as those of South Asia. [201] Within Finland, a North – South gradient in the prevalence of UC has been shown; interestingly vitamin D levels were lower in the population of north Finland. [225] Additionally, a prospective study with a 3 year follow up among the second generation residents of Leicester of South Asian descent showed that they are in fact at higher risk to develop ulcerative colitis than the European residents. [226] A very recent well designed prospective study done among USA nurses with no previous history of IBD, showed that even in populations with great genetic variation (such as the USA), living in a southern state at the age of 30 reduced significantly the incidence of UC and Crohn's (HR 0.62 and 0.48 respectively). [227] This gradient remained significant after adjusting for family history and smoking; unfortunately data on the disease course and vitamin D levels between different latitudes were not collected, however the authors extensively discuss the likelihood of a difference between vitamin D levels between the different latitudes as the possible explanation for their findings. [227] Moreover, a study of 72,719 women, using vitamin D levels as these were predicted by a linear regression

model according to age, sunlight exposure, dietary intake, race and BMI, showed that those who were vitamin D sufficient according to their predicted levels, the risk of developing Crohn's disease was significantly lower [HR 0.38 (95% CI 0.15 – 0.97) p<0.05] and there was also a trend towards a reduced risk for developing ulcerative colitis [HR 0.57 (95% CI 0.19 – 1.70) p=0.2]. [228] Every 100 IU of vitamin D in this population's diet resulted in a 10% reduction in UC risk and 7% reduction in Crohn's disease risk. [228]

1.8.1.2. Vitamin D genetics

Recently, genetic polymorphisms relevant to vitamin D have been shown to be related to the risk of developing IBD. In a case control study of 158 UC patients, 245 CD patients and 164 controls, 3 different VDR polymorphisms were studied: TaqI genotype was significantly related to Crohn's disease [OR 1.99, 95% CI: 1.14 – 3.47, p=0.02] [212] This result was not reproduced in a smaller study from Iran, which showed an association between Fok I polymorphism of the VDR gene and UC [OR 1.57, 95% CI: 1.11 – 2.22, p=0.01], [229] and similar results were obtained in a more extensive Irish study enrolling 1359 patients. [230] However, in another study from New Zealand where 897 IBD patients and 482 controls were genotyped for three VDR SNPs (rs11568820, rs2228570 and rs731236) there was no association between the studied genetic variations and the risk of IBD for the overall population but only an association between rs731236 (TaqI) in males with UC and CD. [231] In a study of Jewish IBD patients, the Bmsl vitamin D receptor polymorphism was associated with UC [OR 2.21, 95%CI (1.06 – 4.9), p=0.04].

[232] A study genotyping 636 IBD cases and 248 non-IBD controls for two common SNPs of the vitamin D binding protein, showed that DBP 420 variant Lys is less common in IBD patients than controls, adding evidence for a role of vitamin D in the pathogenesis of IBD. [233]

In a recent meta-analysis of 15 GWAS studies, a SNP on the VDR was proved to be associated with the risk of IBD. [58] A recent meta-analysis of 9 studies, confirmed an association between the tt genotype of TaqI genotype and the risk of developing Crohn's disease in Europeans and that the ff genotype of the FokI polymorphism is associated with UC in Asians, pointing a role for ethnicity in the susceptibility to IBD perhaps due to under-defined specific gene-sets. The same study suggests that the Aa and aa genotypes of the ApaI polymorphism reduce the susceptibility to Crohn's disease. [234]

1.8.1.3 Vitamin D in animal models of IBD

In two animal models of IBD, the VDR status played a crucial role in the development and severity of intestinal inflammation: in the CD45RB transfer model, transferring T cells from VDR knock-out mice to Rag KO mice resulted in more severe colonic inflammation compared to transferring equal numbers of T cells from VDR wild-type mice. Similarly, in the IL-10 knock-out model, knocking out the VDR resulted in more severe inflammation and 100% mortality in 8 weeks compared to IL-10KO/VDR-wild type mice. [235] In another study, it was documented that high levels of human VDR expression on the intestinal epithelium of mice (transgenic model) had a protective effect when colitis was attempted to be induced with TNBS, DSS or activated T cell transfer.

VDR signalling blocked the activation of NF-kB and was associated with reduced IEC apoptosis. [236]

Another proposed mechanism for the beneficial role of vitamin D in IBD is its role on the intestinal microbiota. VDR KO mice and mice lacking the ability to produce 1,25 $(OH)_2$ vitamin D were shown to have greater numbers of Bacteroides and Proteobacteria and fewer Firmicutes and Defferibacters. [237] A similar modification of the intestinal microbiome was shown in another study comparing mice raised on a vitamin D deficient or sufficient diet: mice that were raised on vitamin D deficient diets had increased population of Bacteroides, Actinobacteria and Gammaproteobacteria compared to thosed raised on a vitamin D sufficient diet. Interestingly and in contrast with the previously described study, the vitamin D deficient mice also had increased population of Firmicutes. [238] However, both these studies suggest that vitamin D status is a regulator of the intestinal microbiota in mice, which plays a role in the development of IBD.

IL-10 KO mice expressed higher levels of TNF-α in the ileum and ascending colon and when treated with a high calcium, vitamin D sufficient diet the degree of inflammation was less, compared to mice kept vitamin D deficient through dietary and light restriction. [239] In another model of IBD, DSS induced colitis, when mice were raised with a vitamin D deficient diet had lower levels of serum vitamin D, more significant weight loss and higher colitis histology scores when compared to mice raised on a vitamin D sufficient diet. [240] Moreover, micro array experiments proved that 27 genes were expressed at a lower level in mice raised on a vitamin D deficient diet, including angiogenin-4 which is a protein with bactericidal activity. [240] In another study using DSS induced colitis as a model for IBD, treatment with a vitamin D analogue

significantly decreased the histologic score of colonic inflammation compared to controls. This was related with a reduction in cytokine production by colonic DCs and downregulation of their co-stimulatory molecules. [241]

A proposed mechanism for the beneficial role of vitamin D in IBD was the enhancement of the epithelial integrity, whose loss is a key event in the pathogenesis of the disease: treatment of Caco-2 monolayers with $1,25(OH)_2$ vitamin D enhanced the expression of ZO-1 and E-cadherin and when the cells were incubated with DSS, the tight junction on the monolayer was significantly disrupted when vitamin D was not presen. [242] This result was replicated in another study that also proved that mice fed with a vitamin D deficient diet had lower serum vitamin D levels and increased intestinal epithelial permeability both at baseline and after C. Rodentium infection when this was assessed in vivo by measuring the serum levels of a FITC-dextran probe that was administered orally. [238]

1.8.2 Therapeutic potential of vitamin D

The emerging evidence about the role of vitamin D in the pathogenesis of IBD and about the prevalence of vitamin D deficiency in IBD has triggered clinical studies about the therapeutic potential of vitamin D. In a double blinded placebo controlled trial of Vitamin D (1200 IU per day) vs placebo in 94 Crohn's disease patients who were in clinical and biochemical remission, those randomised to vitamin D would take longer to relapse compared to those randomised to placebo and according to cox regression analysis there was a statistical trend [HR 0.42, 95% CI 0.16 – 1.10, p=0.06]. [243] However, subgroup analysis from the same study of 10 patients receiving vitamin D supplementation, that

have been selected based on the largest vitamin D increase from week 0 to week 26, and 10 patients receiving placebo (seasonally matched) showed that the PBMCs of those receiving vitamin D, would produce more IL-6 than those receiving placebo. [244] This would advocate against a possibly immunosuppressive role of vitamin D but it could represent bias relevant to the fact that the patients receiving vitamin D supplementation could possibly have subclinical inflammation that was not identified by the CRP or the CDAI. A smaller clinical trial in Crohn's disease patients comparing two different doses of vitamin D administration (10000 IU vs 1000 IU daily) showed superiority of the 10000 IU dose in clinical score improvement, implying that optimizing the dosing regime of vitamin D makes a difference in the clinical outcome. [201] In a recent publication on quality improvement interventions for paediatric IBD, among 505 paediatric patients those who had their vitamin D levels optimized through ergocalciferol administration had significantly better chance of having inactive disease than those who had not; similarly vitamin D levels were significantly higher among IBD patients with inactive disease. [245]

Data from in vitro experiments suggest a potential role for vitamin D in the treatment of intestinal fibrosis; CARD – 024, a vitamin D analogue could repress fibrotic changes induced on human colonic myofirbroblasts by TGF-β stimulation or culture on high stiffness substrates. [246] Given the fact that intestinal fibrosis leads to surgery and morbidity associated with its complications and the relevant loss of intestinal surface this novel role of vitamin D may be promising.

1.8.3 Vitamin D in cancer prevention in IBD:

As patients with IBD are at increased risk of colorectal cancer and vitamin D has been shown to prevent cancer growth and proliferation by inhibiting growth factors and inducing apoptosis, its role as a possibly chemopreventive agent for colorectal cancer has been investigated. [201] In mice, colonic tumour development was inhibited by increasing the dietary intake of vitamin D. [247] Additionally, cyclin D1 levels – a protein linked with early colorectal tumours - have been documented to be higher in the descending colon of the VDR KO mice in comparison to the heterozygote and wild type mice. [248]

In a large prospective case control study, the serum levels of vitamin D were inversely related to the incidence of colorectal cancer. In fact, patients whose levels were at the highest quartile, were 40% less likely to develop colorectal cancer in comparison to those of the lowest quartile. Although serum levels were related with the risk of colorectal cancer, this was not related to the dietary intake of vitamin D and calcium. [249] Additionally, higher vitamin D levels were associated with reduced colorectal cancer specific [HR 0.69, (95% CI 0.50 – 0.93), p=0.04] and overall mortality [HR 0.67 (95% CI 0.50 – 0.88), p=0.01]. [250] However, these results were not reproduced in a cohort of Finnish male smokers. [251] Furthermore, a prospective trial of oral supplementation with small dose (400 IU) of vitamin D per day in 36282 post menopausal women, showed no difference in the incidence of colorectal cancer between the two groups. [252]

However published studies on vitamin D in IBD do not fully explore important aspects of its possible role on the response to the already applied treatments and the potential role of ethnicity.

1.9 The medical management of IBD

The medical management of IBD traditionally includes strategies of induction to remission and maintenance of clinical remission. This can be achieved with a variety of medication. In this section I will summarize the most important aspects of the evidence base for the current use of medical treatments. As this represents a large volume of literature, I will mostly concentrate on EEN.

1.9.1 5-ASAs

Treatment with sulphasalazine or 5-ASAs represents the most common current treatment for induction to remission in patients newly diagnosed with ulcerative colitis. These can be delivered orally or rectally according to the extent of the disease and can be successful in inducing clinical remission. [3] The ASCEND 3 trial proved that among patients with moderately active disease, 4.8 g of mesalamine per day can improve disease activity in up to 70% of patients and induce clinical remission in 43% of them. [253] A recent study evaluating the usefulness of prolonged release mesalazine in patients with mildly to moderately active UC proved that remission can be induced in up to 60% of patients with the rate of complete endoscopic remission being 47% among those who received 4g once daily and a favorable safety profile. [254] Reassuringly a meta-analysis of the mucosal healing rates of UC patients treated with 5-ASAs, showed a cumulative rate of 44%. [255]

Mesalamine has been proved to be more efficacious than placebo in maintaining remission in ulcerative colitis patients with a success rate of 59% vs 40% for placebo. [256] This is in agreement with previously published results that show a maintenance of

remission rate of 64% for mesalazine vs 38% for placebo [257] and confirmed by a Cochrane analysis. [258]

The outcomes of the use of 5-ASA compounds for the induction and maintenance of remission in Crohn's disease have been extensively studied and are more controversial. Some studies have proved that 5-ASAs are not superior to placebo in improving the disease activity. [259, 260] Others proved that a high dose of oral aminosalicylates [261] can be more efficacious than placebo (placebo response 18%) in inducing remission to 43% of patients with mild or mild to moderately active ileal or ileocolonic disease. However, a later meta-analysis of three trials of high dose oral mesalamine (4g/day) showed that in comparison to placebo, mesalamine reduces the CDAI by a net of 18 points, proving that the evidence for the use of mesalamine in inducing remission among Crohn's disease patients is at best equivocal. [262]

Similarly, the data on the use of 5-ASAs in maintaining remission in Crohn's disease patients are conflicting. In a multicenter randomized control study, sulfasalazine was of no benefit when compared to steroids, combination of steroids and sulfasalazine and placebo. [263] Another study comparing high dose mesalamine with placebo for the maintenance of remission in Crohn's disease patients who were induced to remission with steroids showed that the mesalamine group did not gain any benefit in terms of numbers of patients in successfully maintained in remission and time to first relapse. Contrary to the previous study, a trial including 125 Crohn's disease patients in remission proved that 2.4g of 5-ASA per day are superior to placebo in maintaining remission for at least 12 months. However, a Cochrane review analyzing the results of 8 placebo

controlled studies concluded that there is no evidence to suggest a benefit for 5-ASAs over placebo for the maintenance of remission in Crohn's disease. [264]

1.9.2 Corticosteroids

The usefulness of steroids for the treatment of ulcerative colitis was first described in a landmark randomized controlled trial in 1954, [265] and further validated by others. [266] Their success in managing active inflammation is achieved by the modulation of genes involved in the regulation of the innate immune response, the suppression of Th1 and Th2 responses, [267] the inhibition of white cell migration and adhesion through the down regulation of L-selectin, chemokine release and pro-inflammatory cytokine release. Additionally steroids block the maturation of dendritic cells to a mature phenotype expressing MHC II molecules and co-stimulatory molecules such as B7.1, B7.2 and ICAM-1 blocking T cell activation. [268] Despite the high success rate of steroids in managing active ulcerative colitis, their role in maintenance of remission is limited to the point of being considered as a not acceptable strategy by their dose and duration dependent adverse events as osteoporosis, osteonecrosis, hyperglycaemia, glaucoma, cataracts, cushingoid appearance and the increased risk of infections. [269]

Steroids have been proved more effective than placebo in inducing remission in Crohn's disease patients, [270] and the addition of sulphasalazine did not provide a therapeutic benefit, [271] hence they have been the mainstay of treatment for Crohn's disease for decades. Their efficacy in treating active inflammation was confirmed on a meta-analysis that quotes a benefit of up to 30% more than placebo in inducing remission. [272] However, when penetrative complications are present (perianal fistulas, Crohn's mass)

steroids can be associated with increased risk of sepsis, perioperative complications and death. [273]

Apart from the risks associated with the use of steroids in Crohn's disease, there are other issues that limit their use as a maintenance treatment. About 70% of patients who achieve remission with corticosteroids do not achieve mucosal healing, perhaps because of the inability of steroids to control submucosal inflammation. Hence about 50% of patients who entered remission with steroids will clinically relapse within a year. [274] In clinical trials, both prednisolone [270] and budesonide [275] have failed as maintenance strategies, although budesonide was better than placebo, but with a median time to relapse of 179 days.

1.9.3 Exclusive enteral nutrition

The use of steroids is a particular challenge among paediatric Crohn's disease patients. Almost 30% of paediatric patients experience growth retardation [276] which has been proved to be relevant to chronic active inflammation. [277] Given the proven negative impact of steroids in children's bone mineral density [278] and growth [279], managing Crohn's disease with minimal or no use of steroids at all would be beneficial.

1.9.3.1 The use of enteral nutrition as primary therapy for Crohn's disease

The use of enteral diets was initially described as a means of support, rather than treatment among patients hospitalized post small bowel resections, acute pancreatitis and acute flares of IBD. [280] The first report of the use of elemental diet in IBD patients was

in 1973: 13 IBD patients were treated with an elemental diet for an average of 22 days achieving a significant nutritional benefit with very good tolerability. [281] Subesequently, in 1980, it was used for the management of growth retardation in 4 children with ileal or ileocolonic disease. The growth velocity achieved during the 6 weeks of enteral nutrition was impressive and the disease activity reduced, although according to the presented data, 2 of the patients had inactive disease and 2 had mild to moderate active disease. [282] In a study of the effect of exclusive elemental diet in gastrointestinal protein loss, exclusive elemental diet was trialed open label on 7 patients with extensive jejunoileal Crohn's disease. There was a 47% reduction in plasma protein loss with a corresponding increase in plasma protein levels and although clinical response was not formally assessed, all patients reported increased energy levels and improvement of their overall well-being. [283] At the same time, a French publication reported that exclusive enteral nutrition was effective in treating active inflammation and bypassing steroid dependency in 17 children with active Crohn's disease based on clinical and laboratory criteria. [284] The results were repeatable in a further cohort of 10 children treated with enteral nutrition for three weeks by the same group; all children went into clinical remission from a moderate or moderate to severe disease activity on baseline. [285] The results of these trials were put in context when exclusive elemental diet as a means of induction to remission in paediatric and adolescent patients with Crohn's disease was compared to high dose steroids in a randomized controlled trial. 17 patients were recruited and treated with either high dose steroids or exclusive enteral nutrition for 6 weeks and the outcome in achieving clinical remission as measured by the Lloyd – Still disease activity index, biochemical markers and body weight was similar between the 2

groups. Interestingly, although the weight gain was similar between groups, the height velocity was significantly higher among the nutritionally treated patients, after six months of starting treatment. [286] The results of this trial were replicated in two metachronous randomized trials: One including 19 paediatric patients with Crohn's disease that were randomized to an exclusive whole protein enteral diet (n=10) or prednisolone [287] and one including 20 adult patients with mild to moderate active Crohn's disease thay were randomized to receive corticosteroids or exclusive enteral nutrition for 2 weeks. [288] In both trials, EEN was proved to be at least as efficacious as treatement with corticosteroids in inducing clinical remission. Similar results were produced by a more recent retrospective study of 105 paediatric Crohn's disease patients (36 having received EEN and 69 corticosteroids) showing a remission rate of about 90% for both groups. [289]

The studies described above provide enough evidence to support the use of EEN in Crohn's disease patients. However, there is heterogeneity in the disease phenotype of patients included and the type of feed used making it difficult to extract safe conclusions regarding the best patient selection and practice in EEN in order to achieve optimal results.

1.9.3.1.1 Response to EEN and disease location

A prospective open label study of the effect of EEN in 65 children with Crohn's disease suggested that EEN is less efficient in inducing remission to patients with colonic disease compared to those with ileal or ileocolonic (50% vs 91% and 82% respectively). It is noteworthy that post treatment improvement in endoscopic scores of disease activity was

not observed in patients with colonic Crohn's disease. [290] However, a different study using EEN in 110 children with Crohn's disease for 8 weeks suggested that remission rates in patients with colonic disease are about 80% and not different to the rest of patients included in the study and therefore that disease location does not influence the achievement of remission with EEN. [291]

1.9.3.1.2 Are all formulas similarly efficient?

The composition of the feed administered has been a significant point of interest in research attempting to optimize the results of nutritional therapy for Crohn's disease. The favorable result of enteral nutrition in achieving clinical remission was achieved with elemental but not necessarily with polymeric diets in a randomized control study of 30 patients (75% vs 36% respectively). [292] However, this result was not reproducible in other studies. In a randomized trial including 32 patients, Gonzalez-Huix et al report similar efficacy of a polymeric diet that was administered through a naso-gastric tube with high dose corticosteroids in achieving clinical remission (80% vs 88%, p=0.4). Of note, one of the two patients that failed treatment with corticoteroids during the first week of treatment, was treated with a polymeric diet and went into remission. [293] Similarly, a trial comparing an amino-acid based low fat diet to a peptide based high fat diet in adults with Crohn's disease reported similar efficiency in inducing remission between the two treatment arms. [294] The same result was reproduced in a study comparing an amino-acid with whole protein enteral feeding. [295] Further trials confirmed the equal efficiency of polymeric and elemental diets in treating active Crohn's disease in children: Ludvigsson et al performed a trial of elemental vs polymeric diet for active Crohn's

disease in 33 children showed similar efficiency between the two formulas in achieving remission and favored the polymeric diet in terms of nutritional benefit. [296] Grogan et al compared elemental with polymeric diet in 34 children with newly diagnosed Crohn's disease. Elemental and polymeric diet achieved similar rates of clinical remission (90% and 80% respectively, p = ns) and within a two year follow up the time to clinical relapse was also similar between groups. [297] Rigaud et al reported equal and high rates of remission in difficult to treat adult patients with Crohn's (n=30) who had failed treatment with corticosteroids and were randomized to receive an exclusive diet with either an elemental or a polymeric formula. 53% of patients were in clinical remission after two weeks of treatment and 70% after four weeks of treatment with no difference in remission rates between the two groups. Interestingly, most of the patients taking part in this study suffered from colonic Crohn's disease. [298] The equality in efficiency between elemental and polymeric diets has been confirmed by a Cochrane review in 2007. [299]

1.9.3.2 EEN in adults with Crohn's disease

Traditionally, enteral nutrition has been a treatment reserved for the paediatric patients. More recently its role has been investigated for the management of active mucosal inflammation among adult patients; In 1984 O'Morrain et al reported comparable disease activity improvement with corticosteroids or exclusive elemental diet among adult patients with Crohn's disease. [300] In a study of exclusive polymeric diet among adult Crohn's disease patients that required hospitalization in 3 centers in UK, steroids and enteral nutrition had similar efficiency in inducing remission in the per protocol population. However, 40% of the patients that were initially randomized to receive EEN

did not tolerate enteral nutrition due to either its poor palatability or due to the discomfort associated with a nasogastric tube. [301] A multi-center controlled trial from East Anglia involving 124 patients showed that in the intention to treat population, elemental diet was shown to be efficient at inducing remission in 84% of patients who tolerated it. However 30% of patients initially enrolled in the study did not tolerate it. [302]

At a higher level of evidence, several meta-analyses attempted to confirm the benefit of EEN in inducing remission in children with Crohn's disease with variable results. A meta-analysis of 8 trials comparing EEN with corticosteroids showed that on an intention to treat basis, corticosteroids are more efficient than EEN in inducing clinical remission. However, this was influenced to a certain degree by a 21% intolerance / non-adherence rate of the population receiving EEN. [303] A later mata-analysis including 571 patients that were treated with EEN reported a cumulative remission rate of 70% which is similar to that of corticosteroid treatment. [304] This was confimed by another meta-analysis of 5 trials [305] and a meta-analysis of 11 clinical trials proving similar efficacy of corticosteroids with EEN in inducing clinical remission in Crohn's disease patients. [306] However, a Cochrane review concluded that corticosteroids are more effective than EEN in inducing clinical remission in Crohn's disease patients. This was according to the authors possibly influenced by the significant variability between the trials included in the reported remission rates, likely secondary to the differences between the included populations, the disease activity measures, the interventions employed to administer EEN and the trial methodology. [299]

1.9.3.3 Enteral nutrition for maintenance of remission

Having been proven that exclusive enteral nutrition is an excellent option for the induction of remission in patients with Crohn's disease the possibility of using enteral nutrition for the maintenance of remission has attracted analogous interest. When children that received EEN achieved clinical remission, the probability of maintaining remission was significantly higher for those who continued receiving enteral nutrition supplementary to their normal diet. At 12 months 79% (15/19) of children who received supplementary enteral nutrition were in clinical remission compared to 43% (12/28) of those who didn't and this was statistically significant. [307] In the same study, patients who carried on with partial enteral nutrition achieved better height velocities within the 12 months of follow up. [307] In a study of partial EN in steroid dependent patients who were in remission, administration of supplementary EN helped to wean off steroids and 43% of patients ramained in steroid free remission at 12 months. [308] In a trial of "half enteral nutrition" in adults, patients were randomized to receive either unrestricted diet or 50% of their daily requirements through an enteral feed. Remission had been induced with steroids, EEN, TPN, Infliximab and/or surgery and within the two years of follow up 64% of the patients having an unrestricted diet presented with a relapse compared to 35% of patients who received "half enteral nutrition". [309] These two studies were included in a Cochrane review suggesting that supplementary enteral nutrition may be effective in maintaining remission. [310] In another study among adult Crohn's disease patients that required hospitalization for the management of their Crohn's, administration of at least 900 Kcal daily in the form of enteral nutrition was an independent factor predicting non-hospitalisation, especially for those with ileal involvement. [311]

Similarly, among 145 patients that were treated with total parenteral nutrition to achieve remission, the recurrence rate was significantly lower among those that continued to receive partial enteral nutrition during the maintenance period. [312]

In the era of biologic therapies for Crohn's disease, partial EN has been investigated as a possible factor that could enhance or maintain for longer the effect of anti-TNF therapy and the data are mostly conflicting. The first published prospective clinical trial of enteral nutrition during maintenance treatment with Infliximab in patients who had been induced to remission with Infliximab included 56 patients who were randomized to receive a night EN infusion with a day low fat diet, or carry on their usual dietary pattern. Although the percentage of patients who were still in remission was higher in the EN group that result did not reach statistical significance. [313] However, a retrospective study from the same country (Japan) included 102 patients with a mean follow up of 18 months and reported significantly higher cumulative remission rates among patients who received concomitant EN compared to those who didn't. 31% of patients on concomitant EN had their inflammatory markers raised or required IFX treatment intensification as compared to 58% of patients who didn't receive concomitant EN ($p<0.01$) and multivariate analysis confirmed that partial EN was the only factor associated with maintenance of remission. [314] Similar results were obtained from another retrospective Japanese study including 85 patients. Sustained remission was associated with the amount of EN used concomitantly to IFX and patients receiving at least 600 Kcal of EN per day were more likely to sustain remission. [315]

The studies described above provide very encouraging results on the usefulness of partial EN on the maintenance of remission in Crohn's disease with or without the concomitant

use of biologics. However, they are heterogeneous in their definition of relapse and lack of endoscopic data. In this context the studies of EN in the prevention of post operative recurrence become even more meaningful. Yamamoto et al conducted a randomized control study of post operative partial EN in patients who had undergone ileal resection or right hemicolectomy due to obstructive or penetrative complications of Crohn's disease and had clear margins post-operatively: patients who were randomized to receive about 50% of their daily calorific needs through EN had significantly lower rates of clinical (5% vs 35%, $p<0.05$) and endoscopic (30% vs 70%, $p<0.05$) recurrence 12 months post surgery. [316] When the follow up of this study was extended to 5 years, the EN group presented significantly reduced recurrence requiring treatment with biologics, however the cumulative recurrence and reoperation rates, although lower in the EN group, did not reach statistical significance. [317] Similarly, Esaki et al reported significantly less cumulative post – operative recurrence rates in patients receiving at least 1200 Kcal of enteral nutrition after surgery. [318]

1.9.3.4 Enteral nutrition and mucosal healing

Exclusive enteral nutrition significantly improves patients' nutritional status. Following treatment with EN, the weight z scores of paediatric patients normalize and this is related to an improvement in body water, fat and protein composition. Similarly, an increase in height velocity and the normalization of insulin like growth factor – 1 (IGF – 1) has been described. [319] However, it is proved that the clinical benefits achieved with enteral nutrition are beyond nutritional improvement. Clinical remission in Crohn's disease patients is associated with mucosa healing and a decrease in the mucosal expression of

pro-inflammatory cytokines mRNA such as IL-1, IL-8 and IFN-γ and an increase in mucosal anti-inflammatory cytokines such as TGF-β [320] The effect of mucosal healing was not observed in patients receiving steroids when compared to enteral nutrition. [321] In an excellent study of 37 children randomized to enteral nutrition or oral corticosteroids for 10 weeks, which was followed by endoscopic re-evaluation, it was demonstrated that although the rates of clinical remission were similar, only 33% of steroid treated patients achieved mucosal healing as compared to 74% of patients treated with exclusive enteral nutrition. (figure 1.14) Similarly the histological activity scores were lower among the EEN treated patients. [322] The effect of EEN in achieving endoscopic improvement in patients with Crohn's disease was reconfirmed by another study of 34 paediatric patients reporting a 60% rate of early endoscopic response with a 42% rate of complete mucosal healing among patients who tolerated treatment. [323] These results imply an interaction between enteral nutrition and the mucosal immune system in Crohn's disease patients.

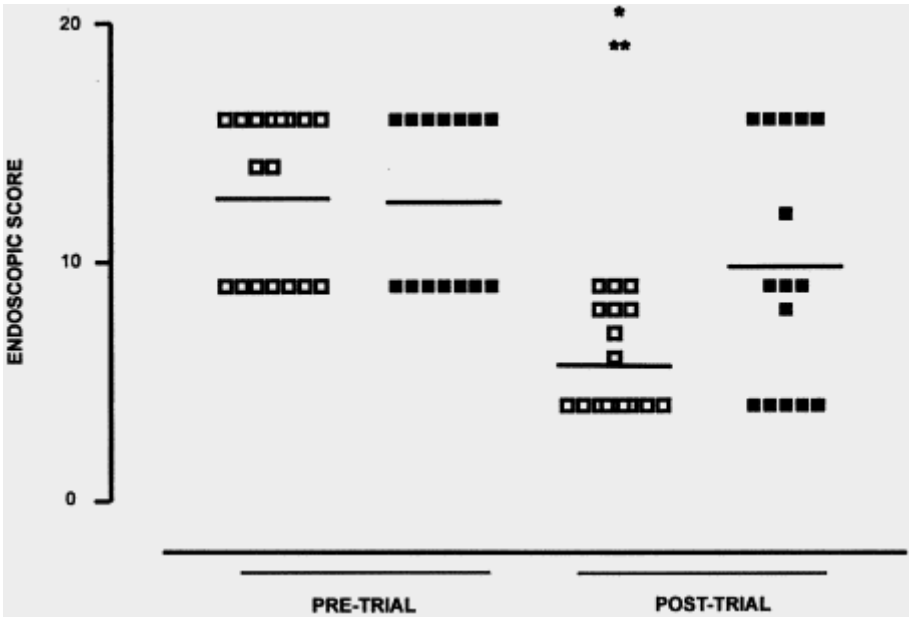


Figure 1.14: Crohn's disease endoscopic severity index scores in two groups of patients pre and post treatment with exclusive enteral nutrition (open squares) or oral corticosteroids (filled squares). (Borrelli O et al, Clinical Gastroenterology and Hepatology, 2006. **4**(6): p. 744-53) [322]

In ulcerative colitis, although an improvement in the degree of diarrhea has been observed, it was not linked with any mucosal improvement. [324]

1.9.3.5 Long term outcomes of exclusive enteral nutrition

Given its efficiency in inducing remission, achieving mucosal healing and improving nutrition, the favorable long term outcomes with EN come as a natural consequence. In a retrospective British study of 44 paediatric patients it was shown that in a median follow up of 3 years 38% of patients did not relapse and the median time to relapse was 54 months (and almost double if the large bowel is not involved). The use of steroids was

avoided in almost 50% of cases and when required their use would be postponed by 68 weeks. [325] Similar results were obtained in a randomized controlled study of elemental versus polymeric diet in paediatric Crohn's disease where a third of the patients remained in remission at the end of a two-year follow up, [297] and a retrospective study reporting a 40% relapse free survival at two years for paediatric patients treated with EEN. [326] Another study attempted to elucidate any differences in the outcome of EEN when this was used more than once in order to treat active Crohn's disease. [327] In this study of 52 paediatric patients (of whom 40 were newly diagnosed) 92% achieved clinical remission at the end of the first course of EEN and the remission rates were similar between newly diagnosed and previously treated patients. Of those, 67% relapsed within the subsequent 12 months and when a second course of EEN was used to treat their relapsing disease, remission was achieved in 62% of cases. [327]

These results show a very promising outcome in the long term, however the follow up period was not even for all patients and some of the patients included would be followed up for just 1 year. Additionally important aspects of the disease evolution as the need for treatment escalation and surgery were not described.

The long term outcomes of induction to remission with EEN have not been extensively described in adult patients. A prospective study from 3 London hospitals suggests that more than 90% of adult patients induced to remission with EEN will remain in remission after a year. [301]

1.9.3.6 The therapeutic mechanisms of enteral nutrition

Although the use of enteral nutrition as an induction strategy is established knowledge and in many institutions practice, it is still not well known how enteral nutrition delivers its therapeutic benefit. Possible mechanisms apart from bowel rest and nutritional improvement include the modulation of the intestinal microflora and the modulation of the patient's immune response.

1.9.3.6.1 The effect of enteral nutrition on the intestinal microbiota

The role of the composition of intestinal microflora in the pathogenesis of IBD, is well established given the role of antibiotics in treating Crohn's disease, the improvement of colonic inflammation with faecal diversion and the data from germ free animal studies suggesting the necessity of the microbiota for the occurrence of IBD. A study of 6 newly diagnosed paediatric Crohn's disease patients treated with EN, using the faecal 16s DNA to describe the faecal microbiota proved that the diversity of the Eubacteria, Bacteroides and coccoides of patients is significantly changing on the course of time with treatment administration compared to controls. The change in microbiota composition remains after discontinuation of treatment and is related to the decrease in the disease activity index. [328] Similar results were obtained by others. [329] The correlation of change in microbiota with the disease improvement with EN poses the question on whether the alteration in the microbiota is the outcome of EN through which EN exerts its anti-inflammatory action, or whether the shift in microbiota pattern is the result of the improvement of mucosal inflammation. In fact a study of 15 patients who were on enteral nutrition with a polymeric diet due to upper GI obstructive lesions or dysphagia

secondary to neurological deficit proved that the composition of the microbiota did not change over time with EN, however the concentration of acetate, butyrate and total short chain fatty acids significantly increased with EN. [330] This positive effect of EN on the short chain fatty acids is opposite to the results obtained from other studies that have proved a negative or neutral effect. [331] These results imply that possibly EN does not achieve its therapeutic benefit through the modification of the microbiota, although it must be noted that these studies characterize the luminal microbiota which could be different to the mucosal microbiota.

However there is a possibility that at least a part of the anti-inflammatory effect of EN occurs through its impact on the metabonomic profile of the intraluminal bacteria: In a study using faecal samples of 18 paediatric patients having undergone treatment with EEN, there was a significant decrease in the pro-inflammatory acetic acid and an increase in the anti-inflammatory butyrate. [332]

1.9.3.6.2 Enteral nutrition and the intestinal epithelium

Given the limitations of the "microbiota" hypothesis, taking into account the direct and inevitable interaction of EN with the intestinal epithelium and the fact that intraluminal contents as butyrate have been proved to alter the expression of genes that co-ordinate the mucosal immune response, such as IL-8 and MCP-1, [333] the action of EN through a direct interaction with the intestinal epithelium is a very attractive hypothesis and the data supporting it are so far are encouraging but very limited. Incubation of Caco2 cells with a polymeric formula has been proved to protect the tight junction integrity from the adverse effect of TNF-α. [334] The protective effect of a polymeric formula in this experimental

setting was equally effective to that of Infliximab and more effective to that of hydrocortisone. [334] Incubation of colonic biopsies from Crohn's disease patients with elemental diet resulted in a significant increase in the ratio of IL-1 receptor antagonist to IL-1β when compared to controls. This increase in the anti-inflammatory cytokines did not occur when tissue from UC patients or non-IBD patients was incubated with elemental diet proving a direct link between enteral nutrition and the treatment of active Crohn's inflammation. [335] Another study using a variety of epithelial cell lines proved that the incubation of epithelial cells with a polymeric formula feed significantly decreased the inflammatory response (as measured through supernatant IL-8 levels) when these were stimulated with IL-1 or TNF-α. Pre-treatment with EN reduced the subsequent inflammatory response of the intestinal epithelial cells and treatment with EN after exposure to pro-inflammatory stimuli "treated" the inflammatory response successfully. This was mediated by the potentiation or inhibition of degradation of IκB and possibly the subsequent inhibition of NF-κB translocation. [336] Consistent with the in vitro data, a preceding study of patients with Crohn's disease pre and post treatment proved that the reduction in IL-2 producing cells was best achieved with enteral nutrition. [337] These data suggest that EN has a direct effect on the immunological function of the intestinal epithelium which may mediate its short and long term benefits. Additionally, given the role of IL-2 in T cell activation they imply a possible link between enteral nutrition and the inhibition of T cell activation. We will examine this possibility in the present thesis.

1.9.4 Thiopurines

It is clear that recurrent use of steroids should not be the treatment plan for either UC or Crohn's disease and that although they are effective in induction of remission a substantial number of patients will relapse. Hence in patients who require repeated courses of steroids or who become steroid dependent the development of an escalated maintenance strategy is the appropriate next step. The drugs of choice in this situation belong to the group of thiopurines and are azathioprine and 6-mercaptopurine. There is strong evidence that they can result in successful weaning of steroids and endoscopic improvement in previously steroid dependent UC patients for almost 60% of those who tolerate it. [338] Maintenance with thiopurines resulted in decreased need for further steroids and decreased requirements for hospital admissions and surgery. [339] In Crohn's disease patients, thiopurines constitute excellent options for the maintenance of remission. A Cochrane analysis confirmed that using thiopurines patients with Crohn's disease are 2-3 times more likely to be in steroid free remission. [340] However, non-adherence to thiopurines has been highlighted as a particular problem, especially in adolescents and young adults; among our patients at Barts and The London NHS Trust, 29% of adolescents/young adults were non adherent as compared to 8% of the adults. [341]

Thiopurines exert their cytotoxic activity by being incorporated into the DNA duplex, halting the cell cycle and blocking the action of some restriction enzymes and finally cell proliferation. [342] Their immune modulatory effect in IBD is also supported by the induction of apoptosis and the selective inhibition of inflammatory gene expression in activated T cells such as the α4 integrin. [343] At least a part of its apoptotic effect is

mediated through the diversion of a co-stimulatory pathway – that of CD28 – in to a pro-apoptotic pathway. [344] These data apart from confirming the role of T cell activation in the pathogenesis of IBD show that its inhibition directly or through the co-stimulatory pathways can be treatment strategies.

1.9.5 Anti-TNF-α inhibitors

The most recently developed and utilized drugs for IBD are the monoclonal antibodies against TNF-α which in literature are often described as biologics. The ones most commonly used are infliximab (which was the first to be discovered) and its fully humanized version, adalimumab. The efficiency of infliximab as a treatment option for immune mediated inflammatory disorders was initially described in rheumatoid arthritis patients [345] and soon after in Crohn's disease. [346] Infliximab as means of induction to remission can achieve remission or response for about 70% of patients reducing the need for steroid administration. [347, 348] In a landmark study, the ACCENT I trial, infliximab was proven to be efficacious as means of maintenance for Crohn's disease. Patients on infliximab were about 2-3 times more likely to be in remission at week 30 and 54 compared to those on placebo with remission rates reaching 40%. This was accompanied by weaning of steroids and improvement in quality of life scores. [349] The ACCENT II trial proved that infliximab is very effective in inducing and maintaining remission in fistulizing Crohn's disease. 70% of the 282 patients with perianal, enterocutaneous or rectovaginal fistulas achieved improvement of their fistulating disease on induction treatment with 3 doses of infliximab at weeks 0, 2 and 6. From those who achieved an initial response concerning their fistulas, 36% would be in complete response

and 46% in response from the perspective of fistulation if they were randomized to carry on treatment with infliximab as compared to 19% and 23% respectively for placebo. [350]

Similar encouraging results were obtained for adalimumab in Crohn's disease. The CLASSIC I trial showed that after 2 doses of infliximab 36% of patients with moderate to severe Crohn's disease according to CDAI who were anti-TNF inexperienced achieved clinical remission and 50% a reduction of at least 100 points in their disease activity score. [351] The superiority of adalimumab to placebo in inducing clinical remission remained in the more challenging to treat patients who had been previously intolerant to infliximab or had a secondary loss of response, with a 21% remission rate on induction compared to only 7% for placebo. [352] In another study including primary non responders to infliximab, the rate of remission on induction with adalimumab was reported to be 38% for the overall population and 29% for the primary non responders, a very encouraging result. [353] Adalimumab has been shown to be an effective treatment strategy for refractory paediatric CD as well; in a recent study 61% of paediatric patients achieved remission with adalimumab. [354]

As well as infliximab, adalimumab was proved to be an effective maintenance strategy. The CLASSIC II trial proved that about 80% of patients who achieved remission on induction would still be in remission after a year of treatment compared to 44% of patients randomized to placebo. From the patients not achieving remission with the induction regime of the CLASSIC I trial and were enrolled for open label administration of adalimumab, 35% discontinued treatment (25% of them for lack of efficacy) and 46% of the intention to treat population was in remission at week 54. [355] Another study, the

CHARM trial showed similar good results irrespectable of previous exposure to anti-TNFs. [356]

Infliximab is now licensed for use in the treatment of active ulcerative colitis as well. According to the results of the ACT-1 trial 39% of patients can achieve remission and 69% a clinical response after 3 doses of infliximab compared to 15% and 37% respectively for placebo. At the end of one year of treatment the remission and response rates fell to 20% and 37% respectively but still achieved statistical significance when compared to placebo. [357] Less encouraging were the results for adalimumab in UC. On induction treatment, adalimumab failed to achieve a statistical significance for remission compared to placebo. At the end of year 1, 22% of adalimumab treated patients were in remission compared to 12% of the placebo treated patients. The corresponding percentages were 10% and 3% respectively for patients having previously failed infliximab. [358]

Infliximab was developed as an anti-cytokine agent and its success in Crohn's disease came as no surprise given that the number of TNF-α secreting molecules are increased in active Crohn's disease. [352] Interestingly another anti-TNF drug, etanercept which is a soluble receptor to TNF-α rather than a monoclonal antibody such as infliximab is not effective in treating active mucosal inflammation when compared to placebo. [359] This shows that the healing action of infliximab is not mediated solely by cytokine blockage. In a study of 10 patients who underwent lower GI endoscopy immediately before and 24 hours after infliximab infusion it was proved, that although the number of peripheral blood lymphocytes was not influenced by treatment, in the lamina propria infliximab induced T cell apoptosis. Studies in Jurkat cells proved that incubation with infliximab

decreases their ability to produce IFN-γ and makes them prone to apoptosis by increasing the Bax/Bcl-2 ratio. [360] Although both infliximab and etanercept neutralize TNF-α effectively only infliximab induces lamina propria T cell apoptosis. [361] Similarly the population of the highly inflammatory Th17 lineage in the lamina propria of Crohn's disease patients, is significantly reduced after treatment with infliximab, [362] while the population of regulatory T cells is increased in the peripheral blood. [363] These results suggest that targeting the mucosal activated T cells can be an effective way to treat chronic active inflammation.

The mucosal immune modulation achieved with anti-TNFs influences other immune molecules as well. Recently it has been proved that MAdCAM-1, which is essential for the recruitment of lymphocytes in the colonic mucosa, is down-regulated in patients who respond to treatment with infliximab or adalimumab. This down-regulation is not the result of mucosal healing and management of mucosal inflammation but a direct pharmacological effect as it occurs when inflamed colonic biopsies are cultured in the presence of infliximab. [364] Another study of the effect of Infliximab on 69 Cell Adhesion Molecules in 61 patients with colonic IBD proved that treatment with Infliximab restores to normal levels most of the molecules that are overexpressed prior to therapy. [365]

1.10 Questions to be examined

In this thesis, we will attempt to explore the nutrient effects in inflammatory bowel disease from the angle of enteral nutrition and vitamin D. We study the role of enteral nutrition in the long term outcome of Crohn's disease. We use the real life criteria of clinical relapse and treatment escalation as markers of disease progression as our data stem from real life experience. In an attempt to examine the possibility of enteral nutrition directly affecting the T cell activation and possible ways of achieving this, we investigate the effect of enteral nutrition on the expression of CD58 in the intestinal epithelium.

We elect to study both adult and paediatric populations. I am an adult physician - gastroenterologist; however, the long term influence of EEN can be best described in children. EEN is the standard clinical practice of our Trust and therefore, selection bias which could be a limitation in the adult population – given that not all adults diagnosed with Crohn's disease receive EEN as primary treatment – is eliminated by studying a more uniform population such as our paediatric cohort. However, we also describe the course of Crohn's disease in a group of adult patients in an attempt to discuss its outcomes and the intolerance rate that has been described as a major limitation for the use of EEN among adults in the so far published literature.

Finally, given the link between ethnicity and vitamin D levels and taking into advantage the fact that our local population includes immigrants of a specific area of South Asia (Bangladesh) we aim to compare the disease course and phenotype between patients of British and Bangladeshi descent.

Chapter II: The long term outcomes of exclusive enteral nutrition when used as the primary treatment for children with newly diagnosed Crohn's disease

2.1 Background

Enteral nutrition has been proved to be at least as effective as corticosteroids in inducing remission in children with Crohn's disease [286] and in our paediatric gastroenterology unit it is used as first line treatment for children diagnosed with Crohn's disease. The data available regarding the long term outcomes when using enteral nutrition as induction treatment are limited. There is only one study including 44 patients with a median follow up of 3 years. The range of follow up was heterogeneous, between 1 and 7 years and the study analyses the data in a solely descriptive way. [325] One recently published prospective study of 34 paediatric patients that were newly diagnosed with Crohn's disease, suggested that EEN improved clinical, endoscopic and radiological disease activity. Of the 26 children that completed the course of EEN, 22 achieved clinical remission and of those, 15 achieved mucosal healing at 6 weeks. Mucosal healing at 6 weeks predicts a better outcome at 1 year in terms of endoscopic relapse, need for anti-TNF use and hospitalization. [323]

We therefore devised a retrospective study to describe the long term outcome of enteral nutrition in children diagnosed with Crohn's disease for the 5 years after their diagnosis. We sought to define the time to clinical relapse and time to and need for treatment escalation as well as time to progression of disease phenotype according to the Montreal classification. Additionally we attempted to investigate if vitamin D levels play a role.

2.2 Methods

2.2.1 Patients

All the patients diagnosed with Crohn's disease during their childhood under the care of paediatric IBD clinic at Barts and The London NHS Trust between 2003 and 2006 were included (incident cohort). Patients who did not remain under our department and for whom data were not available for a follow up of 5 years at the time of the study were excluded. All patients underwent bi-directional endoscopy and multiple biopsies and small bowel studies (MRI small bowel or small bowel follow through) in order to confirm the diagnosis of Crohn's disease according to the PORTO criteria. [366] We did not collect data for patients diagnosed with Crohn's disease prior to 2003 as Infoflex, our prospective database was introduced in 2003. We included only patients diagnosed with Crohn's disease at the Royal London Hopsital. The catchment area of the Royal London Hospital includes East London, Kent, Essex and East Hertfordshire..

2.2.2 Data collection

This was a retrospective study examining the first five years after the diagnosis of Crohn's disease in children receiving EEN for induction to remission. The patients to be included for the study were collected through the gastroenterology unit database (Infoflex). Infoflex was established by Dr NM Croft for the needs of the paediatric IBD team of the Royal London Hospital and is now expanding in the transition and adult IBD clinics. A number of variables are collected and saved prospectively in the database. These include date and source of referral, date, type of diagnosis and Montreal

classification, surgery, past medical history and family history, medication at each visit and disease activity.

Additional clinical data were collected through the outpatient clinics letters that were electronically available at the electronic patients records (EPR). Biochemistry and haematology data were collected through the electronic databases of EPR, Winpath and Clinical records system (CRS). All data were collected by two medically qualified investigators who were both working as clinical research fellows at Barts and The London to ensure the validity of the data (NK and AR). A list of all the collected variables is available in Appendix II.

The data collection for this chapter took place between March 2010 and December 2011.

2.2.3 Treatments

All patients were treated with exclusive enteral nutrition (Modulen IBD Nestle) as induction strategy under the joint care of a paediatric gastroenterology consultant and the paediatric dietician. Treatment was administered orally, but in a minority who did not tolerate it, or when concerns regarding inadequate intake existed a small bore NG tube was used. The volume of the feed was initially 1 litre per day and would gradually increase to cover the patients' needs according to dietician review. Enteral nutrition was administered as an exclusive diet; no other food apart from water and flavouring powder for the feed (made from the feed manufacturer) was allowed. Treatment duration was 6 weeks for all patients according to the British Society of Paediatric Gastroenterology, Hepatology and Nutrition Guidelines. [367] Patients were assessed about their response

to treatment and subsequently a normal diet was re-introduced under dietician follow up within the next 1-2 weeks. Gradual food re-introduction allowed for any food intolerances to be identified. The exact protocol of EEN and food re-introduction is shown in Appendix III.

Subsequent medical treatments included further courses of enteral nutrition, 5-ASAs, corticosteroids, antibiotics, thiopurines and anti-TNFs and were prescribed according to local and national guidelines by paediatric gastroenterologists of our unit.

2.2.4 Definitions of remission and relapse

Clinical remission was defined according to physician's global assessment as described in clinic letters provided that the patient was not on corticosteroids. Clinical relapse was defined according to physician's global assessment from clinic letters and / or need for treatment with corticosteroids or escalation of treatment to azathioprine, need for surgery or need for escalation to anti-TNF. We elected to use physician's global assessment in our definition for remission/relapse and not an arithmetic measure such as the paediatric Crohn's disease activity index (PCDAI) [368] mainly for two reasons: Due to the retrospective data collection from clinic letters, a number of the variables included in the PCAI would be missing. Additionally the clinical decision making regarding our patients in the clinic setting is based on the global assessment of an experienced paediatric gastroenterologist; the use of a numerical activity index such as the PCAI is more relevant in a clinical trial setting, where patients are not necessarily seen by an experienced specialty physician in order to ensure uniformity rather than our clinical setting.

Patients who achieved complete remission with exclusive enteral nutrition are characterized as responders and patients who did not achieve that are characterized as non responders.

2.2.5 IBD phenotype and disease course

Crohn's disease was phenotyped according to the Montreal Classification [369] and was recorded at diagnosis and at 5 years post diagnosis. The Montreal classification has now been superseded by the Paris classification, [23] however this work has been undertaken prior to the development of the later. The natural history of Crohn's disease was assessed by comparing the time to change in Montreal Classification; the treatments used including numbers of courses of steroids, and use of and time to use of immunosuppressant treatments and surgeries.

2.2.6 Vitamin D levels

Vitamin D status was estimated through the measurement of serum 25(OH)vitamin D levels. The optimal vitamin D levels are a matter of debate in literature as there is no clear consensus of opinions and a recent systematic review of the relevant literature suggests different cut off values for every functional endpoint of vitamin D. [370] In our study, vitamin D insufficiency was defined as 25 (OH) vitamin D levels of less than 50 ng/L as this has been described as the threshold of pharmacological intervention and deficiency as levels of less than 25 ng/L as this threshold is reportedly associated with osteomalacia. [371]

Our endpoint of disease course was analysed twice: First, according to vitamin D deficiency against non deficient patients and second, according to vitamin D

insufficiency against sufficient patients. For this part of the study only patients who had their 25-hydroxy-vitamin D levels checked during the course of follow up were included.

2.2.7 Data analysis

Data were analysed to provide results describing the time to first clinical relapse, to first use of steroids, thiopurines or anti-TNFs and the time to first surgery. Our population was divided in those who achieved clinical remission with exclusive enteral nutrition on induction and those who did not. Further analysis according to vitamin D deficiency was undertaken. The statistical tests used for univariate analysis of baseline demographic and disease specific variables were student's t-test (for continuous variables) and the chi-square test (for categorical variables). Kaplan Mayer analysis with log rank test was conducted to determine the time to clinical relapse or treatment escalation in the two groups. All analyses were 2 tailed and p-values of less than 0.05 were considered as statistically significant. All statistical tests were performed electronically with the use of statistical package for social sciences (SPSS version 16.0). Prism – GraphPad (GraphPad Prism 6) software was used for the generation of graphs.

2.2.8 Ethical approval

The data for this component of the thesis were collected as part of an audit for Barts and The London NHS Trust (Clinical Effectiveness Unit, audit number 992) and therefore did not require formal ethical approval according to the UK National Research Ethics Service (NRES) guidelines.

2.3 Results:

2.3.1 Demographics – disease characteristics at diagnosis

64 patients were diagnosed with Crohn's disease between 2003 and 2006. 6 did not have EEN as induction treatment and there were minimal data recorded for 2 other patients as follow up was not carried on with our Trust after diagnosis due to patient relocation. (Fig 2.1)

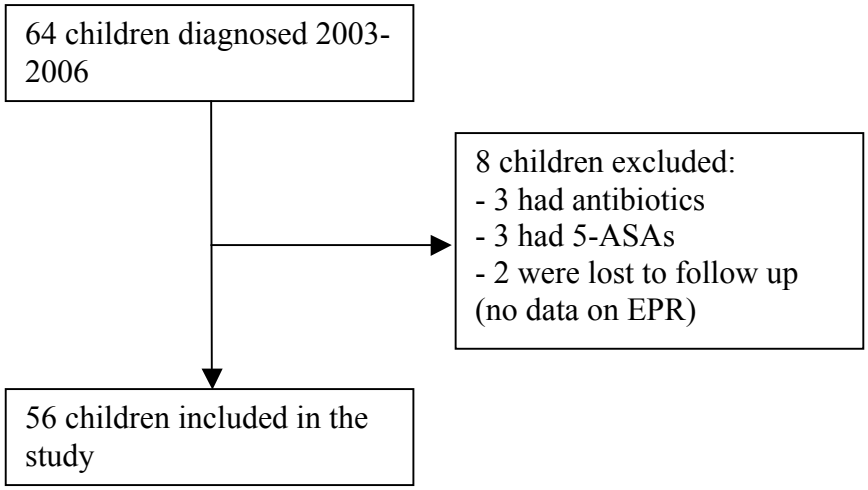


Figure 2.1: Patients included in the study

56 patients were included in the study and 71% (40/56) were male. The duration of follow up was 5 years for all patients as established by the study criteria. The mean [SD] age and CRP at diagnosis were 12.2 [2.6] years and 46.1 mg/dl [39.7] respectively. 64% (36/56) of patients were Caucasian, 21% (12/56) Asian, 9% (5/56) Afro-Caribbean and 5% (3/56) of other or mixed ethnic decent. 25% (14/56) of patients had ileal disease, 12.5% (7/56) colonic and 62.5% (33/56) ileocolonic disease. Upper GI involvement was

present in 48% (27/56) of our patients. 84% (47/56) of patients had inflammatory disease phenotype at diagnosis, 5% (3/56) stricturing and 11% (6/56) penetrative. Perianal disease was present in 30% (17/56) of our patients. The baseline demographic and disease specific characterisitics of the patients included are summarized in table 2.1. EEN was administered to all the patients included. Only 20% (11/56) of the patients required administration of the feed through NG tube and 5% (3/56) did not tolerate EEN. No patient with Crohn's disease received corticosteroids as primary treatment.

2.3.2 Disease course

After six weeks of treatment, 57% (32/56) of patients were in clinical remission, 25% (14/56) experienced a clinical response and 18% (10/56) failed to improve on exclusive enteral nutrition. Among patients who achieved clinical remission, 28% (9/32) had EEN administered via NG tube compared to 8% (2/24) of those who didn't (p=0.06) The mean [SD] CRP at 6 weeks was 12.9 mg/dl [20.4] and was significantly lower when compared to the CRP at diagnosis pairwise. Other inflammatory markers also descreased in concert; ESR from 34.4 [31.3] on diagnosis to 18.1 [20.3] after treatment ($p<0.01$) and platelet count from $564*10^3/\mu/L$ [183] to $486*10^3/\mu/L$ [140] post treatment ($p<0.01$). Serum albumin increased at the same interval from 31.4 [8.0] to 39.1 [5.0] ($p<0.01$).

By the end of year 1 and 5, 45% and 80% of patients had clinically relapsed respectively. The mean [SD] time to clinical relapse was 23.7 [20.8] months. 62.5% of our patients required treatment with corticosteroids over the 5 years of follow up. The median [range] number of courses of corticosteroids required overall was 2 [0 – 6] and the mean [SD] time to first course of steroids was 29.9 [25.3] months. 79% of patients required treatment

with thiopurines, 19% required treatment with anti-TNFs and 32% required surgical management with mean [SEM] time to treatment escalation 30.7 [20.0], 51.8 [16.0] and 51.1 [16.1] months respectively. The data described above are summarized on Table 2.1

Characteristics		All patients
Gender male, n (%)		40 (71.4)
Ethnicity	Caucasian, n (%)	36 (64.3)
	Asian, n (%)	12 (21.4)
	Afrocaribbean, n (%)	5 (8.9)
	Other, n (%)	3 (5.4)
CRP at diagnosis, mg/dl		46.1 (39.7)
Alb at diagnosis, mg/dl		31.4 (8.0)
Age at diagnosis, years		12.2 (2.6)
Vitamin D checked ever, n (%)		42 (75)
Vitamin D levels, ng/L		36.6 (25.2)
Vitamin D insuficiency, n (%)		33 (78.6)
Vitamin D deficiency, n(%)		19 (45.2)
Location	Ileal, n(%)	14 (25)
	Colonic, n(%)	7 (12.5)
	Ileocolonic, n(%)	35 (62.5)
	+ L4, n(%)	27 (48.2)
	+ perianal, n(%)	17 (30.4)
Behaviour	Inflammatory, n(%)	47 (83.9)
	Strictuting, n(%)	3 (5.4)
	Penetrating, n(%)	6 (10.7)
Clinical relapse in 1 year, n (%)		25 (44.4)
Clinical relapse in 5 years, n (%)		45 (80.4)
Time to relapse, months		23.7 (20.8)
Need for corticosteroids, n (%)		35 (62.5)
Time to corticosteroids, months		29.9 (25.3)
Number of corticosteroid courses in 5 years, median (range)		2 (0-6)
Need for thiopurines, n (%)		44 (78.6)
Time to thiopurines, months		30.7 (20.0)
Need for anti-TNFs, n (%)		16 (28.6)
Time to anti-TNFs, months		51.8 (16.0)
Need for surgery, n (%)		18 (32.1)
Time to surgery, months		51.1 (16.1)

Table 2.1 The baseline demographics and disease specific characteristics and the disease course after primary treatment with EEN of our population

All values express Mean [SD] unless otherwise indicated

2.3.3 What is the meaning of the initial response to enteral nutrition?

The clinical course of Crohn's disease differed significantly between the patients who achieved clinical remission at diagnosis when compared to those who did not. The baseline demographics and disease characteristics did not differ significantly between the two groups apart from that perianal disease was more prevalent among those who did not achieve remission with EEN. (Table 2.2) 58% (14/24) of the responders and 34% (11/32) of the non responders to EEN relapsed clinically within the first year of diagnosis (p=0.07). Within the five years follow up period more non-responders would relapse clinically and this would be sooner in comparison to patients who achieved clinical remission with EEN as primary treatment. (Figure 2.2)

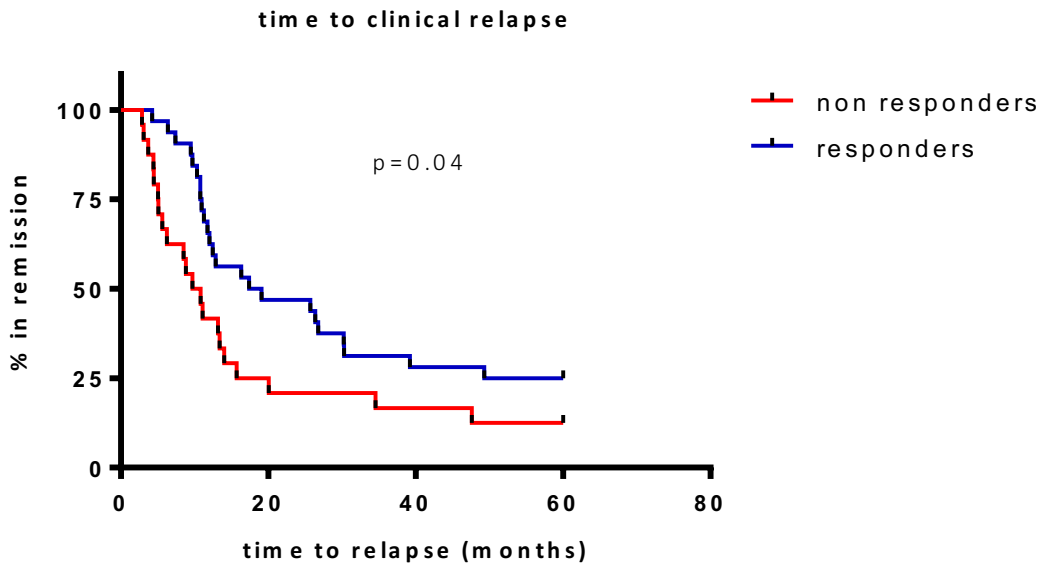


Figure 2.2: Survival analysis of time to clinical relapse between responders and non responders to EEN. Within the five years follow up period more non-responders would relapse clinically and this would be sooner in comparison to patients who achieved clinical remission with EEN as primary treatment

Significantly less responders (50%, 16/32) required treatment with corticosteroids over the course of 5 years when compared to non responders (79%, 19/24) (p=0.03). Moreover, the time to first steroid use was significantly longer among the responders and the total number of steroid courses over 5 years was significantly lower in this group, confirming that achievement of remission with EEN as the primary treatment results in less frequent, less likely and later exposure to corticosteroids. (Table 2.2, figure 2.3)

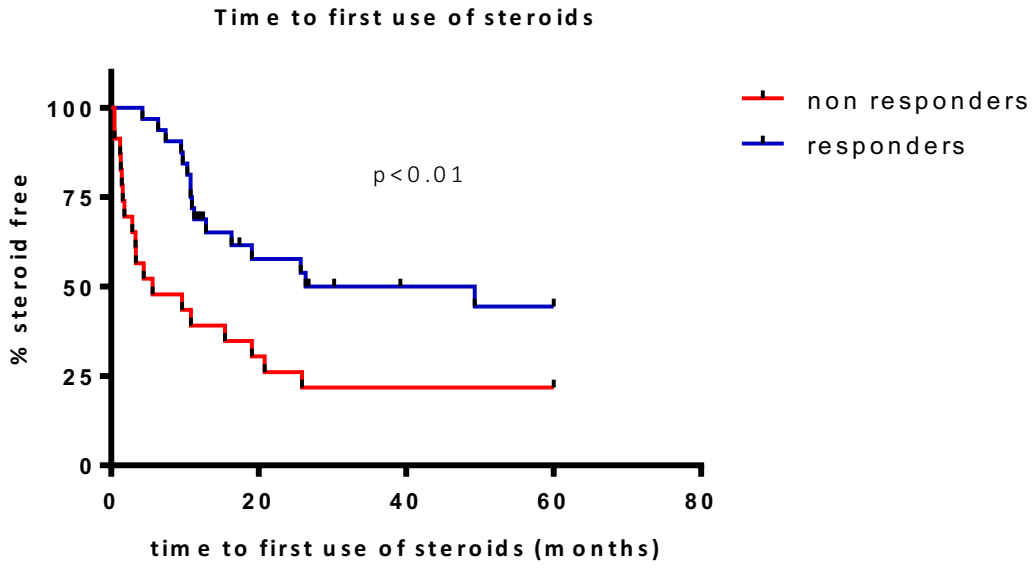


Figure 2.3: Survival analysis of time to first use of corticosteroids among responders and non responders to EEN. The need for use of corticosteroids was less among responders to EEN as primary treatment for Crohn's disease. The time to first use of corticosteroids was longer in that group.

The proportion of patients who required treatment with thiopurines, anti-TNFs or surgical intervention did not differ significantly between groups (Table 2.2), neither did the time required for the patients to be escalated to these treatments. However, there was a trend towards earlier use of thiopurines among patients who did not achieve clinical remission with EEN (25.3 months [20.0] for non responders vs 34.7 months [19.2] for responders, p=0.06) (figure 2.4), showing the need for early aggressive medical management in those who fail to achieve remission with EEN.

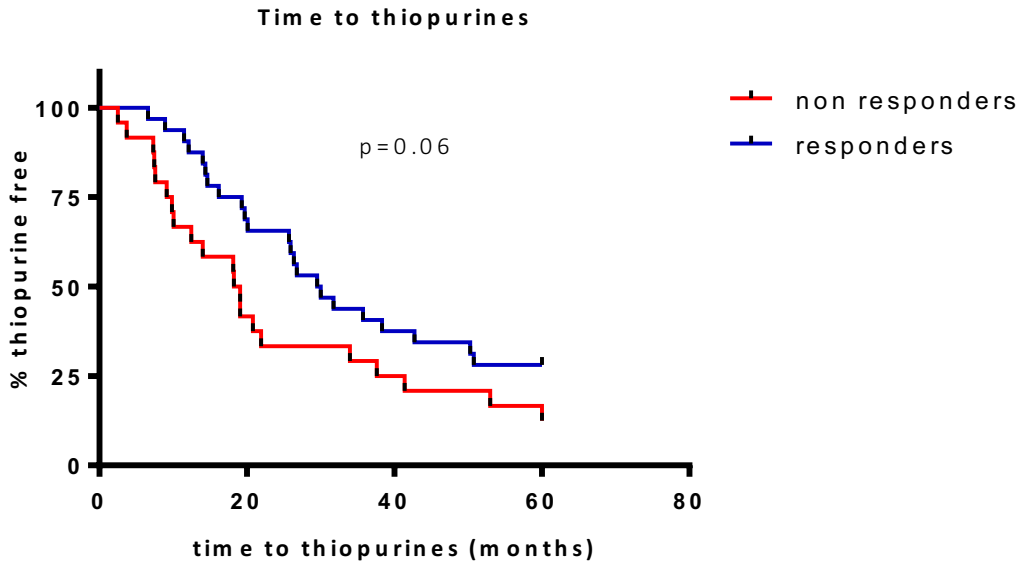


Figure 2.4: Survival analysis of time to first use of thiopurines among responders and non responders to EEN

Characteristics		Remission achieved n=32	Remission not achieved n=24	p - value
Gender male, n (%)		22 (69)	18 (75)	0.61
Ethnicity	Caucasian, n (%)	17 (53.1)	19 (79.2)	0.06
	Asian, n (%)	9 (28.1)	3 (12.5)	
	Afrocaribbean, n (%)	2 (15.6)	0 (0)	
	Other, n (%)	2 (8.3)	1 (3.1)	
CRP at diagnosis, mg/dl		49.8 [45.7]	41.0 [29.5]	0.47
Alb at diagnosis, mg/dl		30.5 [9.6]	32.5 [5.3]	0.39
Age at diagnosis, years		12.0 [2.7]	12.4 [2.6]	0.53
Vitamin D checked, n (%)		25 (78.1)	17 (70.8)	0.53
Vitamin D levels, ng/L		33.4 [4.6]	41.4 [6.9]	0.32
Vitamin D insuficiency, n (%)		20 (80)	13 (76.5)	0.78
Vitamin D deficiency, n(%)		13 (52)	6 (35.3)	0.29
Location	Ileal, n(%)	9 (28.1)	5 (20.8)	0.65
	Colonic, n(%)	3 (9.4)	4 (16.7)	
	Ileocolonic, n(%)	20 (62.5)	15 (62.5)	
	+ L4, n(%)	17 (53.1)	10 (41.7)	0.40
	+ perianal, n(%)	6 (18.8)	11 (45.8)	0.03
Behaviour	Inflammatory, n(%)	27 (84.4)	20 (83.3)	0.89
	Strictuting, n(%)	2 (6.2)	1 (4.2)	
	Penetrating, n(%)	3 (9.4)	3 (12.5)	
Clinical relapse in 1 year, n (%)		11 (34.4)	14 (58.3)	0.07
Clinical relapse in 5 years, n (%)		24 (75)	21 (87.5)	0.24
Time to relapse, months		28.2 [21.0]	17.8 [19.2]	0.04†
Need for corticosteroids, n (%)		16 (50)	19 (79)	0.03
Time to corticosteroids, months		21.7 [15.9]	6.8 [7.9]	0.002†
Number of corticosteroid courses in 5 years, median [range]		0.5 [0-4]	3 [0-6]	0.008
Need for thiopurines, n (%)		23 (71.9)	21 (87.5)	0.16
Time to thiopurines, months		34.7 [19.2]	25.3 [20.0]	0.06†
Need for anti-TNFs, n (%)		7 (21.9)	9 (37.5)	0.2
Time to anti-TNFs, months		53.1 [15.4]	50.2 [16.9]	0.23†
Need for surgery, n (%)		9 (28.1)	9 (37.5)	0.46
Time to surgery, months		53.0 [13.7]	48.5 [18.8]	0.42†

Table 2.2: Demographic and disease specific characteristics of responders and non responders to EEN. † denotes Kaplan – Meier analysis, log rank test. All variables are expressed as mean [standard deviation], unless differently indicated.

2.3.4 Disease course among responders and non responders excluding patients who did not tolerate EEN

We further analysed the data according to the viva comments, after excluding the three patients who did not tolerate EEN to eliminate any bias relevant to patients' intolerance to EEN. Time to clinical relapse and time to first use of corticosteroids remained statistically significantly longer among responders to EEN. The results are shown on Appendix IV.

2.3.5 Progression of disease phenotype among responders and non responders to EEN in 5 years

We compared the two groups according to the progression of their disease location and behavior. Progression of phenotype is a major complication of Crohn's disease [28] as this can lead to escalation of medical management, need for surgery and severe implications in patients' quality of life.

There was no difference in the disease phenotype as this was defined according to the Montreal classification between responders and non responders to EEN. There was a trend towards more frequent progression of disease location and behavior among the patients who did not respond to the initial induction with EEN (62.5%, 15/24) compared to those who did (37.5%, 12/32) (p=0.06) (table 2.3). This trend was mostly led by the development of new stricturing or penetrating complications which were more prevalent among the non responders (45.8%, 11/24), compared to responders (25%, 8/32) (p=0.10) and which can cause severe morbidity and disability in paediatric patients with Crohn's disease. In fact, data sub-analysis confirmed that 6.2% (2/32) of responders to EEN

developed new stricturing over the course of follow up as compared to 29.2% (7/24) of the non responders (p=0.02). Given the fact that stricturing is associated with the development of intestinal failure, [34] this result may point the need for a more proactive management of children failing their primary treatment with EEN, but a randomized study of more aggressive therapy at an earlier point will be needed before safe recommendations on this can be made. There was no difference between groups in the proportion of patients who developed new penetrative complications over the course of 5 years (18.8% of responders vs 16.7% of non responders, p=0.84).

		Remission achieved n=32	Remission not achieved n=24	p - value
Location	Ileal, n (%)	8 (25.0)	5 (20.8)	
	Colonic, n (%)	1 (3.1)	3 (12.5)	0.40
	Ileocolonic, n (%)	23 (71.9)	16 (66.7)	
	+ L4, n (%)	18 (58.1)	12 (50.0)	0.55
	+ perianal, n (%)	9 (29.0)	11 (45.8)	0.20
Behaviour	Inflammatory, n (%)	19 (59.4)	9 (37.5)	0.13
	Stricturing, n (%)	4 (12.5)	8 (33.3)	
	Penetrating, n (%)	9 (28.1)	7 (29.2)	
MC progress		12 (37.5)	15 (62.5)	0.06
	Location progress, n (%)	5 (15.6)	2 (8.3)	0.41
	New L4, n (%)	4 (12.5)	2 (8.3)	0.62
	New perianal, n (%)	5 (15.6)	2 (8.3)	0.41
	Behaviour progress, n (%)	8 (25)	11 (45.8)	0.10

Table 2.3: The final Montreal classification (MC) and the disease progression of responders and non responders to EEN

2.3.6 Disease course comparison between Caucasian and South Asian patients.

The phenotype of Crohn's disease according to the Montral Classidication did not differ between Caucasian and South Asian patients. Similarly the need for and time to steroids and treatment escalation to thiopurines, anti-TNFs or surgery did not differ between the two ethnic groups. (Table 2.4) However, more South Asian patients presented new perianal disease during follow up [4 (33.3%) compared to 3 (8.3%) Caucasian patients (p=0.03)]. Also more South Asian patients presented expansion of their disease location during follow up compared to Caucasian patients. (Table 2.5)

The vitamin D levels –when these were checked- did not differ between the two groups, neither did the incidence of vitamin D insufficiency or deficiency. (Table 2.4)

Characteristics		Caucasian n=36	Asian n=12	p - value
Gender male, n (%)		25 (69.4)	10 (83.3)	0.35
CRP at diagnosis, mg/dl		43.1 (35.6)	34.1 (27.9)	0.51
Alb at diagnosis, mg/dl		31.1 (9.2)	32.2 (6.8)	0.73
Age at diagnosis, years		12.8 (2.4)	11.1 (2.7)	0.04
Vitamin D checked, n (%)		16 (44.4)	6 (50)	0.74
Vitamin D levels, ng/L		34.7 (18.3)	26.8 (22.7)	0.41
Vitamin D insuficiency, n (%)		13 (81.2)	5 (83.3)	0.91
Vitamin D deficiency, n(%)		8 (50)	4 (66.7)	0.48
Location	Ileal, n(%)	7 (19.4)	3 (25)	0.4
	Colonic, n(%)	4 (11.1)	3 (25)	
	Ileocolonic, n(%)	25 (69.4)	6 (50)	
	+ L4, n(%)	16 (44.4)	7 (58.3)	0.4
	+ perianal, n(%)	11 (30.6)	4 (33.3)	0.86
Behaviour	Inflammatory, n(%)	30 (83.3)	10 (83.3)	0.64
	Strictuting, n(%)	2 (5.6)	0 (0)	
	Penetrating, n(%)	4 (11.1)	2 (16.7)	
Remission with EEN, n(%)		17 (47.2)	9 (75)	0.09
Clinical relapse in 1 year, n (%)		19 (52.8)	6 (50)	0.87
Clinical relapse in 5 years, n (%)		32 (88.9)	9 (75)	0.24
Time to relapse, months		20.5 (18.6)	23.2 (2.7)	0.57
Need for corticosteroids, n (%)		26 (72.2)	8 (66.7)	0.71
Time to corticosteroids, months		24.9 (24.6)	28.4 (24.3)	0.62
Number of corticosteroid courses in 5 years, median [range]		2 (0-6)	1.5 (0-3)	0.26
Need for thiopurines, n (%)		32 (88.9)	8 (66.7)	0.07
Time to thiopurines, months		26.3 (17.8)	34.6 (23.8)	0.16
Need for anti-TNFs, n (%)		10 (27.8)	5 (41.7)	0.37
Time to anti-TNFs, months		54.4 (12.4)	41.7 (23.5)	0.21
Need for surgery, n (%)		14 (38.9)	3 (25)	0.38
Time to surgery, months		48.9 (17.6)	52.5 (15.4)	0.42

Table 2.4: Demographic and disease characteristics of Caucasian and South Asian paediatric patients who received EEN as primary treatment for Crohn's disease.

		Caucasian n=36	Asian n=12	p - value
Location	Ileal, n (%)	7 (19.4)	2 (16.7)	0.45
	Colonic, n (%)	4 (11.1)	0 (0)	
	Ileocolonic, n (%)	25 (69.4)	10 (83.3)	
	+ L4, n (%)	19 (54.3)	7 (58.3)	0.81
	+ perianal, n (%)	12 (34.3)	6 (50)	0.33
Behaviour	Inflammatory, n (%)	19 (52.8)	4 (33.3)	0.27
	Strictuting, n (%)	8 (22.2)	2 (16.7)	
	Penetrating, n (%)	9 (25)	6 (50)	
MC progress				
	Location progress, n (%)	3 (8.3)	4 (33.3)	0.03
	New L4, n (%)	3 (8.3)	2 (16.7)	0.41
	New perianal, n (%)	3 (8.3)	4 (33.3)	0.03
	Behaviour progress, n (%)	11 (30.6)	6 (50)	0.22

Table 2.5: Montreal classification and phenotype progression in Caucasian and South Asian paediatric patients receiving EEN as primary treatment for Crohn's disease.

2.3.7 Response to exclusive enteral nutrition and Vitamin D

Vitamin D levels were checked in 41% (13/32) of the responders and 50% (12/24) of the non responders at some point during their follow up. There was no difference between the two groups in the mean vitamin D levels (mean [SD] ng/L 34.8 [21.1] for responders vs 31.0 [15.6] for the non-responders, p=0.62) , neither in the percentage of patients who were insufficient (76.9% [10/13] of the responders vs 91.7% [11/12] of the non-responders, p=0.32) or deficient in vitamin D (46.2% [6/13] of the responders vs 50% [6/12] of the non-responders, p=0.85).

2.3.7.1 The phenotype and course of Crohn's disease in vitamin D deficient vs vitamin D sufficient patients (vitamin D > 25ng/L)

The phenotype of Crohn's disease was similar in patients who were sufficient and deficient in vitamin D. Similar proportions of vitamin D sufficient and deficient patients presented clinical relapse and required treatment with corticosteroids within the follow up period (Table 2.6). However, there was a trend towards earlier need for treatment with corticosteroids among the vitamin D deficient patients who tended to require more courses of corticosteroids during their follow up. (Table 2.6) Our data suggest that more patients who were vitamin D deficient required earlier treatment with a thiopurine during their disease course, another indication of more aggressive disease progress, but there was no difference in the need and time to escalation of treatment to anti-TNFs and surgery (Table 2.6)

.

		Vitamin D deficient (n=12)	Not Vitamin D deficient (n=13)	p - value
Age at diagnosis, years		12.8 [2.5]	12.4 [2.2]	0.67
Location	Ileal, n (%)	3 (25.0)	5 (38.5)	0.15
	Colonic, n (%)	3 (25.0)	0 (0)	
	Ileocolonic, n (%)	6 (50.0)	8 (61.5)	
	+ L4, n (%)	6 (50.0)	6 (46.2)	0.85
	+ perianal, n (%)	6 (50.0)	5 (38.5)	0.56
Behaviour	Inflammatory, n (%)	11 (91.7)	9 (69.2)	0.16
	Stricturing, n (%)	0 (0)	0 (0)	
	Penetrating, n (%)	1 (8.3)	4 (30.8)	
Clinical relapse in 1 year, n (%)		7 (58.3)	5 (38.5)	0.32
Clinical relapse in 5 years, n (%)		11 (91.7)	10 (76.9)	0.32
Time to relapse, months		16.9 [18.5]	27.8 [22.2]	0.12
Need for corticosteroids, n (%)		10 (83.3)	8 (61.5)	0.10 †
Time to corticosteroids, months		17.1 [21.2]	34.5 [25.6]	
Number of corticosteroid courses in 5 years, median [range]		3 [0 – 6]	1 [0 – 4]	0.07 †
Need for thiopurines, n (%)		11 (91.7)	8 (61.5)	0.03 †
Time to thiopurines, months		21.9 [18.0]	39.4 [20.6]	
Need for anti-TNFs, n (%)		2 (16.7)	4 (30.8)	0.38 †
Time to anti-TNFs, months		56.0 [13.2]	47.0 [20.6]	
Need for surgery, n (%)		5 (41.7)	6 (46.2)	0.92 †
Time to surgery, months		45.0 [20.1]	47.1 [20.9]	

Table 2.6: Disease course and phenotype of patients who were vitamin D deficient or not.

† denotes Kaplan - Meier analysis, log rank test. All variables are expressed as mean [standard deviation], unless differently indicated.

2.3.7.2 The phenotype and course of Crohn's disease in vitamin D insufficient vs vitamin D sufficient (vitamin D > 50 ng/L) patients

The phenotype of Crohn's disease according to their Montreal classification on diagnosis was similar between the two groups at diagnosis. Similar proportions of patients in both groups relapsed, required treatment with corticosteroids, thiopurines and biologics and there was no difference in the time required for treatment escalation. Similar proportions of patients required surgical intervention between the two groups. (Table 2.7)

It is of note that most of the patients have been insufficient in vitamin D and only 4 of the 25 patients in total who had their vitamin D levels checked during their follow up were sufficient in vitamin D and hence our results are subject to type II error.

		Vitamin D insufficient (n=21)	Vitamin D sufficient (n=4)	p - value
Age at diagnosis, years		12.8 [2.4]	11.5 [1.8]	0.31
Location	Ileal, n (%)	8 (38)	0 (0)	0.15
	Colonic, n (%)	3 (14)	0 (0)	
	Ileocolonic, n (%)	10 (48)	4 (100)	
	+ L4, n (%)	10 (48)	2 (50)	0.93
	+ perianal, n (%)	8 (38)	3 (75)	0.17
Behaviour	Inflammatory, n (%)	18 (86)	2 (50)	0.10
	Stricturing, n (%)	0 (0)	0 (0)	
	Penetrating, n (%)	3 (14)	2 (50)	
Clinical relapse in 1 year, n (%)		10 (48)	2 (50)	0.93
Clinical relapse in 5 years, n (%)		18 (86)	3 (75)	0.59
Time to relapse, months		20.6 [20.0]	33.0 [25.4]	0.38 †
Need for corticosteroids, n (%)		15 (71)	3 (75)	0.76 †
Time to corticosteroids, months		24.9 [24.9]	32.7 [26.4]	
Number of corticosteroid courses in 5 years, median [range]		2 [0-6]	1 [0-1]	0.17 †
Need for thiopurines, n (%)		17 (81)	2 (50)	0.20 †
Time to thiopurines, months		28.1 [20.0]	46.2 [21.9]	
Need for anti-TNFs, n (%)		4 (19)	2 (50)	0.15 †
Time to anti-TNFs, months		53.6 [15.9]	39.4 [24.5]	
Need for surgery, n (%)		10 (48)	1 (25)	0.49 †
Time to surgery, months		45.5 [20.3]	49.1 [21.8]	

Table 2.7: The Montreal classification and disease course of vitamin D sufficient and vitamin D insufficient patients.

† denotes Kaplan - Meier analysis, log rank test. All variables are expressed as mean [standard deviation], unless differently indicated.

2.4 Discussion

This is one of the few studies with long enough follow up (5 years) showing that EEN is overall well tolerated and that 57% (32/56) of paediatric patients with Crohn's disease achieved clinical remission with EEN as primary treatment. These patients tended to have a better clinical outcome over the first five years from their diagnosis.

The disease extent of our population according to the Montreal Classification is comparable to results of others: Similar to the patients of the Scottish cohort [28] most of our patients were diagnosed with ileocolonic disease and about half of them had upper GI involvement.

2.4.1 The 5 year outcome of paediatric patients with CD treated with EEN as primary therapy

In our paediatric population with newly diagnosed Crohn's disease treated with exclusive enteral nutrition to induce remission, 57% of patients achieved clinical remission. Our result is in agreement to the results reported in another recent study from UK. [326] Knight et al in one of the first studies describing the long term outcomes of enteral nutrition quoted a 90% response rate, which is very close to our 82% rate of combined response or remission. [325] The mean time to clinical relapse in our patients has been 28.7 months if we include in our calculation the patients who did not relapse during the 5 year follow up. The mean [SEM] time to relapse for the patients that relapsed during the course of follow up was 14.9 [1.7] months which contrasts to the recently quoted 6.5 months [326] but is closer to the 54.5 weeks reported by others in a group of patients with similar age of diagnosis and Montreal classification to our patients. [325] This difference

could be possibly explained by the ethnic composition of our population. 22% of our patients are of Asian origin, mainly Bangladeshi – and they presented a more aggressive disease course, with a higher proportion of them developing new perianal disease and overall advancing their disease phenotype according to Montreal Classification over the course of follow up compared to white Caucasian patients. Also, we demonstrated in a study described in this thesis that patients of Bangladeshi descent with Crohn's disease have a more aggressive disease course than White Caucasian patients. Although Cameron et al do not describe the ethnic diversity of their population, taking into account demographic data of the population of Glasgow suggesting that Asian ethnic minorities represent about 7% of the population [372], we can safely assume that our study includes a greater proportion of South Asian patients.

2.4.2 Achievement of clinical remission with EEN is associated with better long term outcomes – interpretation and clinical implications

Previous studies have raised the question about whether induction to remission with exclusive enteral nutrition delays the need for treatment with corticosteroids. [325] Our results give an answer to this question and confirms that achievement of remission with enteral nutrition reduces the need for and delays the time to treatment with corticosteroids. This is explained by the associated prolonged time to clinical relapse.

Although responders to EEN who achieved remission were less likely to suffer from perianal disease, this is unlikely to account for the difference observed in the two groups in the need for and the time to first treatment with corticosteroids as in our clinical practice we avoid using steroids in patients with perianal disease. Cox regression analysis

reconfirmed that adjusting for patients with perianal disease, patients who achieve remission with exclusive enteral nutrition have less need for steroid treatment and if they do, take longer to require it (HR 0.31, [95% CI 0.15 – 0.64], p<0.01). In our patients there was a statistical trend towards less cumulative need for azathioprine for those who achieved clinical remission with EEN. This could possibly reflect the effect of perianal disease among the non responders which in clinical practice requires more often treatment with immunomodulators or anti-TNFs. However, adjusting for perianal disease, Cox regression analysis showed that achievement of remission with EEN is an independent predictor of less cumulative need for thiopurines over five years (OR 0.50, [95% CI 0.26 – 0.95], p=0.03) while perianal disease was an independent factor associated with more often and shorter interval time to the of use of anti-TNFs (HR 3.9, [95% CI 1.4 – 11.3], p=0.01).

A likely explanation of the difference observed in the long term outcome in patients who achieve remission with EEN is the achievement of mucosal healing by this group of patients as has already been described by others, [322] although this was not directly assessed in our study. Additionally, taking into account our data (described in the following chapter) about the effect of EN to the expression of CD58, the effect of EN on the luminal microbiota [328] and the proven impact of the luminal microbiota on the mucosal immunology and specifically the cytokine profile of the dendritic cells, [373] we can speculate that the beneficial impact of EEN on the long term outcome of Crohn's disease might be relevant to a direct downregulation of the pro-inflammatory response

Our results could suggest that patients who do not achieve remission with EEN should be followed up and treated more aggressively; however the trial data to support this opinion

are not adequate. In a small study of early Infliximab vs conventional management in paediatric patients who had been diagnosed with Crohn's disease for a year, there was an initial benefit from the group that received early Infliximab. However this was not evident by the end of 3 years of follow up. [374] In a larger study of paediatric Crohn's disease patients, early use of azathioprine was associated with less exposure to corticosteroids and less hospitalizations, but the follow up period of 1 year in this study is not adequate to pre-define the long term outcome. [375] Moreover, neither of these clinical trials uses any kind of risk stratification of the patients included.

2.4.3 Strengths

Our study has several strengths. First of all, although retrospective in design, it includes a consecutive number of patients maintained in a prospectively collected database with equal follow up that were treated with EEN as primary therapy for Crohn's disease. The total number of patients included is comparable to the sample of previous other studies, however the follow up period is significantly longer than what has been described by others [326] [325] investigating the long term follow up after EEN. Moreover, this is the first study attempting to investigate the impact of the response to induction treatment with EEN to the long term outcome of five years.

2.4.4 Limitations

Conversely, this work also has a number of limitations. Because of our retrospective data collection our results are potentially subject to interpretation bias and bias due to missing data. Therefore, outcome measures such as quality of life and social function, both very

important in paediatric Crohn's disease were not collected. Similarly, information on adherence and feed volume are not available. Our data represent a population of children treated in a tertiary center and are possibly subject to tertiary center bias although taking account the complexity of paediatric IBD cases and the fact that local district general hospitals in most cases are not able to deliver a paediatric endoscopy and specialist IBD care this risk is probably overrated. We based our assessment of the response to enteral nutrition on the physician's global assessment which is a relatively subjective measure and likely to be subject to inter-observer variability. Due to the retrospective data collection, an objective measure of response and relapse could not be assessed. However, we used steroid prescription, escalation of medical treatment, need for surgery and physician's global assessment in order to describe the natural history of Crohn's disease in our cohort of patients in an attempt to provide a detailed description of the disease course of our patients. Unfortunately data on mucosal healing and endoscopic scores were not available because children are not evaluated endoscopically in remission. Moreover, measurements of faecal calprotectin which could provide an objective measurement of clinical and endoscopic disease activity, [376] are not available as faecal calprotectin testing is not routinely available in our Trust.

2.4.5 Vitamin D levels and their impact on the long term outcome of paediatric Crohn's disease

44% [25/56] of our patients had their vitamin D levels checked at least once at some point during the time of follow up. This is lower than the percentage of patients who had

their vitamin D level checked in other studies, [245] likely due to the fact that there is no strict protocol concerning the measurement of Vitamin D levels in adult or paediatric IBD patients in our clinical setting.

Vitamin D levels and vitamin D insufficiency did not impact on the clinical course of Crohn's disease in our paediatric patients. However, patients who had vitamin D levels of 25ng/L or less tended to require corticosteroids more often and earlier in their disease course. They also required more often and earlier during their disease course treatment with a thiopurine. It is difficult to precisely interpret this result given the fact that most of our patients had a single measurement of their vitamin D level, which is variable in time and dependent on external factors such as sunlight. Not having serial vitamin D levels for our patients it is difficult to establish their vitamin D status at the time of a disease flare. The results on the impact of vitamin D levels on the clinical course of Crohn's disease are mixed in the studies published so far, with some reporting a reduced likelihood of disease flare for those who are sufficient in Vitamin D levels over a year's follow up [245] and a direct direct effect of vitamin D levels in disease activity and surgery [377, 378] while others assessing the role of vitamin D in the paediatric setting, suggest that vitamin D levels are not related to disease activity. [379]

Moreover, one should take into account the fact that these studies do not provide any pathophysiological link between vitamin D plasma levels and disease activity. Vitamin D deficiency in Crohn's disease could possibly represent a surrogate marker of mucosal disease activity and associated malabsorption that would lead to the clinical manifestation of active disease in the future and not necessarily the primary regulator of the disease course. Also, one would expect that the exposure of patients' to sunlight is limited during

periods of active disease due to their daily activities being compromised. In fact, a study on the effect of vitamin D supplementation in the immunological response of PBMCs proved that supplementation and normalization of vitamin D levels in Crohn's disease patients resulted in increased production of IL-6, a proinflammatory cytokine from PBMCs and CD4+ T cell proliferation. [244]

Overall, the data presented in this chapter, give useful insights on the long term outcomes of EEN when it is used among children with Crohn's disease as primary therapy. However, the part of the study assessing the role of Vitamin D is limited by the lack of serial measurements and the data presented may be indicative but are not conclusive of the role of vitamin D in the course of Crohn's disease among paediatric patients.

Chapter III: The effect of enteral diet on the expression of CD58 in intestinal epithelial cell lines

3.1 Background

In the previous chapter we discussed the long term outcome of paediatric patients with Crohn's disease receiving EEN as their induction treatment, proving the favorable outcomes of patients responding to EEN. Although EEN is – at least in the UK – the first line induction treatment for children with Crohn's disease, the current mechanisms of its action are largely unknown. Apart from providing patients with a period of bowel rest and nutritional support, EEN impacts on the composition of the luminal microbiota and alters the host immunological response.

The first point at which any direct interaction between EEN and the mucosal immune system could possibly lead to a down-regulation of T cell response is at the interface with intestinal epithelial cells. Enteral nutrition has been shown to have direct immunoregulatory activity on the intestinal epithelium: it down – regulates the IL-8 response when added to the medium of Caco-2 cells, [336] as well as the MHC II expression on the intestinal epithelium in animal studies [380] possibly through the direct inhibition of the Class II transactivator gene mRNA. [381] The effect of enteral nutrition on the expression of MHC II molecules and secretion of IL-8 could possibly affect negatively the T cells activation.

Given the fact the T cell activation depends on MHC II as well as on the signaling provided by co-stimulatory molecules and taking into account the evidence on the role of co-stimulatory molecules in the pathogenesis of IBD we sought to establish whether enteral nutrition can affect the expression of a co-stimulatory molecule: CD58. CD58 has been shown to be expressed on Caco-2 cells however its role in the pathogenesis and management of IBD has not been extensively studied. In this chapter we investigate if

CD58 is expressed on the intestinal epithelium of patients with Crohn's disease and study the impact of EEN on the expression of CD58 on the intestinal epithelial cells.

3.2 Methods:

3.2.1 Intestinal epithelial cells (ex-vivo)

Intestinal epithelial cells from areas with active and inactive inflammation of patients undergoing colonoscopy or colectomy as part of their standard management for Crohn's disease and from healthy mucosa (patients undergoing colectomy for colorectal cancer) were isolated with standard treatment with 1mM EDTA [382] and have been a kind donation of Anna Vosenkamper and Neil McCarthy, post doctoral fellows.

3.2.2 Intestinal epithelial cell lines and culture conditions

Caco-2 cells and HT-29 cells obtained from the Jackson's laboratory were cultured in culture medium at standard conditions (37˚ C and 5%CO2) at the Blizard Institute of cell and molecular science. Both lines used are cancer cell lines as there is lack of a good model in non cancer cell lines. The medium used was Dulbecco's Modified Eagles Medium (DMEM) supplemented with Fetal Calf Serum (FCS) (10% per volume), penicillin and streptomycin mix (1% per volume), non essential amino acids (1% per volume) and HEPES buffer solution (1% per volume). The cells were split with trypsin and passages 20 – 35 were used for experiments.

For experimentation, cells were seeded in 6 or 12 well plates and when they reached a confluence of 80-90% they were kept in serum free medium for 48h. Subsequently, half of the wells were cultured in serum free medium for further 48h or in enteral diet formula

(Modulen IBD®) diluted in serum free medium at the concentration recommended by the manufacturer for use in Crohn's disease patients. After this incubation, the supernatants were removed and cells were washed with PBS three times before the protein was harvested.

3.2.3 Protein extraction (for both cell lines and ex-vivo cells)

Cells were inclubated with 150ul of lysing solution at 4˚ C for 30 mins. The lysing solution is made of cell-lytic reagent enriched with phosphatase inhibitors (1:1000) and phosphatase inhibitor. Subsequently, samples were sonicated for 15 secs each and then protein was qualtified. Protein quantification took place through spectophotometry against known standards with a standard curve. After protein quantification, PBS was added to each sample in different amounts to reach a final equal concentration. Laemli Buffer was added (10ul per sample) and samples were further incubated at 65˚C for 15 mins before being stored at -80˚C.

3.2.4 SDS PAGE and Western blot

3.2.4.1 Materials

SDS PAGE was used to separate proteins according to their molecular weights on polyacrilamide gels. Each gel was made of 1.65 ml 30% Acrylamide, 1.9 ml Tris HCl 1M pH 8.8, 50ul 10% SDS, 1.35 ml of water, 50 ul of 10% Ammonium persulphate and 3 ul TEMED. The running buffer was made of Glycine (192mM), 0.1% SDS and Tris base diluted in water and normalized to a pH of 8.3. Each of the stacking gels was made of

30% Acrylamide (335 ul), Tris – HCl 1M pH 6.8 (250 ul) water (1.35 ml), 10% SDS (20 ul), ammonium persulphate (25ul) and TEMED (2.5 ul).

The primary antibody was a mouse IgG2 anti-human CD58 antibody purchased from Sigma Aldrich matched with a secondary antibody. In order to normalize the CD58 blot and ensure that similar amounts of protein were loaded to each well, the CD58 antibody was removed with Restore Western Blot Stripping Buffer (Thermo Scientific) and the blots were re - stained with antibodies to GAPDH.

3.2.4.2 Assay

The gels were put into the gel tanks and sufficient running buffer was added to cover the wells. Samples were added and electrophoresed at 110 V until the gel front reached the bottom of the gel (approximately 1 hour).

A semi – dry trans – blotter was used for the western blot. This involved making "a sandwich" of Whatman paper soaked in transfer buffer, the membrane (pre wet in methanol), the gel and more Whatman paper soaked in transfer buffer. The semi dry bloater was electrophoresed for 80 mins at 200 V. Membranes were subsequently removed and incubated in 5% albumin in washing buffer for 1h to block. Membranes were then incubated overnight in 3ml 1% albumin in washing buffer and the primary antibody in the concentration recommended by the manufacturer. The following morning, the blots were washed 6 times (10 mins each) in washing buffer and were then incubated with the secondary antibody in 1% albumin solution for 1 hour and then re-washed for 1 hour as before. The blots were then incubated for 5 mins with ECL, wrapped in cling film

and exposed to film in the dark room. The film was developed using an automated developer.

3.2.4.3 Western blot quantification

We used Adobe Photoshop CS6 for western blot quantification. The colors were inverted and the bands were selected using the "lasso application" according to software instructions. Using the histogram analysis the quantification of the area surface and intensity were extracted.

The quantification of CD58 was defined as :

Intensity CD58 * Area CD58 / Intensity GAPDH * Area GAPDH

The results of this formula were normalized for each blot in order to allow comparability. To achieve this, the results of each western blot gel were multiplied to a factor so as the sum of the qualntification results of each gel would be equivalent to 100.

3.2.5 Statistics

The data obtained from the quantitation study were tested for Gaussian distribution with the Kolmogorov-Smirnov test. Comparisons were made using 2-tailed student's t-test. All statistical analysis was performed with SPSS software version 16.0

3.3 Results

3.3.1 CD58 expression in the human intestinal epithelium

Intestinal epithelial cells from 2 healthy controls, 3 patients with inactive Crohn's disease and 2 patients with active Crohn's disease were obtained. CD58 protein was expressed on the intestinal epithelium in all cases. (Figure 3.1) Further to this result, we attempted to investigate whether CD58 is expressed at a protein level in human intestinal epithelial cell lines.

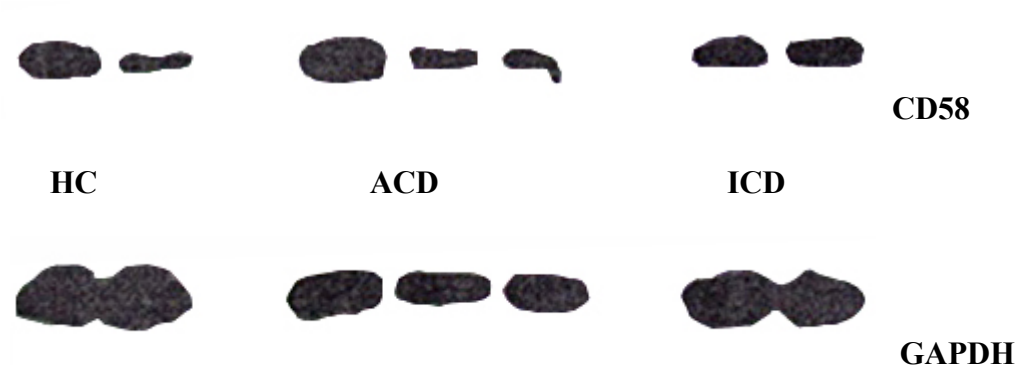


Figure 3.1: Experiment 1: The CD58 expression on intestinal epithelial cells from healthy controls (HC), patients with active Crohn's disease (ACD) and patients with inactive Crohn's disease (ICD): CD58 is expressed in the intestinal epithelial cells of healthy volunteers as well as patients with active or inactive Crohn's disease

3.3.2 CD58 expression in Caco-2 cells

CD58 protein, was expressed in Caco-2 cells. Further to this result, we attempted to investigate whether when Caco-2 cells were cultured in the presence of Modulen, CD58 expression was significantly down regulated. This result was reproduced in 3 independent experiments as these are shown to figures 3.2 – 3.4. Statistical analysis of the quantification of the western blot bands through Photoshop confirmed the statistical significance of the results. This is presented in figure 3.5. Similar results were obtained in an experiment using HT-29 instead of Caco-2 cells (figure 3.6).

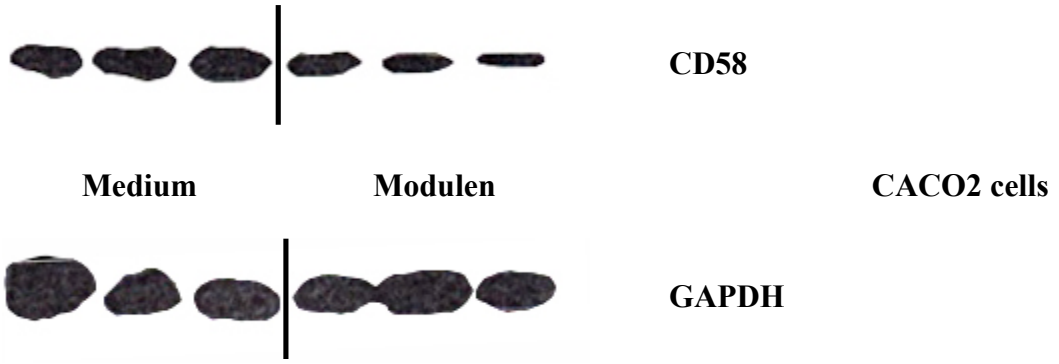


Figure 3.2: Experiment 2: The CD58 expression in untreated (medium) and modulen treated (Modulen) CACO2 cells

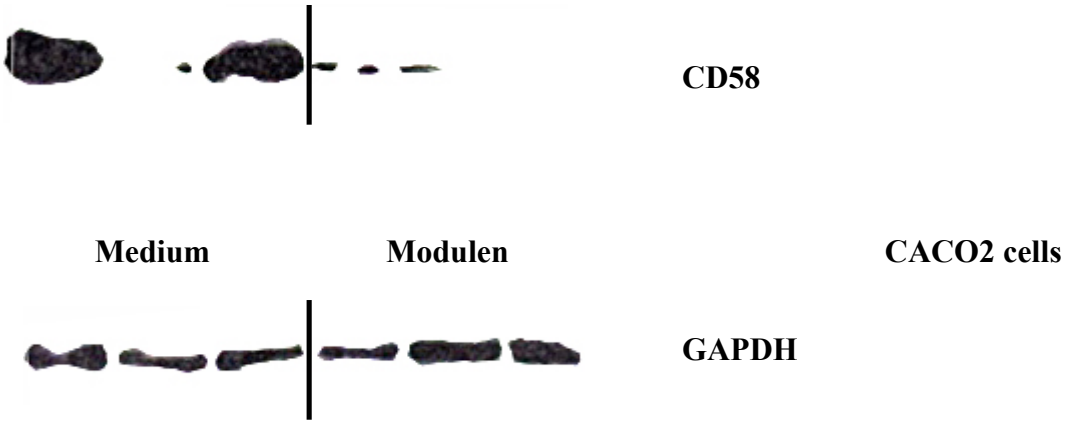


Figure 3.3: Experiment 3: The CD58 expression in untreated (medium) and modulen treated (Modulen) CACO2 cells

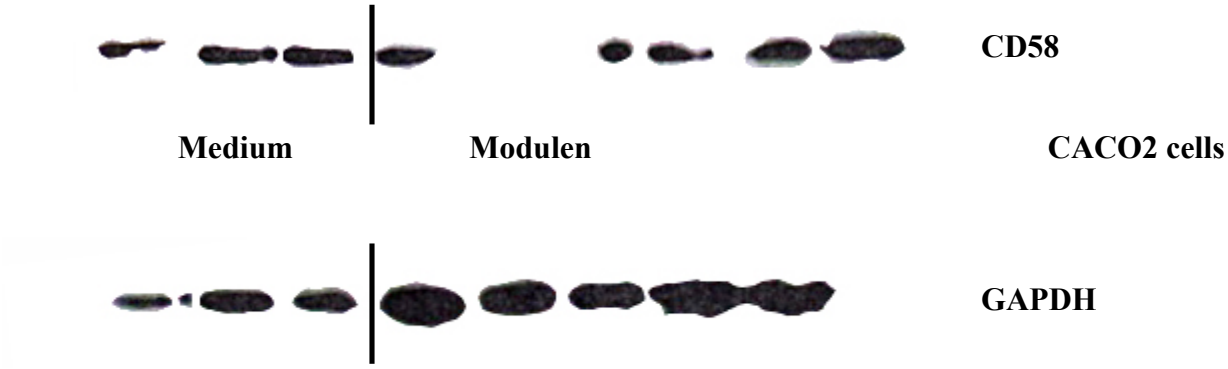


Figure 3.4: Experiment 4: The CD58 expression in untreated (medium) and modulen treated (Modulen) CACO2 cells

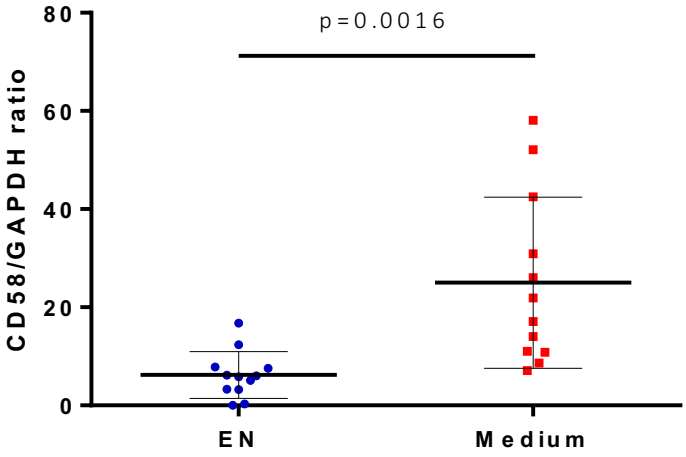


Figure 3.5: The quantitative comparison of CD58 protein expression among Caco-2 cells treated or not treated with Modulen. Modulen significantly reduces the expression of CD58 in Caco-2 cells (p=0.0016).

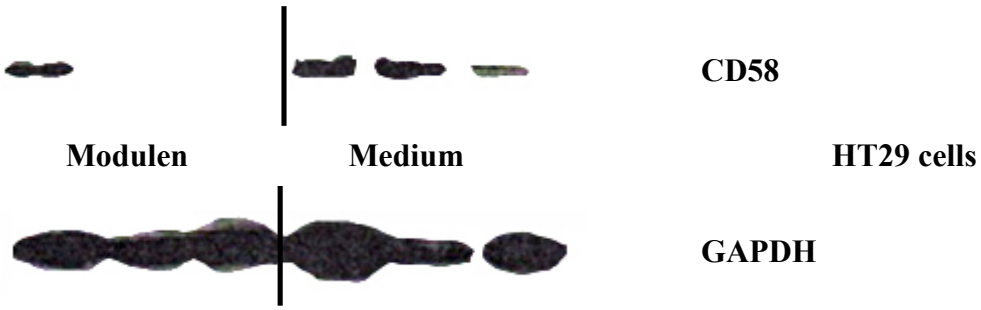


Figure 3.6: Experiment 5: The CD58 expression in untreated (medium) and modulen treated (Modulen) HT29 cells. CD58 is expressed on HT29 cells and is downregulated after treatment with Modulen

3.3.3 Summary of findings:

The co-stimulatory molecule CD58 was expressed in the epithelial cells of patients with active or inactive Crohn's disease as well as in the intestinal epithelial cells of healthy controls. CD58 was expressed in intestinal epithelial cell lines, however incubation with enteral diet resulted in significant downregulation of the CD58 expression at protein level.

3.4 Discussion

This is the first study confirming the presence of the co-stimulatory molecule CD58 in the intestinal epithelium of patients with Crohns disease, UC and healthy volunteers as well. This extrapolates the findings of previous studies that proved the presence of CD58 in intestinal epithelial cell lines to the human gut. [98] Our study was the first to attempt to and successfully downregulate the expression of CD58 on intestinal epithelial cell lines with the use of enteral nutrition. We propose that a mechanism by which enteral nutrition could possibly exert its beneficial impact, is on the intestinal inflammation through the down-regulation of CD58 on the intestinal epithelium. This mechanism is not the only means by which this happens as enteral diet could act in a number of ways. A recent study suggested that the expression of CD58 is decreased in the mucosa of patients with Crohn's disease and is normalized after treatment of these patients with infliximab. [365] However, the results obtained from this study did not investigate the expression of this molecule on the intestinal epithelium but in whole biopsies. Given the previously shown pro-inflammatory interaction between intraepithelial lymphocytes and CD58 on intestinal

epithelial cells [99] which was inhibited upon blockage of CD58, we provide additional data regarding CD58 on the intestinal epithelium as a target for therapeutic intervention in IBD. This is further supported by the data on the successful management of psoriasis – another T cell mediated discorder – by alefacept, an antibody inhibiting the CD58-CD2 interaction. [121]

The idea of dietary treatment influencing the immunology of IBD is a very interesting aspect in IBD research. Apart from the immunomodulation achieved through EEN, a recent study has shown that VSL3, a probiotic mostly used for the management of pouchitis, is able to increase the production of IL-10 and decrease the expression of TLR-2 in colonic dendritic cells. [383]

Our study does not provide data on whether CD58 is up-regulated or not in the intestinal epithelium of patients with IBD. The main reason for this, is the fact that the experiments were designed to determine the presence or not of this molecule on the intestinal epithelium. The number of patients included were insufficient for quantitative analysis. Attempting to assess the expression of CD58 in IBD patients compared to healthy controls would be logistically difficult given the fact that most of our IBD patients who are undergoing colonoscopy are on treatments that could possibly affect the expression of CD58 in any direction. Hence only newly diagnosed patients should be included. Moreover, assessing the impact of enteral nutrition in the intestinal epithelium of IBD patients would mean the undertaking of a repeat colonoscopy in patients after the end of treatment. Given the risks and discomfort associated with colonoscopy and the fact that EEN is mainly used according to the current clinical practice in children and adolescents with Crohn's disease it would be difficult to ethically justify such an undertaking for

research purposes. Therefore, it is technically difficult to assess and extrapolate our results as these are shown on cell lines to patients.

In order to assess the effect of enteral nutrition on the CD58 expression on Caco-2 cells we elected to use western blot as it is a method that directly assesses protein expression. Although our blots clearly indicate the down-regulation of CD58 on Caco-2 cells after exposure to Modulen the only way to quantify our findings was through assessing the area and intensity of the bands by using Adobe Photoshop®. This technique confirmed our findings which were visually evident on the transfers, however we acknowledge that this is prone to selection and interpretation bias. In an attempt to minimize the relevant bias data were quantified and analysed with the method described according to area and intensity and also by comparing the mean intensities of preselected and equal areas (data not shown) with similar results. Another limitation of western blot is the fact that very small differences on time exposure of films to the blots can result in significant differences that are observed in the intensity of the blots making any comparison of proteins isolated in different blots technically challenging and difficult to interpret. In order to overcome this limitation we normalized our results against the expression of GAPDH and internally for every blot as described in the relevant methods section.

Chapter IV: Exclusive enteral nutrition among adult patients with Crohn's disease

4.1 Background

Exclusive enteral nutrition has been traditionally regarded as the treatment of choice for the management of paediatric Crohn's disease. However, a growing body of evidence has suggested that it can also be a valid choice in the management of adults. A UK study suggested that EEN can be as efficient as the treatment with corticosteroids if tolerated. However, the same study reported a 40% intolerance rate suggesting that adherence to EEN among adults may be a challenge. [301] A landmark East Anglia study suggested that it can be effective in inducing remission in up to 85% of a patients, but reported similarly high rates of intolerance mounting to 30%. [302] The rates of intolerance and subsequent non adherence may be associated with the unfavorable outcomes of a meta-analysis [303] and a Cochrane review [299].

The evidence described above, highlights the issue of adherence and tolerance in the use of EEN among adult patients with Crohn's disease. The intention-to-treat population in London is socially and ethnically diverse, making adherence a challenge. Our catchment area includes patients of mostly Caucasian or South Asian background. Ethnic diversity is associated with different socioeconomic and education backgrounds, different patient beliefs and possibly attitudes and different genetic backgrounds. These factors may influence the rates of tolerance/adherence and the short and the long term outcomes of EEN.

Therefore we conducted a retrospective study in our adult patients with Crohn's disease aiming to document the efficacy and long term outcomes of EEN among adults with Crohn's disease. We also aimed to investigate whether ethnicity if a factor associated with our study endpoints.

4.2 Methods

4.2.1 Patients

Adult patients with either established or newly diagnosed Crohn's disease that received care from the department of dietetics of Barts and The London NHS Trust between February 2012 and May 2014 with a view to be established on exclusive enteral nutrition were identified with the support of Lydia Hill, dietician. Patients that did not receive further follow up by our department or who migrated or were lost to follow up for any reason were excluded from this study. All patients had been diagnosed with Crohn's disease based on standard histopathological and radiological criteria. [384] Patients with indeterminate colitis and patients who were diagnosed elsewhere with Crohn's disease but the diagnosis had not been clearly confirmed were excluded from the study. The catchment area of the adult IBD service of the Royal London Hospital includes East London and London suburbs in Essex within the M25.

4.2.2 Data collection

This is a retrospective study examining the disease course among adult patients with Crohn's disease after receiving EEN. The patients to be included for the study were identified by Lydia Hill, dietician at the Royal London Hospital through the dietetics department database.

Clinical data on the disease phenotype, concurrent and previous treatments and disease course and need for further medical or surgical therapies were collected through the outpatient clinics letters that were electronically available at the electronic patients

records (EPR). Biochemistry and haematology data were collected through the electronic Clinical records system (CRS). All data were collected by the author of this thesis.

The data collection for this chapter took place between 01 June 2015 and 15 September 2015.

4.2.3 Treatments

All patients were treated with exclusive enteral nutrition in order to induce remission under the joint care of an adult gastroenterology consultant and a dietician. Treatment was administered orally, but in a minority who did not tolerate it, or when concerns regarding inadequate intake arose, a small bore NG tube was used. The volume of the feed was initially 1 litre per day and would gradually increase to cover the patients' nutritional requirements, according to dietician review. Enteral nutrition was administered as an exclusive diet; no other food apart from water and flavouring powder for the feed (made from the feed manufacturer) was allowed. Treatment duration was 6 to 8 weeks for all patients. Patients were assessed on their response to treatment and subsequently a normal diet was re-introduced with dietician's advice follow up within the next 1-2 weeks.

Medical treatments after the course of EEN included corticosteroids, antibiotics, thiopurines and anti-TNFs and were prescribed according to local and national guidelines by the gastroenterologists of our unit.

4.2.4 Follow up

The follow up period included the time from initiation of EEN up until the date of the last documented follow up appointment that was attended by the patient.

4.2.5 Definitions of remission and relapse

Clinical remission and clinical response was defined according to physician's global assessment as described in clinic letters provided that the patient was not on corticosteroids. Clinical relapse was defined according to physician's global assessment from clinic letters and / or need for treatment with corticosteroids or escalation of medical treatment or need for surgery. We elected to use physician's global assessment in our definition for remission/relapse and not an arithmetic measure such as the Crohn's disease activity index (CDAI) mainly for two reasons: Due to the retrospective data collection from clinic letters, a number of the variables included in the CDAI would be missing. Additionally the clinical decision making regarding our patients in the clinic setting is based on the global assessment of an experienced paediatric gastroenterologist; the use of a numerical activity index such as the CDAI is more relevant in a clinical trial setting, where patients are not necessarily seen by an experienced specialty physician in order to ensure uniformity rather than our clinical setting.

4.2.6 Data analysis

Data were analysed to provide results describing the time to clinical relapse, to use of steroids, thiopurines or anti-TNFs and the time to first surgery after administration of EEN. Our population was divided in those who achieved clinical remission with

exclusive enteral nutrition on induction and those who did not. The statistical tests used for univariate analysis were student's t-test (for continuous variables) and the chi-square test (for categorical variables). Kaplan Mayer analysis with log rank test was conducted to determine the time to clinical relapse or treatment escalation in the two groups. All analyses were 2 tailed and p-values of less than 0.05 were considered as statistically significant. All statistical tests were performed electronically with the use of statistical package for social sciences (SPSS version 16.0). Prism – GraphPad (GraphPad Prism 6) software was used for the generation of graphs.

4.2.7 Ethical considerations

As we have been evaluating the disease course of patients using our IBD service, we did not require formal ethical approval according to the UK National Research Ethics Guidelines. [385]

4.3 Results

4.3.1 Patients

55 patients were identified in the dietetics department database and from them 33 were excluded. Among the excluded patients, 14 patients did not have a confirmed diagnosis of Crohn's disease (disease diagnosed abroad but not confirmed in UK, diagnosis of Tuberculosis or Indeterminate colitis), 10 patients were lost to follow up very soon after administration of EEN, 4 patients received partial enteral nutritional support or advice on a low residue diet, 3 had EEN co-administered with oral or IV corticosteroids and 2 had EEN administered for short term (1 week) as a bridge to surgery. (Fig 4.1)

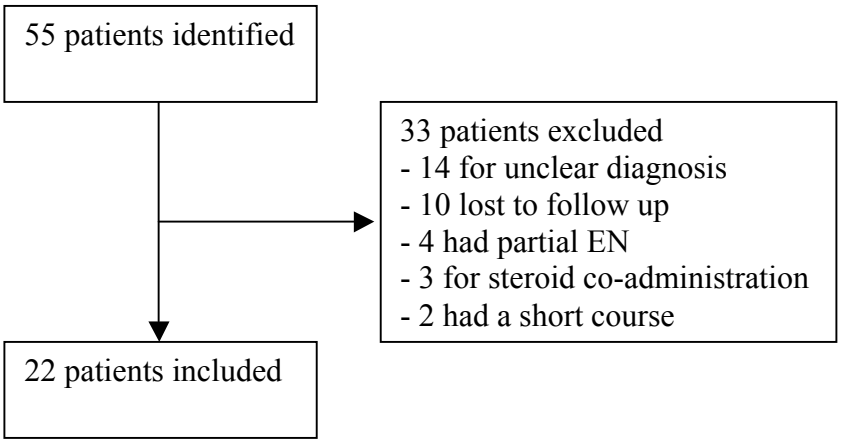


Figure 4.1: Patient population included in the study

4.3.2 Demographics – disease characteristics at the time of EEN

73% (16/22) of the patients included were male. The mean [SD] age at diagnosis and age at the time of EEN administration and were 20.6 [7.3] and 30.8 [8.8] years respectively. The mean disease duration at the time of EEN was 7.5 [8.8] years. The mean [SD] duration of follow up was 1.9 [0.8] years. 40.9 % (9/22) of the patients were Caucasian, 54.5 % (12/22) South Asian and 4.5% (1/22) African – Caribbean.

31.8% (7/22) of patients were diagnosed with Crohn's disease at age younger than 17 years, 63.8% (14/22) between 17 and 39 years and 4.5% (1/22) at the age of 40 or older. 31.8% (7/22) of patients had ileal disease, 4.5% (1/22) colonic and 63.6% (14/22) ileocolonic disease. Upper GI involvement was present in 22.7% (5/22) of our patients. 31.8% (7/22) of patients had inflammatory disease phenotype, 36.4% (8/22) stricturing and 31.8% (7/22) penetrative. Perianal disease was present in 31.8% (7/22) of our patients. There was no significant difference in the baseline demographic and disease specific characteristics between Caucasian and South Asian patients. (see table 4.1)

All patients received exclusive enteral nutrition. An NG tube was administered in only 9.1% (2/22) of patients and 18.2% (4/22) did not tolerate the diet. There was no difference in the rates of intolerance and the need for NG placement between Caucasian and South Asian patients [22.2% (2/9) vs 16.7% (2/12), p=0.75 and 11.1% (1/9) vs 8.3% (1/12), p=0.83 respectively]. 27.3% (6/22) of patients had EEN administered in the past. The mean [SD] duration of EEN was 6.1 [2.6] weeks.

Characteristics		All patients n=22	Caucasian n=9	S. Asian n=12	p value
Gender male, n (%)		16 (72.8)	7 (77.8)	8 (66.7)	0.58
Age		30.8 [8.8]	30.5 [6.6]	31.7 [8.7]	0.73
Disease duration		7.5 [8.8]	8.4 [9.1]	7.2 [9.3]	0.77
Age at dianosis, years		20.6 [7.3]	19.5 [6.1]	21.8 [8.2]	0.49
Age at diagnosis (years)	≤ 16, n (%)	7 (31.8)	3 (33.3)	3 (25)	0.65
	17-39, n (%)	14 (63.6)	6 (66.7)	8 (66.7)	
	≥ 40, n (%)	1 (4.5)	0 (0)	1 (8.3)	
Location	Ileal, n (%)	7 (31.8)	4 (44.4)	3 (25)	0.49
	Colonic, n (%)	1 (4.5)	0 (0)	1 (8.3)	
	Ileocolonic, n (%)	14 (63.6)	5 (55.6)	8 (66.7)	
	+ L4, n (%)	5 (22.7)	1 (11.1)	4 (33.3)	0.24
	+ perianal, n (%)	7 (31.8)	1 (11.1)	5 (41.7)	0.13
Behaviour	Inflammatory, n (%)	7 (31.8)	2 (22.2)	5 (41.7)	0.65
	Strictuting, n (%)	8 (36.4)	4 (44.4)	4 (33.3)	
	Penetrating, n (%)	7 (31.8)	3 (33.3)	3 (25.0)	
Previous treatments	EEN, n (%)	6 (27.3)	1 (11.1)	4 (33.3)	0.24
	Corticosteroids, n (%)	13 (59.1)	6 (66.7)	6 (50)	0.45
	Thiopurines, n (%)	15 (68.2)	6 (66.7)	8 (66.7)	1.0
	Methotrexate, n (%)	3 (13.6)	1 (11.1)	2 (16.7)	0.72
	Anti-TNF, n (%)	7 (31.8)	3 (37.5)	3 (25)	0.55
	Surgery, n (%)	7 (31.8)	3 (33.3)	4 (33.3)	1.0

Table 4.1 Baseline demographic and disease specific characteristics. All values express Mean [SD] unless otherwise specified.

4.3.3 Disease course

After treatment, 22.7% (5/22) of patients went into remission, 77.3% (15/20) experienced a clinical response and 9.1% (2/22) did not have any benefit. There was no difference in the clinical response and remission rates between Caucasian and South Asian patients. (See table 4.2) The mean [SD] CRP after treatment was 17.6 mg/dl [22.5] and was significantly lower compared to the CRP prior to initiation of EEN 60.2 mg/dl [66.8] ($p<0.01$). In concert, the mean [SD] platelet count decreased from $363*10^3/\mu L$ [117] to $346*10^3/\mu L$ [124] ($p=0.38$) and serum albumin levels increased from 36.4 g/L [7.3] to 40.4 g/L [5.5] ($p<0.01$)

By the end of follow up 63.6% (14/22) of patients had clinically relapsed, 55.6% (5/9) of the Caucasian and 66.7% (8/12) of the South Asian patients (p=0.60). The mean [SD] time to clinical relapse was 11.1 [11.2] months and there was no significant difference in duration of remission between the two ethnic groups (see table 4.2). 31.8% (7/22) of patients required treatment with corticosteroids after EEN and the mean [SD] time to corticosteroids was 18.9 [11.1] months. 36.4% (8/22) of patients required de novo treatment with thiopurines after EEN and18.2% (4/22) continued with thiopurine treatment that was prescribed prior to EEN. The mean [SD] time to treatment with thiopurine was 16.2 [12.8] months. 50% (11/22) of patients required treatment with anti-TNF after EEN and the mean [SD] time to treatment was 14.7 [10.5] months. 36.4% (8/22) of patients required surgical treatment after EEN and the mean [SD] time to surgery was 18.0 [10.0] months.

There were no differences between the two ethnic groups in the disease outcomes during follow up between the two ethnic groups. Similar proportion of patients clinically relapsed, required steroids, treatment with thiopurine, anti-TNF or surgery. Also, the time to treatment escalation did not differ between the two ethnic groups.

Clinical course and need for treatment escalation after EEN	All patients n=22	Caucasian n=9	S. Asian n=12	p value
Remission after EEN, n (%)	5 (22.7)	1 (11.1)	4 (33.3)	0.24
Response after EEN, n (%)	15 (77.3)	4 (44.4)	6 (50.0)	0.80
Clinical relapse during follow up, n (%)	14 (63.6)	5 (55.6)	8 (66.7)	0.60
Time to clinical relapse (months)	11.1 [11.2]	12.3 [13.1]	10.3 [10.8]	0.57†
Need for corticosteroids, n (%)	7 (31.8)	2 (22.2)	5 (41.7)	0.35
Time to corticosteroids (months)	18.9 [11.1]	20.4 [9.3]	18.2 [13.0]	0.37†
Need to initiate thiopurines, n (%)*	8 (36.4)	3 (42.9)	5 (50.0)	0.77
Time to thiopurines (months)	16.2 [12.8]	16.3 [11.8]	16.2 [14.5]	0.77†
Need to initiate anti-TNF, n (%)	11 (50)	6 (66.7)	5 (41.7)	0.26
Time to anti-TNF (months)	14.7 [10.5]	9.7 [8.6]	18.6 [11.1]	0.07†
Need for abdominal surgery, n (%)	8 (36.4)	5 (55.6)	3 (25.0)	0.15
Time to abdominal surgery (months)	18.0 [10.0]	14.7 [11.2]	20.9 [8.9]	0.13†

Table 4.2: Response to EEN and need for treatment escalation during follow up. All values express Mean [SD] unless otherwise stated

*4 patients already on azathioprine exluded

† denotes Kaplan-Meier, log rank test

4.4 Discussion

This study attempts to record the outcomes to EEN in adult patients with Crohn's disease. Also, this is the first study to our knowledge attempting to compare the response to EEN and the need for treatment escalation after EEN between Caucasian and South Asian adult patients with Crohn's disease.

The rate of remission after treatment of EEN in our adult patients was only 22.7% (5/22) and is significantly lower than that of our paediatric patients of whom 57.1% (32/56) went into clinical remission after treatment with EEN ($p<0.01$). Comparing patients of

South Asian descent to Caucasian patients did not identify any significant differences in the clinical outcome of EEN or the need for treatment escalation after EEN.

An important factor that may explain the lack of difference between the two ethnic and age groups is the fact that these patients represented adults with advanced disease phenotypes and not treatment naïve newly diagnosed patients, as was the case in our previous study (Chapter 2) on paediatric patients. Many of them had received treatment with corticosteroids or anti-TNF in the past and the majority of them had been on previous treatment with thiopurines. Also 1 in 3 of the patients included had undergone previous surgery. The advanced disease phenotype and failure of previous medical therapies could explain the fact that the rate of intolerance to EEN in our study is lower than the rates reported by others [301] as these patients had been exposed to corticosteroids in the past and possibly had ongoing penetrative complications that would preclude the use of corticosteroids. Their advanced disease phenotype could also set the grounds for the treating physicians to pro-actively escalate medical management rapidly in order to avoid further disease complications. Therefore, one could question whether the description of the treatments that were initiated after EEN are representative of the actual disease course. While in the paediatric setting treatment with immunomodulators would be initiated if absolutely clinically indicated as part of the long term risk management of these patients, in our adult patients with complicated disease it would be undertaken more promptly in order to prevent further complications. However, escalation to anti-TNF therapy, according to the NICE guidelines requires the presence of clinically active disease. The proportion of Caucasian patients that required treatment with anti-TNF or escalation to a new anti-TNF after EEN numerically exceeded the proportion of

South Asian patients and there was a trend for earlier administration of anti-TNF to Caucasian patients. Similar were the trends in the requirement for and time to abdominal surgery after EEN, however the numerical difference observed did not reach statistical significance.

This study has a number of limitations. The patients included where identified through the database of the dietetics department. The data input in the database is relying on the dieticians' meticulousness and it is possible that a number of patients has not been recorded posing a degree of selection bias. Additionally, the total number of patients included is small, making the study underpowered. We did not do preliminary sample size calculations as the final sample size would be entirely dependent on the number of patients that we could identify from the database of the department of dietetics. However a post hoc calculation of the statistical power using a simple online calculator (http://clincalc.com/Stats/Power.aspx) suggests that the statistical power of this study to detect the observed difference in the remission rates, need for anti-TNF and surgery is 12%, 20% and 13% respectively. Therefore our sample can be useful to describe the response to EEN and need for treatment escalation in the overall population. However, the numbers of patients included in each ethnic group is too small to allow for any safe conclusions to be drawn. This may explain the fact that the trends described in the need for anti-TNF and surgery in between patients of South Asian and Caucasian descent contrast the clinical impression from our practice, that overall the South Asian patients have a more aggressive disease course. Therefore we designed a retrospective study aiming to investigate the disease phenotype and course between the two populations that is described in the chapter to follow.

Chapter V: Is there a difference in the clinical course of IBD in adult Bangladeshis and Caucasian patients living in East London? Is vitamin D a contributing factor?

5.1 Background

The data presented so far are based on patients who are being followed up in the paediatric, adolescent and adult IBD clinics at the Royal London and St Bartholomew hospitals which are both based in East London. One of the particularities the local population served by Barts Health is the presence of a significant number of Asian immigrants the majority of whom come from Bangladesh, which is reflected in our patient population and poses a question regarding whether conclusions drawn from our patients can be applicable to the general population. According to census data, in our borough, the Tower Hamlets, individuals of Bangladeshi descent represent 33.2% (67,200 out of a total population of 202,100) and individuals of Indian or Pakistani descent represent 2.2% of the population. [386]

The incidence of IBD in Asian ethnic minorities in the UK has risen. [18]. Data from patients of South Asian descent living in the UK suggest that, compared with Northern Europeans, South Asians are more frequently diagnosed with extensive ulcerative colitis (UC) than with Crohn's disease and that the latter is characterized by a more benign inflammatory non-stricturing non-penetrating phenotype [387]. Moreover, there is evidence to suggest that hypovitaminosis D is more prevalent among patients of South Asian descent. [388]

Our impression when discussing patients in weekly MDTs has been that Bagladeshi patients with IBD in East London have a more aggressive course of disease, compared to white Caucasian patients, but this has not so far been scrutinized scientifically. Therefore in this chapter we investigate the differences in disease course between Bagladeshi and

white Caucasian IBD patients living in East London and whether hypovitaminosis D is a co-founding factor.

5.2 Methods

5.2.1 Study design & clinical setting

We conducted a retrospective case-controlled study to evaluate the phenotype and disease course of IBD patients of Bangladeshi descent, comparing it to that in white Caucasian patients, matched for age at diagnosis and disease duration and investigating the possible role of vitamin D in any differences noted. Barts and the London NHS Trust is a tertiary referral centre in East London, UK, that serves approximately 2000 paediatric, adolescent and adult patients with IBD from the local catchment area and beyond. Only patients of Bangladeshi descent and white Caucasian patients were included. Mixed race patients of either descent were excluded from the study.

5.2.2 Screening

The self-reported ethnicity, age at diagnosis, disease duration and postcode of each consecutive patient attending our specialist IBD outpatient clinics between March and August 2010 was recorded. Only patients living in postcodes from the inner East London area (boroughs of Tower Hamlets, City, Hackney ane Newham) were included in an attempt to minimize tertiary referral bias.

5.2.3 Defining age at diagnosis

In order to investigate if Bangladeshi patients develop IBD at an earlier age than white Caucasian patients, we assessed age at diagnosis in all patients of inner East London, including those who were not subsequently matched. Because the age distribution in our local Bangladeshi patients was skewed towards a younger age compared to that of white Caucasians, for this part of the study we studied the comparison of age at diagnosis in these groups using their respective age distributions in our local healthy population as denominator.

5.2.4 Matching

For the remainder of the study, all patients of Bangladeshi descent who were formally diagnosed with IBD based on standard endoscopic, histological and radiological criteria attending our specialist IBD outpatient clinics were included. These were subsequently matched to a Caucasian IBD patient of English, Scotish or Welsh descent attending our clinics in terms of disease duration (± 5 years) and age at diagnosis (± 3 years) in order to compare the natural history of the disease. When more than one match was available, the individual with the closest disease duration was included.

5.2.5 Demographic and socio-economic data

Demographic data, including place of birth and year of migration were collected from our Electronic patient records online system (EPR) and our IBD database. Socio-economic data was obtained from the ACORN database. [389] The ACORN database provides information on the socioeconomic status based on an individual's postcode, by analyzing

demographic data, social factors, population and consumer behaviour. This online database segments the UK population into 56 ordinal socioeconomic types: where 1 is categorized as “affluent mature professionals, living in large houses” and 56 as “multi-ethnic populations living in crowded flats” and is postcode specific. The area of inner East London is characterized by a significant demographic diversity concerning the socioeconomic status of its residents and the postcode specificity provided by the ACORN database ensures that differences will be identified even within the same geographic area.

5.2.6 IBD phenotype and disease course

IBD was phenotyped according to the Montreal Classification [369] and was recorded at diagnosis and the last follow up. The natural history of UC and Crohn’s disease were assessed by assessing and comparing the time to change in Montreal Classification; the treatments used including numbers of courses of corticosteroids, and use of and time to use of immunosuppressant treatments and surgeries. In an attempt to minimize bias in data collection, we prospectively decided that the relevant data will be collected by 4 researchers: 2 for the data of the Bangladeshi patients and 2 for the data of the white Caucasian patients; The ones collecting data on the Bangladeshi patients (JRG and NMJ) were blinded to the results of the white Caucasian (NK and MW) patients.

5.2.7 Extra-intestinal manifestations and complications

We recorded the incidence of organ specific extra-intestinal manifestations such as arthropathy, skin or ocular involvement and primary sclerosing cholangitis. We also recorded the prevalence of anaemia and vitamin D deficiency at the last outpatient attendance.

Anaemia was defined according to WHO criteria [390]: in men as Hb <13.0 g/dL, in non pregnant women as Hb <12 g/dL, in pregnant women as Hb <11.0 g/dL, in children of 12 - 13 years old as Hb < 12 g/dL and in children of 5-12 years old as Hb<11.5 g/dL. Iron deficiency was defined as a ferritin <30ug/L when the corresponding CRP was <10 mg/L, or a ferritin <100ug/L when C-reactive protein (CRP) was >10 mg/L and/or transferrin saturation (TNSat) <16% [391]. Anaemia of chronic disease was defined as anaemia in the presence of a CRP-adjusted, normal or increased serum ferritin and/or a low plasma iron and iron binding capacity [176]. B12 deficiency was defined as <191 ng/L and folate deficiency as serum folate <3.8 mcg/L. Blood test results were obtained from the electronic patient record.

Vitamin D status was estimated through the measurement of serum 25 – hydroxyl – vitamin D levels. The optimal vitamin D levels have been a matter of debate as there is no clear consensus of opinions and a recent systematic review of the relevant literature suggests different cut off values for every functional endpoint of vitamin D. [370] In our study, vitamin D insufficiency was defined as 25 (OH) vitamin D levels of less than 50 ng/L as this has been described as the threshold of pharmacological intervention and deficiency as levels of less than 25 ng/L as this threshold is reportedly associated with osteomalacia. [371]

5.2.8 Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) (version 16, San Diego, California, USA) and Prism (version 4, Chicago, Illinois, USA) software. All analyses were two tailed and p values <0.05 were considered significant. Univariate analysis of baseline demographic data comparing patients of Bangladeshi descent and white Caucasians was carried out using chi-squared test for categorical data, Student's t-test for continuous normally distributed variables and Mann-Whitney U tests for discrete or nonparametric data. Univariate survival analyses using Kaplan-Meier curves and the log-rank test were used to identify differences in the proportions of patients and the time to change in Montreal Classification, escalation in therapy and need for surgery between the ethnic groups. Where ethnicity was found to be linked with a worse prognosis was undertaken.

5.2.9 Ethical considerations

As we have been evaluating the disease course of patients using our IBD service, we did not require formal ethical approval according to the UK National Research Ethics Guidelines. [385]

5.3 Results

5.3.1 Screening

We screened 2449 appointments of 1247 patients (figure 5.2). 192 patients were excluded because they did not have a confirmed diagnosis of IBD according to standard criteria. 13% [132/1055] and 59% [623/1055] patients reported that they are of Bangladeshi and Caucasian descent respectively and of these significantly more Caucasian than Bangladeshi lived outside the inner East London boroughs (62% [389/623] vs 8% [11/132], $p<0.0001$).

5.3.2 Age at diagnosis

In the population available for matching, only 7% (9/121) Bangladeshis compared with 26% (60/234) Caucasians had IBD diagnosed at age >40 years ($P<0.001$). However, after adjusting for the age distributions in the Bangladeshi compared with the local white Caucasian population, no differences were seen in the age at diagnosis (figure 5.1).

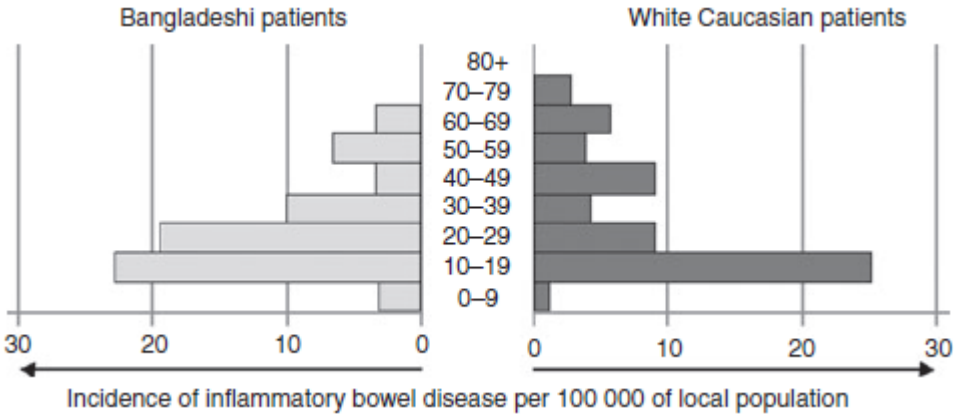


Figure 5.1: Age at diagnosis of the Bangladeshis and white Caucasians in the screening population. There was no difference in the age distribution of IBD incidence between white Caucasian and Bangladeshi patients.

5.3.3 Matching

We were unable to match 2% [2/121] of the Bangladeshi patients because their disease durations were less than 4 months and a similar white Caucasian match was unavailable (see Figure 5.2). As a consequence of our matching there was no difference in the mean [SD] age at diagnosis (23.4 [11.2] vs 24.0 [11.5] yrs, p=0.7) or disease duration (6.1 [4.7] vs 6.9 [4.8] yrs, p=0.43) between the Bangladeshi and white Caucasian groups respectively (Table 5.1).

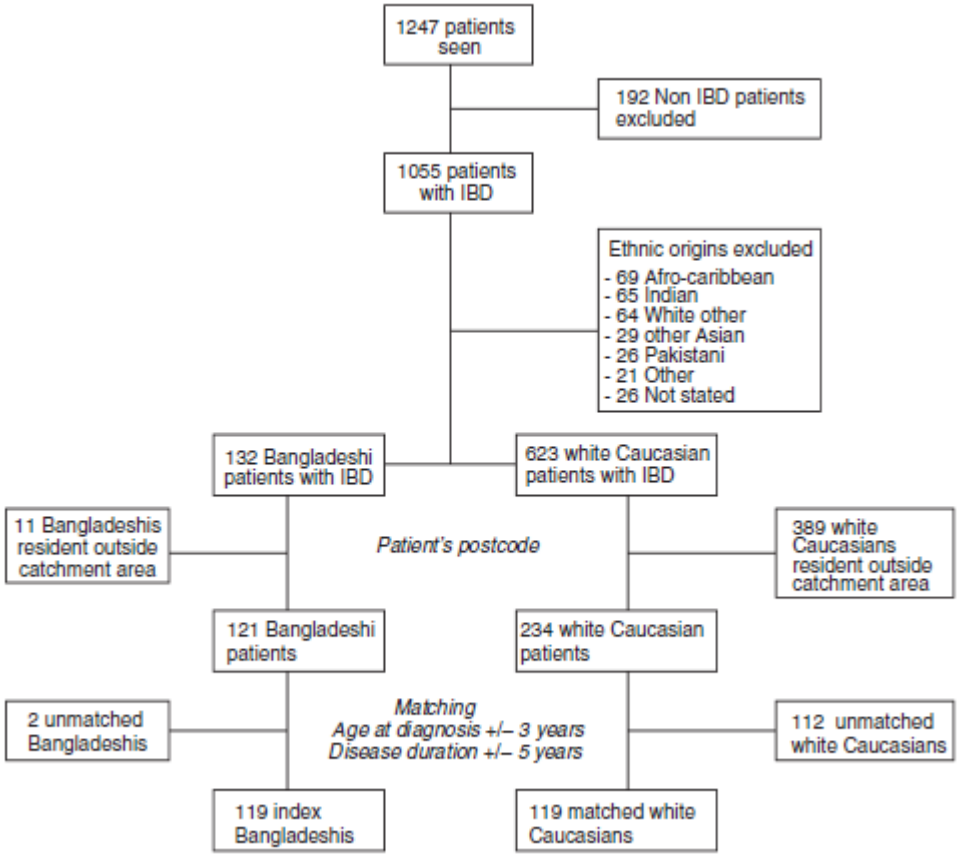


Figure 5.2: Diagram outlining the screening, matching and inclusion of Bangladeshi and white Caucasians. Each index case was then matched on the grounds of age at diagnosis (to within 3 years) and then disease duration (to within 5 years) to a white Caucasian patient of English, Scottish or Welsh descent. Where more than one potential match on the grounds of age at diagnosis was available, the individual with the closest disease duration was included.

5.3.4 Baseline demographics

There was no difference in the mean age between groups (Table 5.1). Overall, more patients of Bangladeshi descent were male than female (p=0.05) (Table 5.1). There were no differences in the proportions of patients who were ex- or current smokers or in the

duration of smoking, between groups. However, and allowing for missing data, more Bangladeshi men (41% [26/63]) than women (10% [4/40], p<0.01) were, or had been smokers; in contrast, there was no difference in smoking status of patients according to gender in the white Caucasians (53% [17/32] men vs 48% [16/33] women, p=1.0) (Table 5.1). Based on their postcodes and according to the ACORN classification type (median [range]), patients of Bangladeshi (55 [9-56]) descent lived in more socioeconomically deprived areas of East London than patients of white Caucasian (21 [1-56]) descent (p<0.0001).

Characteristic		**Bangladeshi n = 119**	**White Caucasian n = 119**	**p-value**
Sex	Male, n (%)	72 (61)	57 (48)	0.05
Age	Mean [SD] age (years)	29.6 [11.6]	30.9 [11.8]	0.37
Cigarette Smoking	Lifelong non-smoker, n(%)	73/103 (71)	65/98 (66)	0.55
	Smoker, n (%)	15/103 (15)	13/98 (11)	
	Ex-smoker, n (%)	15/103 (15)	20/98 (20)	
	Pack years	3.1 [0.7-15.0]	4.5 [0.3-42]	0.18
ACORN type	ACORN type	55 [9-56]	21 [1-56]	<0.001
Duration	Mean [SD] age at diagnosis (years)	23.4 [11.2]	24 [11.5]	0.69
	Mean [SD] disease duration (years)	6.1 [4.7]	6.9 [4.8]	0.16
	Time to diagnosis (months)	5 [0-172]	5 [0-134]	0.59
Disease type	Crohn's disease, n (%)	79 (66)	62 (52)	0.004
	Ulcerative Colitis, n (%)	40 (34)	49 (41)	
	IBD Unclassified, n (%)	-	8 (7)	

Table 5.1: Demographic characteristics, disease duration and type of the included white Caucasian and Bangladeshi patients. Values are expressed as median [range] unless otherwise indicated.

5.3.5 Disease type and time to diagnosis

More patients of Bangladeshi descent were diagnosed with Crohn's disease than UC or inflammatory bowel disease of uncertain type (IBDU) than their matched patients of white Caucasian descent (Table 5.1). There was no difference in the time to diagnosis between Bangladeshis or white Caucasians for either UC or Crohn's disease (Table 5.1).

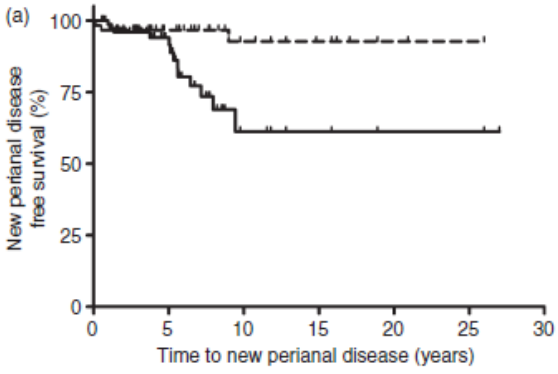
5.3.6 Phenotype and natural history of Crohn's disease

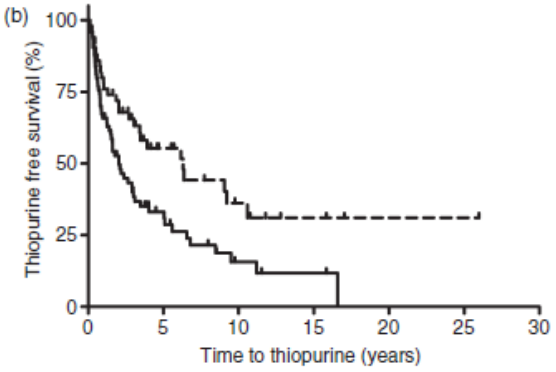
There were no differences in the phenotype of Crohn's disease according to the Montreal Classification between Bangladeshis and white Caucasian descent at diagnosis (Table 5.2). Over the period of follow up, there were no differences in the time to, or the proportions of, patients whose Crohn's disease became more extensive (increased Location (L) or developed upper GI disease + L4) or changed behaviour (increased B) between groups. However, patients of Bangladeshi descent (Hazard ratio (HR) [95%CI] 9.2 [1.7, 48.1], p=0.009) were more likely to develop perianal disease earlier in their disease course than white Caucasians (Figure 5.3a).

Characteristic		Bangladeshi n=79	White Caucasian n=62	p – value
Crohn's Disease (Montreal Classification)	A1: Age≤16, n (%)	33 (42)	29 (48)	0.74
	A2: 17-40, n (%)	43 (54)	29 (48)	
	A3: >40, n (%)	3 (4)	2 (3)	
	L1: Ileal, n (%)	26 (33)	20 (32)	0.47
	L2: Colonic, n (%)	19 (24)	14 (23)	
	L3: Ileocolonic, n (%)	30 (38)	26 (42)	
	+ L4: Upper GI, n (%)	14 (18)	13 (21)	0.39
	B1: Inflammatory, n (%)	50 (63)	34 (55)	0.93
	B2: Stricturing, n (%)	12 (15)	7 (11)	
	B3: Penetrating, n (%)	16 (20)	9 (15)	
	+ p: Perianal, n (%)	18 (23)	10 (16)	0.46
Disease course	Increase in Montreal Classification location (L)	3 (4)	4 (6)	0.92‡
	Time to increase in L (months)	55 [6-227]	92 [17-251]	
	New upper GI disease (+L4)	4 (5)	1 (2)	0.09‡
	Time to develop L4 (months)	56 [6-324]	79 [13-312]	
	Increase in Montreal Classification behaviour (B)	17 (22)	12 (19)	0.81‡
	Time to increase in B (months)	56 [6-324]	64 [1-311]	
	New perianal disease (+p)	13 (16)	2 (3)	0.002‡
	Time to develop +p (months)	61 [6-324]	88 [1-312]	
Medications ever	5-ASA, n (%)	74/79 (94)	49/57 (86)	0.13
	Budesonide, n (%)	25/78 (32)	15/53 (28)	0.65
	Prednisolone, n (%)	52 (66)	38 (61)	0.35
	Number of courses of corticosteroids	1 [0-5]	0 [0-7]	0.02
	Thiopurines, n (%)	60/79 (76)	39/62 (63)	0.01‡
	Time to thiopurines (months)	20 [1-199]	37 [0-312]	
	Methotrexate, n (%)	14 (18)	6 (10)	0.12‡
	Time to methotrexate (months)	54 [6-324]	77 [8-312]	
	Anti-TNF, n (%)	26/79 (33)	13/60 (22)	0.02‡
	Time to anti-TNF (months)	20 [1-199]	37 [0-312]	
Surgery	Intestinal surgery, n (%)	18 (30)	27 (44)	0.03‡
	Time to intestinal surgery (months)	47 [0-190]	42 [0-312]	
	Perianal surgery, n (%)	16 (20)	5 (8)	0.04‡
	Time to perianal surgery	51 [0-324]	75 [1.5-312]	
Vitamin D insufficiency, n (%)		44/57 (77)	16/36 (44)	p<0.01
Vitamin D deficiency, n (%)		21/57 (37)	3/36 (8)	p<0.01
Mean [SD] Vitamin D levels, ng/L		34.5 [22.4]	56.1 [25.3]	p<0.01

Table 5.2: Phenotype and disease course of Crohn's disease patients of white Caucasian and Bangladeshi descent. ‡ denotes Kaplan-Meier analysis, log rank test. Values are expressed as median [range] unless otherwise indicated.

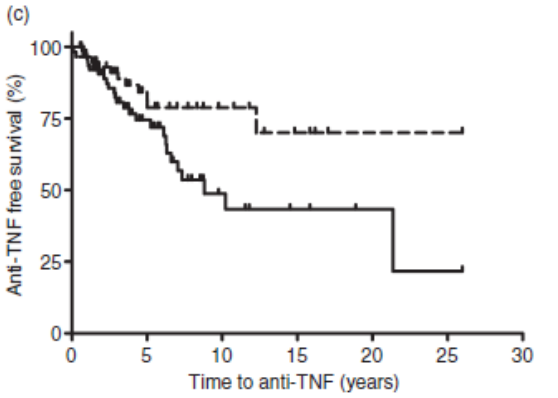
The disease course, as described by the need for treatment escalation differed between the two ethnic groups. Bangladeshi patients required more courses of corticosteroids than white Caucasians (table 5.2). They also tended to require thiopurines and anti-TNFs earlier in their disease course as compared to white Caucasians (figure 5.3b and 5.3c). Interestingly, White Caucasian patients required surgery (mostly a right hemicolectomy) earlier in their disease course than Bangladeshi patients and patients of Bagladeshi descent were more likely to have undergone surgery for perianal disease (figure 5.3d).

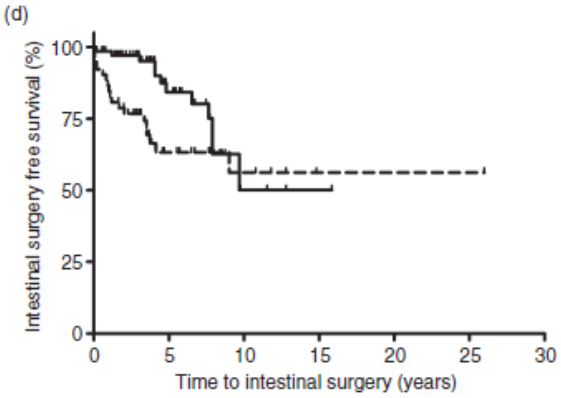




Characteristic [Moderator]	HR	95% CI	*P*-value
Bangladeshi [white]	8.6	1.4, 53.1	0.02
Smoking ever [lifelong non-smoker]	2.3	0.7, 7.8	0.17
ACORN socioeconomic type	1.0	0.98, 1.06	0.29
Age at diagnosis <17 years [≥17]	12.7	2.6, 62	0.002
Ileocolonic Crohn's [ileal or colonic disease]	1.2	0.4, 3.7	0.69
Steroids at diagnosis [no steroids at diagnosis]	2.3	0.2, 23.5	0.47
Thiopurines [no thiopurines]	2.2	0.5, 10.3	0.33

Characteristic [Moderator]	HR	95% CI	*P*-value
Bangladeshi [white]	1.7	1.0, 3.0	0.06
Smoking ever [lifelong non-smoker]	1.3	0.7, 2.1	0.40
ACORN socioeconomic type	1.0	0.99, 1.02	0.64
Age at diagnosis <17 years [≥17]	0.9	0.5, 1.5	0.59
Ileocolonic Crohn's [ileal or colonic disease]	1.0	0.6, 1.7	0.92
Perianal disease [no perianal disease]	1.7	0.9, 2.9	0.08
Steroids at diagnosis [no steroids at diagnosis]	3.0	1.5, 2.9	0.02





Characteristic [Moderator]	HR	95% CI	*P*-value
Bangladeshi [white]	3.0	1.2, 7.7	0.02
Smoking ever [lifelong non-smoker]	0.9	0.4, 1.9	0.75
ACORN socioeconomic type	1.0	0.96, 1.01	0.15
Age at diagnosis <17 years [≥17]	2.5	1.2, 5.1	0.02
Ileocolonic Crohn's [ileal or colonic disease]	0.9	0.4, 1.8	0.77
Perianal disease [no perianal disease]	1.8	0.7, 4.3	0.20
Steroids at diagnosis [no steroids at diagnosis]	1.3	0.4, 4.2	0.65
Thiopurines [no thiopurines]	4.6	1.4, 15.6	0.01

Characteristic [Moderator]	HR	95% CI	*P*-value
Bangladeshi [white]	0.4	0.2, 0.9	0.03
Smoking ever [lifelong non-smoker]	0.5	0.2, 1.4	0.19
ACORN socioeconomic type	1.0	0.99, 1.04	0.19
Age at diagnosis <17 years [≥17]	0.7	0.3, 1.4	0.28
Ileocolonic Crohn's [ileal or colonic disease]	1.1	0.5, 2.3	0.85
Perianal disease [no perianal disease]	0.3	0.1, 1.3	0.12
Steroids at diagnosis [no steroids at diagnosis]	0.6	0.2, 2.1	0.40
Thiopurines [no thiopurines]	1.8	0.8, 4.3	0.16

Figure 5.3: Kaplan Meier analysis (graph) according to ethnicity and Cox regression analysis (table) of time to thiopurine (a), time to biologic (b), time to intestinal surgery (c) and time to new perianal disease (d) of patients with Crohn's disease. The solid lines represent the disease course among patients of Bangladeshi descent and the dotted lines represent the disease course among white Caucasian patients.

5.3.7 Vitamin D and Crohn's disease course

Taking into account previous data supporting that vitamin D deficiency is prevalent among South Asian patients [388] and the possible role of vitamin D in the pathogenesis [228] and course of Crohn's disease [245] we attempted to define the prevalence of vitamin D deficiency and insufficiency in our patients and whether this plays a role in the disease outcome.

The mean [SD] vitamin D levels (ng/L) were significantly lower among Crohn's disease patients of Bangladeshi descent (34.5 [22.4]) when compared to white Caucasian patients (56.1 [25.3]) $p<0.01$. Similarly the prevalence of Vitamin D deficiency and insufficiency were both significantly higher among patients of Bangladeshi descent (Table 5.2). (Please see discussion)

However, the disease progression, described according to progression in phenotype according to the Montreal classification, need for corticosteroids, number of courses of corticosteroids, time to medical treatment escalation and time to surgery was similar between Crohn's disease patients who were deficient or sufficient in vitamin D. (Table 5.3 and 5.4)

		Vit D deficient (n=24)	Not Vit D deficient (n=69)	p value
Disease course	Progress in MC location (L), n (%)	1 (4.2)	5 (7.2)	0.63‡
	Time to progress in L (months)	65.8 [18-311]	68.3 [6.5-324]	
	New upper GI disease (+L4), n (%)	2 (8.3)	2 (2.9)	0.84‡
	Time to develop L4 (months)	74.5 [18-311]	68.3 [6.5-324]	
	Progress in MC behaviour (B), n (%)	4 (16.7)	20 (29)	0.23‡
	Time to progress in B (months)	65.8 [18-311]	56.8 [5.1-324]	
	New perianal disease (+p), n (%)	4 (16.7)	7 (10.1)	0.43‡
	Time to develop +p (months)	62.2 [12-312]	66.3 [6-324]	
Medication ever	Prednisolone, n (%)	17 (70.8)	42 (60.9)	0.38
	Number of courses of steroids	1 [0 - 4]	1 [0-5]	0.69
	Thiopurines, n (%)	18 (75)	50 (72.5)	0.43‡
	Time to thiopurines (months)	20.5 [0-134]	30.1 [0-204]	
	Methotrexate, n (%)	5 (20.8)	6 (8.7)	0.11‡
	Time to methotrexate (months)	61.8 [8-312]	66.3 [6-324]	
	Anti-TNF, n (%)	7 (29.2)	17 (24.6)	0.74‡
	Time to anti-TNF (months)	19 [0-106]	34.3 [0-256]	
Surgery	Intestinal surgery, n (%)	11 (45.8)	32 (46.4)	0.94‡
	Time to intestinal surgery (months)	51 [0-178]	44 [0-190]	
	Perianal surgery, n (%)	3 (12.5)	10 (14.5)	0.82‡
	Time to perianal surgery	65.8 [0-324]	66.3 [0-324]	

Table 5.3 Disease course of Crohn's disease patients who are deficient or not deficient in vitamin D. ‡ denotes Kaplan – Meier analysis, log rank test, Values are expressed as median [range] unless otherwise indicated. MC stands for Montreal Classification

		Vit D insufficient (n=60)	Vit D sufficient (n=33)	p value
Disease course	Progress in MC location (L), n (%)	5 (8.3)	1 (3)	0.24‡
	Time to progress in L (months)	62.2 [6.5-324]	80.5 [19-204]	
	New upper GI disease (L4), n (%)	3 (5)	1 (3)	0.55‡
	Time to develop L4 (months)	64.8 [6.5-324]	80.5 [19-204]	
	Progress in MC behaviour (B), n (%)	15 (25)	9 (27.3)	0.98‡
	Time to progress in B (months)	56.5 [5-324]	67.1 [7-204]	
	New perianal disease (+p), n (%)	8 (13.3)	3 (9.1)	0.41‡
	Time to develop +p (months)	56.7 [6.5-324]	68.3 [19-204]	
Medication ever	Prednisolone, n (%)	37 (61.7)	22 (66.7)	0.63
	Number of courses of steroids	1 [0-5]	1 [0-5]	0.81
	Thiopurines, n (%)	43 (71.7)	25 (75.8)	0.76‡
	Time to thiopurines (months)	25.2 [0-199]	33.5 [0-204]	
	Methotrexate, n (%)	7 (11.7)	4 (12.1)	0.86‡
	Time to methotrexate (months)	56.2 [6.5-324]	77.9 [19-204]	
	Anti-TNF, n (%)	16 (26.7)	8 (24.3)	0.57‡
	Time to anti-TNF (months)	46.6 [6.5-311]	68.3 [11-204]	
Surgery	Intestinal surgery, n (%)	28 (46.7)	15 (45.5)	0.90‡
	Time to intestinal surgery (months)	44.5 [0-190]	45.0 [0-141]	
	Perianal surgery, n (%)	10 (16.7)	3 (9.1)	0.26‡
	Time to perianal surgery	56.3 [0-324]	80.5 [0-204]	

Table 5.4 Disease course of Crohn's disease patients who are insufficient or sufficient in vitamin D. ‡ denotes Kaplan – Meier analysis, log rank test. Values are expressed as median [range] unless otherwise indicated. MC stands for Montreal Classification

5.3.8 Phenotype and disease course of ulcerative colitis

UC patients of Bangladeshi descent were more likely to suffer from extensive disease compared to white Caucasian patients (60% vs 33%, p=0.02) at diagnosis. The likelihood of disease extension during the follow up period was similar between groups. There were no differences in the number of courses of corticosteroids and the time to or proportions

of patients that required escalation of medical therapy or surgical intervention (colectomy) between groups (table 5.5).

Characteristic		**Bangladeshi (n=40)**	**White Caucasian (n=49)**	**p-value**
UC Montreal Classification	E1: Proctitis, n (%)	1 (2.5)	15/45 [31%]	0.002
	E2: Left , n (%)	15 (37.5)	14/45 [29%]	
	E3: Total, n (%)	24 (60)	16/45 [33%]	
Disease course	Extension of UC, n (%)	2 (5)	6/49 [12%]	0.61‡
	Time to extension of UC (months)	40 [9-239]	48 [5-288]	
Medication ever	5-ASA, n (%)	34 (85)	47 [96%]	0.07
	Prednisolone, n (%)	29 (43)	38 [78%]	0.58
	Number of courses of steroids	2 [0-6]	1 [0-13]	0.86
	Thiopurines, n (%)	25 (63)	24 [49%]	0.23‡
	Time to thiopurines (months)	25 [3-239]	54 [3-288]	
	Methotrexate, n (%)	9 (23)	7 [14%]	0.34‡
	Time to methotrexate (months)	49 [9-239]	56 [5-288]	
	Calcineurin inhibitor, n (%)	3 (8)	6 [12%]	0.55‡
	Time to calcineurin inhibitor (months)	52 [0-239]	56 [3-288]	
Surgery	Intestinal surgery, n (%)	3 (7.5)	5 (10.2)	0.68‡
	Time to intestinal surgery (months)	48 [9-239]	56 [3-288]	
Vitamin D insufficiency, n (%)		21/27 (77.8)	8/17 (47.1)	0.04
Vitamin D deficiency, n (%)		14/27 (51.9)	5/17 (29.4)	0.14
Mean [SD] Vitamin D levels, ng/L		33.5 [23.1]	49.4 [28.5]	0.048

Table 5.5: Phenotype and disease course of UC patients of white Caucasian and Bangladeshi descent. ‡ denotes Kaplan – Meier analysis, log rank test. Values are expressed as median [range] unless otherwise indicated.

5.3.9 Vitamin D and the course of UC

There was no difference in the proportion of patients of Bangladeshi descent when compared to white Caucasians who were vitamin D deficient (Vitamin D < 25ng/L) (Table 5.5). However, more patients of Bangladeshi descent were insufficient (Vitamin D < 50 ng/L) in vitamin D when compared to white Caucasians (77.8% vs 47.1%, p=0.04) and the mean [SD] (ng/L) levels of vitamin D were significantly lower among patients of Bangladeshi descent (33.5 [23.1] vs 49.4 [28.5] p<0.05).

Patients who were deficient in vitamin D were significantly more likely to require corticosteroids for the management of their disease compared to those who were not deficient (Table 5.7). There was no difference in the proportion of patients who required or the time to escalation of medical management to immunosuppressants between groups. However, there was a trend towards more often and sooner need for surgery among the vitamin D deficient patients (Table 5.7). Although there was a trend towards more often and sooner disease extension among patients who were not deficient or insufficient in vitamin D, there was no difference in the final Montreal Classification of UC between groups. This is accounted for by the fact that more vitamin D deficient or insufficient patients had extensive disease at the beginning of follow up.

		Vitamin D insufficient (n=29)	**Vitamin D sufficient (n=15)**	**p value**
Disease course	Increase in disease extent, n (%)	1 (3.4)	3 (20)	0.08‡
	Time to increase in extent (months)	92.7 [10-239]	44 [15-239]	
Medication ever	Prednisolone, n (%)	22 (75.9)	12 (80)	0.76
	Number of courses of steroids	1 [0-6]	2 [0-4]	0.45
	Thiopurines, n (%)	16 (55.2)	10 (66.7)	0.41‡
	Time to thiopurines (months)	31.8 [3-239]	21.6 [7-124]	
	Methotrexate, n (%)	6 (20.7)	3 (20)	0.82‡
	Time to methotrexate (months)	72.0 [10-239]	54.7 [9-154]	
Surgery	Surgery, n (%)	4 (13.8)	1 (6.7)	0.56‡
	Time to surgery (months)	66.7 [10-239]	51.1 [10-239]	

Table 5.6 Disease course of patients with UC who are insufficient or sufficient in vitamin D. ‡ denotes Kaplan – Meier analysis, log rank test. Values are expressed as median [range] unless otherwise indicated.

		Vitamin D deficiency (n=19)	No vitamin D deficiency (n=25)	p value
Disease course	Increase in disease extent, n (%)	0 (0)	4 (16)	0.08‡
	Time to increase in extent (months)	93 [10-214]	44 [13-239]	
Medication ever	Prednisolone, n (%)	18 (94.7)	16 (64)	0.02
	Number of courses of steroids	2 [0-6]	1 [0-4]	0.26
	Thiopurines, n (%)	12 (63.2)	14 (56)	0.74‡
	Time to thiopurines (months)	23.4 [5-166]	31.8 [3-239]	
	Methotrexate, n (%)	6 (31.6)	3 (12)	0.18‡
	Time to methotrexate (months)	76.5 [10-214]	54.7 [9-239]	
Surgery	Surgery, n (%)	4 (21.1)	1 (4)	0.10‡
	Time to surgery (months)	76.5 [10-214]	51.1 [9-239]	

Table 5.7 Disease course of patients with UC who are deficient or not in vitamin D.

‡ denotes Kaplan – Meier analysis, log rank test. Values are expressed as median [range] unless otherwise indicated.

5.3.10 Extraintestinal manifestations

A significant cause of morbidity and work disability among patients with IBD is the presence of extra – intestinal manifestations. [392] Moreover, iron deficiency anaemia has been associated with active disease in IBD patients. [176]

The prevalence of organ specific extra-intestinal manifestations of IBD (arthropathy, cutaneous involvement, ocular involvement, PSC) was equal between the two ethnic groups. Significantly more of the patients of Bangladeshi (39% [47/119]) than white Caucasian (20% [24/119]) descent were anaemic (p<0.001). Furthermore, among anaemic patients, haematinics consistent with iron deficiency were more common in the Bangladeshi patients (p=0.03). (Table 5.8)

Characteristic		**Bangladeshi (n=119)**	**White Caucasian (n=119)**	**p - value**
EIM	Prevalence, n (%)	18 (15)	25 (21)	0.2
Type EIM	Arthropathy, n (%)	16 (13)	15 (13)	1
	Cutaneous, n (%)	1 (1)	6 (5)	0.12
	Ocular, n (%)	1 (1)	3 (3)	0.62
	PSC, n (%)	0 (0)	1 (1)	1
	Vitamin D ng/L	34.2 [22.5]	53.3 [26.0]	<0.001
Anaemia	Prevalence, n (%)	47 (39)	24 (20)	0.001
	Haemoglobin, g/dl	12.6 [2.0]	13.4 [1.5]	<0.001
	Haematocrit	0.38 [0.06]	0.4 [0.04]	0.01
	Mean cell volume, fl	84.1 [6.8]	88.6 [6.8]	<0.001
Anaemia type	Iron deficiency, n (%)	38 (81)	12 (50)	0.03
	Anaemia of chronic disease, n (%)	7 (15)	9 (38)	
	Undetermined, n (%)	2 (4)	3 (13)	

Table 5.8: Extra-intestinal manifestations (EIM) of IBD among patients of Bangladeshi and white Caucasian descent. All variables are expressed as mean [standard deviation] unless otherwise indicated

5.4 Discussion

5.4.1 The course of IBD in white Caucasian and patients of Bangladeshi descent living in East London

Compared with patients of white Caucasian descent, patients of Bangladeshi descent are more likely to be diagnosed with Crohn's disease than UC or IBDU. Our findings contrast with previous reports of IBD phenotype in South Asians living in Leicester in the UK [393] , and in a large group of mixed South Asian patients, the majority of whom were of Indian descent, from North-West London: these two ethnic groups, when compared to non-matched Northern European control patients, tended to be more frequently diagnosed with UC. [387] Although Bangladeshis and South Asians have similar genetic backgrounds, our results could be explained by the fact that they may have differences in lifestyle related environmental factors such as smoking habit, alcohol consumption and diet.

More men than women of Bangladeshi descent were diagnosed with Crohn's disease, whereas there was no difference in disease distribution according to genders in white Caucasian patients, possibly because Bagladeshi men are more frequently smokers as compared to women who rarely ever smoke. [394]

5.4.1.1 The natural history and phenotype of Crohn's disease

Previous studies on the phenotype of Crohn's disease in South Asian patients living in North West London suggest a predominant colonic inflammatory phenotype at diagnosis. [387] We found no differences in the phenotype of Crohn's disease at diagnosis between the two ethnic groups, however, more Bangladeshis than white Caucasians developed

new perianal disease. The higher overall prevalence of perianal disease among patients of Bangladeshi descent is likely to be the explanation for the delay in intestinal surgery among this patient group, as in the presence of perianal disease, medical management with thiopurines or anti-TNFs is a more appropriate choice than surgery. In fact, in our patient group, patients of Bangladeshi descent received these treatments more often and earlier in their disease course as compared to white Caucasians. Apart from perianal disease, the earlier escalation of treatment might signify a more aggressive disease course in terms of frequency of exacerbations as this is implied by the higher number of steroid courses among patients of Bangladeshi descent. Overall, the progression of Crohn's disease to a stricturing or penetrating phenotype, regardless of ethnicity and allowing for the duration of disease, appears similar to that frequently cited from Northern Europe. [395]

5.4.1.2 The phenotype and natural history of ulcerative colitis

Patients of Bangladeshi descent were more likely to suffer from extensive ulcerative colitis as compared to white Caucasian patients. Our result is in agreement with other studies of South Asian immigrants in UK. [387, 396]

Despite the difference in the disease extent, no differences were observed between the two ethnic groups in terms of need for escalation of medical management and surgical intervention. Given the fact that the decision making on the need for further investigation and treatment is based on the assessment of symptoms as these are reported in a clinical encounter and taking into account that symptom awareness and reporting is reportedly low among ethnic minorities in England and especially among those for whom English is

not the main language spoken at home, [397] under-reporting of symptoms among UC patients of Bangladeshi descent – and a number of our patients, mainly females do not speak English - could account for the lack of need for treatment intensification compared to white Caucasians who are more likely to suffer from less extensive disease. However, this is speculation.

5.4.1.3 Extra-intestinal manifestations

In agreement with other studies, [398] our results suggest that there is no difference in the prevalence of organ specific extra-intestinal manifestations between the two ethnic groups. However, iron deficient anaemia was more prevalent among patients of Bangladeshi descent. Chronic active inflammation related to more aggressive disease course, [176] dietary deficiency secondary to low socio-economic status and non adherence to oral iron supplementation or treatment of active inflammation, [341] could possibly account for the difference observed.

5.4.2 Vitamin D levels in adult white Caucasian and adult IBD patients of Bangladeshi descent living in East London.

In our population, Bangladeshi patients were more frequently insufficient or deficient in Vitamin D, and had lower mean vitamin D levels when compared to white Caucasian IBD patients. Although there are no other studies investigating vitamin D levels in Bagladeshi IBD patients, our results are in agreement with a recent study that compared white Caucasian IBD patients with South Asians and obtained similar results to ours, with the South Asian patients having lower mean vitamin D levels and being more

frequently vitamin D deficient. [388] Similarly, another recent retrospective study suggests that all South Asian IBD patients were at least insufficient in vitamin D. [399]

Our result could be accounted for by possibly less exposure to sunlight likely related to the adherence of Bangladeshi patients to their traditional dress codes and less dietary intake of vitamin D among the Bangladeshi patients. Although we did not collect data on dietary habits and sunlight exposure among the Bangladeshi patients, it has been documented that South Asian non-IBD controls in UK have a higher prevalence of hypovitaminosis D, which is related to their lower sunlight exposure and dietary intake compared to white Caucasians. [400] These results confirming the vitamin D differences between South Asians and Caucasian patients without IBD in UK validate the originality of the difference in vitamin D levels observed between the two ethnic groups. They indicate that the possibility of the difference being driven by the more aggressive disease course that is documented among the Bangladeshi patients in our study is unlikely.

According to our results, vitamin D deficiency did not affect the course of Crohn's disease; but vitamin D deficient patients with UC were more likely to require treatment with corticosteroids or surgery and had more extensive disease on diagnosis. Our outcome is comparable to that of others that proved a significant association between vitamin D deficiency and the need for treatment with corticosteroids in a series of 34 patients, [401] showing a possible association between vitamin D status and disease course. However in our study almost all vitamin D deficient patients required treatment with corticosteroids while the corresponding percentage in the study of Blanck et al was 47%. [401] This could be possibly explained by the difference in the ethnical

composition of our cohort which included a significant proportion of Bangladeshi patients who have more aggressive disease compared to the group of patients included by Blanck et al where South Asian patients represented only 7% of the whole. [401]

Overall, our study is limited by the lack of serial vitamin D measurements and the possible and likely unrecorded seasonal variation of vitamin D levels which have been documented to peak in August and trough in February. [402]

5.4.3 Strengths

A small number of studies evaluating the difference in the disease course according to ethnicity have been so far published and our study is the first study of ethnicity in groups matched for age and disease duration. We decided, unlike others [387] to compare for a single ethnicity rather than an array of different mixed ethnicities living in the same geographic area and in an attempt to minimize tertiary center bias we only included patients living in the boroughs of inner East London that would receive IBD care in our center irrespectable of our tertiary center status.

5.4.4 Limitations

Because of the retrospective data collection in our study, our results are possibly subject to interpretation bias and bias because of missing data for certain variables, for example the smoking habit of our patients. Our sample size is relatively small and as a consequence of this our results might be subject to type II error. Although our choice to compare matched groups derived from an intension to obtain more easily interpretable results, comparing all the white Caucasians with all the Bangladeshi patients living in

inner East London boroughs and statistically adjusting for age and disease duration could have resulted in better statistical power as 234 white Caucasian and 121 Bangladeshi patients would have been included. We elected not to use the Bonferroni correction for multiple comparisons because our study of hypothesis driven and our endpoints were all part of the natural history of IBD, therefore using the Bonferroni correction to reduce type I error would be likely to result in possibly false negative results.

CHAPTER VI: DISCUSSION

6.1 Summary of findings

This study explores a number of inter-related aspects of the effect of nutrients and environmental factors in the course of inflammatory bowel disease in children and adults. (see figure 6.1) In particular, we focus on enteral nutrition and its role on disease outcome and on a co-stimulatory molecule. We describe data on the outcome of Crohn's disease among adult patients after EEN administration and we also explore the role of ethnicity on the course of IBD taking into account the links between ethnicity and vitamin D deficiency.

We present data that suggest that a co-stimulatory molecule, CD58 is present in the intestinal epithelium of Crohn's disease and UC patients and that in vitro, this molecule is down-regulated in the intestinal epithelial cell lines after exposure to enteral nutrition. Although further study will be required (including animal models), a down-regulation of co-stimulatory activity could be one of the mechanisms by which enteral nutrition exerts its beneficial effect in the treatment of active Crohn's disease and CD58 might also be a novel therapeutic target. More complex mechanistic studies with IEC and T cell co-cultures as well as animal studies are required to give a clearer indication as to whether this effect impacts on T cell activation and IBD activity.

We have shown that 57% of our paediatric Crohn's disease patients achieve clinical remission with exclusive enteral nutrition. These patients have a better clinical outcome over the course of 5 years; they take longer to clinically relapse and longer to require treatment with corticosteroids and escalation of treatment to thiopurines. Additionally, they are less likely to develop stricturing complications of Crohn's disease. However, our conclusion is limited by the fact that we use the physician's global assessment as an

indicator of disease activity and not a more quantitative measure of disease activity such as the CDAI.

We present data to suggest that Vitamin D-deficient paediatric patients treated with EEN are more likely to require treatment with corticosteroids and thiopurines. This is an indication, rather than a robust conclusion as it is based on a single vitamin D level measurement. It has been shown that a single measurement of Vitamin D in epidemiological studies can be predictive of one's status over the course of 1-3 years [403] however our conclusions may be further affected by the effect of reverse causality between disease activity and vitamin D status. [404]

EEN has been well tolerated among adult patients. In our small and underpowered study (due to its rarity of use in adult practice) there was no difference in the disease outcomes of adult patients with CD after EEN when white Caucasian patients were compared with South Asians.

Therefore, we devised another study focusing solidly in the comparison of disease course in different ethnic groups. Patients of Bangladeshi descent were more likely to be diagnosed with IBD at a younger age, were more likely to suffer from Crohn's disease and when diagnosed with UC they were more likely to suffer from extensive disease when compared to their matched white Caucasian patients. Crohn's disease patients of Bangladeshi descent had a more aggressive disease course: they would also more often and sooner require treatment with thiopurines, anti-TNF or surgery. They would more frequently develop new perianal complications. Ethnicity was the only factor independently associated with all markers of disease progression. Crohns disease patients had similar disease course in terms of need for escalation of medical treatment, need for

surgery and progression of phenotype according to their Montreal Classification irrespective of their vitamin D status. However, among UC patients, those who were deficient in vitamin D had more extensive disease on diagnosis and were more likely to require treatment with corticosteroids or surgery.

6.2 Future directions

The studies published on the effects of nutrients in IBD represent a broad spectrum of the current and past research. However, the data presented in this thesis create new directions for further research in the future, as the work presented in this thesis could be expanded in a way that will answer further clinically relevant research questions.

We are currently collecting data on the effect of EEN and the response to EEN on children's growth, but this will be presented in a separate thesis. Although others have shown that EEN improves growth the first two years after initial administration, there are no data to suggest whether this effect is sustained in the longer term and whether the initial response or failure to respond to EEN is a determinant. In our study, we present the impact of the initial response to EEN on the clinical outcomes of the disease. Our measure of disease activity has been the physician's global assessment. We also used treatment escalation as a surrogate marker of disease activity. However, a prospective study would enable the use of more objective markers of disease activity, such as the PCAI and faecal calprotectin. It would also enable the serial measurement of vitamin D levels, enabling a more accurate answer on the impact of vitamin D levels on the initial response to EEN and the course of disease activity. This would allow us to quantify the

time spent in remission over the course of follow up and its comparison between responders and non responders to EEN resulting in a much clearer answer on whether the initial response to EEN defines the long term disease outcome. In this context the connection between EEN administration, cumulative time spent in remission and growth could be more accurately investigated as well.

We used the change in a patient's Montreal classification as a surrogate marker of disease progression. The current concept in the evaluation of Crohn's disease progression considers the Lemann index [405] as a more accurate marker of the cumulative intestinal damage over time. The Lemann index is calculated based on specific endoscopic and imaging findings [405] and has already been used to evaluate the impact of anti-TNF therapy in CD progression. [406] In a prospective longitudinal study the intestinal damage could be assessed accurately with the use of the Lemann score. This would provide accurate quantitation of disease progression over time and a clearer conclusion on the effect of initial response to EEN on the long term clinical outcome.

An important challenging question on EEN is whether it has a direct immunomodulatory role. In a more invasive setting, allowing for ileal biopsies to be taken before and after EEN administration, the role of EEN as an epigenetic factor that interferes in the methylation of different genes in the intestinal mucosa could be investigated. The quality of genes implicated could give an answer on the role of EEN in the immunology of Crohn's disease and could also provide clues on signaling pathways that could be therapeutically targeted.

Work is required to further evaluate the effect of EEN on the expression of CD58 in the intestinal epithelium in order to extract more information on how EEN exerts its beneficial effect on patients with Crohn's disease and in order to determine whether CD58 could be a therapeutic target in Crohn's disease. Studies on animal models of IBD would be particularly helpful on assessing the effect of EEN on the expression of CD48 (the CD58 analogue for mice) in murine models of colitis eg DSS colitis. Additionally, a trial of treatment with blocking anti-CD48 antibodies in murine models of IBD would provide preliminary information on a possible beneficial effect of alefacept in human patients with Crohn's disease or Ulcerative Colitis.

The results of clinical studies of the role of vitamin D in IBD are so far conflicting. Better designed prospective studies using serial vitamin D measurements will provide more information on the role of vitamin D in the clinical course of IBD. However, one of the most important factors influencing the credibility of any clinical result is its pathophysiological explanation: investigating the effect of vitamin D on the expression and function of TLR4 on the intestinal epithelium, on the antigen presentation in the mucosal dendritic cells using animal models of IBD and on the composition and metabonomics of the faecal microbiota would answer important questions. In the clinical setting serial measurements of Vitamin D among patients with IBD would give more convincing answers about the role of serum levels of Vitamin D in the disease course, or may provide a different cut off target range for IBD patients as the current levels of sufficiency or insufficiency have been estimated based on the role of vitamin D in bone health. Additionally, clinical studies on IBD patients who are are in remission or have

active disease and are administered vitamin D supplementation could give valuable information about the role of Vitamin D status and supplementation on the disease activity. At the same time the study of the faecal microbiome and metabonome of these patients in active and inactive disease status and vitamin D sufficient or deficient status would provide interesting data on whether vitamin D modifies the intestinal microbiota and metabonomics Furthermore, taking intestinal or colonic mucosa biopsies and studying their epigenetic profile could give significant information on the role of vitamin D as an epigenetic regulator of disease activity and direct clues on the genes and signaling pathways that it may affect.

Chapter VII: References

1. Baumgart, D.C. and W.J. Sandborn, *Crohn's disease.* Lancet, 2012. **380**(9853): p. 1590-605.
2. Cameron, F.L., R. Hansen, and D. Goudie, *Inflammatory bowel disease: a classic case of Dalziel's disease.* BMJ, 2010. **341**: p. c4932.
3. Danese, S. and C. Fiocchi, *Ulcerative colitis.* N Engl J Med, 2011. **365**(18): p. 1713-25.
4. Ng, S.C., et al., *Geographical variability and environmental risk factors in inflammatory bowel disease.* Gut, 2013.
5. Cosnes, J., et al., *Epidemiology and natural history of inflammatory bowel diseases.* Gastroenterology, 2011. **140**(6): p. 1785-94.
6. Loftus, E.V., Jr., P. Schoenfeld, and W.J. Sandborn, *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**(1): p. 51-60.
7. Steed, H., S. Walsh, and N. Reynolds, *Crohn's disease incidence in NHS Tayside.* Scott Med J, 2010. **55**(3): p. 22-5.
8. Wilson, J., et al., *High incidence of inflammatory bowel disease in Australia: a prospective population-based Australian incidence study.* Inflamm Bowel Dis, 2010. **16**(9): p. 1550-6.
9. Gearry, R.B., et al., *Population-based cases control study of inflammatory bowel disease risk factors.* J Gastroenterol Hepatol, 2010. **25**(2): p. 325-33.

10. Burisch, J., et al., *East-West gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort.* Gut, 2014. **63**(4): p. 588-97.

11. Vegh, Z., et al., *Incidence and initial disease course of inflammatory bowel diseases in 2011 in Europe and Australia: results of the 2011 ECCO-EpiCom inception cohort.* J Crohns Colitis, 2014. **8**(11): p. 1506-15.

12. Zheng, J.J., et al., *Prevalence and incidence rates of Crohn's disease in mainland China: a meta-analysis of 55 years of research.* J Dig Dis, 2010. **11**(3): p. 161-6.

13. Sawczenko, A., et al., *Prospective survey of childhood inflammatory bowel disease in the British Isles.* Lancet, 2001. **357**(9262): p. 1093-4.

14. Henderson, P., et al., *Rising incidence of pediatric inflammatory bowel disease in Scotland.* Inflamm Bowel Dis, 2012. **18**(6): p. 999-1005.

15. Abramson, O., et al., *Incidence, prevalence, and time trends of pediatric inflammatory bowel disease in Northern California, 1996 to 2006.* J Pediatr, 2010. **157**(2): p. 233-239 e1.

16. Chouraki, V., et al., *The changing pattern of Crohn's disease incidence in northern France: a continuing increase in the 10- to 19-year-old age bracket (1988-2007).* Aliment Pharmacol Ther, 2011. **33**(10): p. 1133-42.

17. Turunen, P., et al., *Incidence of inflammatory bowel disease in Finnish children, 1987-2003.* Inflamm Bowel Dis, 2006. **12**(8): p. 677-83.

18. Tsironi, E., et al., *Incidence of inflammatory bowel disease is rising and abdominal tuberculosis is falling in Bangladeshis in East London, United Kingdom.* Am J Gastroenterol, 2004. **99**(9): p. 1749-55.

19. Barreiro-de Acosta, M., et al., *Emigration to western industrialized countries: A risk factor for developing inflammatory bowel disease.* J Crohns Colitis, 2011. **5**(6): p. 566-9.

20. Rutgeerts, P., et al., *Predictability of the postoperative course of Crohn's disease.* Gastroenterology, 1990. **99**(4): p. 956-63.

21. Olaison, G., K. Smedh, and R. Sjodahl, *Natural course of Crohn's disease after ileocolic resection: endoscopically visualised ileal ulcers preceding symptoms.* Gut, 1992. **33**(3): p. 331-5.

22. Gasche, C., 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**(1): p. 8-15.

23. Levine, A., et al., *Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification.* Inflamm Bowel Dis, 2011. **17**(6): p. 1314-21.

24. Schwartz, D.A., J.H. Pemberton, and W.J. Sandborn, *Diagnosis and treatment of perianal fistulas in Crohn disease.* Ann Intern Med, 2001. **135**(10): p. 906-18.

25. Schwartz, D.A., et al., *The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota.* Gastroenterology, 2002. **122**(4): p. 875-80.

26. Thia, K.T., et al., *Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort.* Gastroenterology, 2010. **139**(4): p. 1147-55.

27. Louis, E., et al., *Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease.* Gut, 2001. **49**(6): p. 777-82.

28. Van Limbergen, J., et al., *Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease.* Gastroenterology, 2008. **135**(4): p. 1114-22.

29. Munkholm, P., et al., *Disease activity courses in a regional cohort of Crohn's disease patients.* Scand J Gastroenterol, 1995. **30**(7): p. 699-706.

30. Froslie, K.F., et al., *Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort.* Gastroenterology, 2007. **133**(2): p. 412-22.

31. Solberg, I.C., et al., *Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study.* Clin Gastroenterol Hepatol, 2007. **5**(12): p. 1430-8.

32. Cosnes, J., et al., *Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery.* Gut, 2005. **54**(2): p. 237-41.

33. Sands, B.E., et al., *Risk of early surgery for Crohn's disease: implications for early treatment strategies.* Am J Gastroenterol, 2003. **98**(12): p. 2712-8.

34. Gearry, R.B., et al., *Predictors for developing intestinal failure in patients with Crohn's disease.* J Gastroenterol Hepatol, 2013. **28**(5): p. 801-7.

35. Moum, B., et al., *Change in the extent of colonoscopic and histological involvement in ulcerative colitis over time.* Am J Gastroenterol, 1999. **94**(6): p. 1564-9.

36. Langholz, E., et al., *Course of ulcerative colitis: analysis of changes in disease activity over years.* Gastroenterology, 1994. **107**(1): p. 3-11.

37. Turner, D., et al., *Severe paediatric ulcerative colitis: incidence, outcomes and optimal timing for second-line therapy.* Gut, 2008. **57**(3): p. 331-8.

38. Vernier-Massouille, G., et al., *Natural history of pediatric Crohn's disease: a population-based cohort study.* Gastroenterology, 2008. **135**(4): p. 1106-13.

39. Ruel, J., et al., *IBD across the age spectrum: is it the same disease?* Nat Rev Gastroenterol Hepatol, 2014. **11**(2): p. 88-98.

40. Sanderson, I.R., R.A. Risdon, and J.A. Walker-Smith, *Intractable ulcerating enterocolitis of infancy.* Arch Dis Child, 1991. **66**(3): p. 295-9.

41. Imielinski, M., et al., *Common variants at five new loci associated with early-onset inflammatory bowel disease.* Nat Genet, 2009. **41**(12): p. 1335-40.

42. Cutler, D.J., et al., *Dissecting Allele Architecture of Early Onset IBD Using High-Density Genotyping.* PLoS One, 2015. **10**(6): p. e0128074.

43. Hansen, R., et al., *Microbiota of de-novo pediatric IBD: increased Faecalibacterium prausnitzii and reduced bacterial diversity in Crohn's but not in ulcerative colitis.* Am J Gastroenterol, 2012. **107**(12): p. 1913-22.

44. Quevrain, E., et al., *Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn's disease.* Gut, 2015.

45. Sokol, H., et al., *Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients.* Proc Natl Acad Sci U S A, 2008. **105**(43): p. 16731-6.

46. Drossman, D.A., et al., *Health-related quality of life in inflammatory bowel disease. Functional status and patient worries and concerns.* Dig Dis Sci, 1989. **34**(9): p. 1379-86.

47. Naliboff, B.D., et al., *Gastrointestinal and psychological mediators of health-related quality of life in IBS and IBD: a structural equation modeling analysis.* Am J Gastroenterol, 2012. **107**(3): p. 451-9.

48. Moradkhani, A., L.J. Beckman, and J.H. Tabibian, *Health-related quality of life in inflammatory bowel disease: Psychosocial, clinical, socioeconomic, and demographic predictors.* J Crohns Colitis, 2012.

49. Hill, R., et al., *Quality of life in children with Crohn disease.* J Pediatr Gastroenterol Nutr, 2010. **51**(1): p. 35-40.

50. Marri, S.R. and A.L. Buchman, *The education and employment status of patients with inflammatory bowel diseases.* Inflamm Bowel Dis, 2005. **11**(2): p. 171-7.

51. Loonen, H.J., et al., *Quality of life in paediatric inflammatory bowel disease measured by a generic and a disease-specific questionnaire.* Acta Paediatr, 2002. **91**(3): p. 348-54.

52. Bastida, G., et al., *The effects of thiopurine therapy on health-related quality of life in Inflammatory Bowel Disease patients*. BMC Gastroenterol, 2010. **10**: p. 26.

53. Casellas, F., et al., *Restoration of quality of life of patients with inflammatory bowel disease after one year with antiTNFalpha treatment.* J Crohns Colitis, 2012. **6**(9): p. 881-6.

54. Casellas, F., et al., *Mucosal healing restores normal health and quality of life in patients with inflammatory bowel disease.* Eur J Gastroenterol Hepatol, 2012. **24**(7): p. 762-9.

55. Spehlmann, M.E., et al., *Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study.* Inflamm Bowel Dis, 2008. **14**(7): p. 968-76.

56. Ng, S.C., et al., *Role of genetic and environmental factors in British twins with inflammatory bowel disease.* Inflamm Bowel Dis, 2012. **18**(4): p. 725-36.

57. Bengtson, M.B., et al., *Familial aggregation in Crohn's disease and ulcerative colitis in a Norwegian population-based cohort followed for ten years.* J Crohns Colitis, 2009. **3**(2): p. 92-9.

58. Jostins, L., et al., *Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease.* Nature, 2012. **491**(7422): p. 119-24.

59. Franke, A., et al., *Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci.* Nat Genet, 2010. **42**(12): p. 1118-25.

60. Tsianos, E.V., K.H. Katsanos, and V.E. Tsianos, *Role of genetics in the diagnosis and prognosis of Crohn's disease.* World J Gastroenterol, 2011. **17**(48): p. 5246-59.

61. Baumgart, D.C. and S.R. Carding, *Inflammatory bowel disease: cause and immunobiology.* Lancet, 2007. **369**(9573): p. 1627-40.

62. Cario, E. and D.K. Podolsky, *Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease.* Infect Immun, 2000. **68**(12): p. 7010-7.

63. Rhee, S.H., et al., *Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation.* Proc Natl Acad Sci U S A, 2005. **102**(38): p. 13610-5.

64. Banchereau, J. and R.M. Steinman, *Dendritic cells and the control of immunity.* Nature, 1998. **392**(6673): p. 245-52.

65. Baumgart, D.C., et al., *Patients with active inflammatory bowel disease lack immature peripheral blood plasmacytoid and myeloid dendritic cells.* Gut, 2005. **54**(2): p. 228-36.

66. Baumgart, D.C., et al., *Aberrant plasmacytoid dendritic cell distribution and function in patients with Crohn's disease and ulcerative colitis.* Clin Exp Immunol, 2011. **166**(1): p. 46-54.

67. Hart, A.L., et al., *Characteristics of intestinal dendritic cells in inflammatory bowel diseases.* Gastroenterology, 2005. **129**(1): p. 50-65.

68. Koboziev, I., F. Karlsson, and M.B. Grisham, *Gut-associated lymphoid tissue, T cell trafficking, and chronic intestinal inflammation.* Ann N Y Acad Sci, 2010. **1207 Suppl 1**: p. E86-93.

69. Bouma, G. and W. Strober, *The immunological and genetic basis of inflammatory bowel disease.* Nat Rev Immunol, 2003. **3**(7): p. 521-33.

70. Brand, S., *Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease.* Gut, 2009. **58**(8): p. 1152-67.

71. Bierer, B.E. and W.C. Hahn, *T cell adhesion, avidity regulation and signaling: a molecular analysis of CD2.* Semin Immunol, 1993. **5**(4): p. 249-61.

72. Harris, N.L. and F. Ronchese, *The role of B7 costimulation in T-cell immunity.* Immunol Cell Biol, 1999. **77**(4): p. 304-11.

73. Schultze, J., L.M. Nadler, and J.G. Gribben, *B7-mediated costimulation and the immune response.* Blood Rev, 1996. **10**(2): p. 111-27.

74. Sandborn, W.J., et al., *Abatacept for Crohn's disease and ulcerative colitis.* Gastroenterology, 2012. **143**(1): p. 62-69 e4.

75. Grose, R.H., et al., *Expression of B7 costimulatory molecules by cells infiltrating the colon in experimental colitis induced by oral dextran sulfate sodium in the mouse.* J Gastroenterol Hepatol, 2001. **16**(11): p. 1228-34.

76. Rogler, G., et al., *T-cell co-stimulatory molecules are upregulated on intestinal macrophages from inflammatory bowel disease mucosa.* Eur J Gastroenterol Hepatol, 1999. **11**(10): p. 1105-11.

77. Rugtveit, J., A. Bakka, and P. Brandtzaeg, *Differential distribution of B7.1 (CD80) and B7.2 (CD86) costimulatory molecules on mucosal macrophage subsets in human inflammatory bowel disease (IBD).* Clin Exp Immunol, 1997. **110**(1): p. 104-13.

78. Davenport, C.M., et al., *Inhibition of pro-inflammatory cytokine generation by CTLA4-Ig in the skin and colon of mice adoptively transplanted with CD45RBhi*

CD4+ T cells correlates with suppression of psoriasis and colitis. Int Immunopharmacol, 2002. **2**(5): p. 653-72.

79. Shahinian, A., et al., *Differential T cell costimulatory requirements in CD28-deficient mice.* Science, 1993. **261**(5121): p. 609-12.

80. Yang, S.Y., S. Chouaib, and B. Dupont, *A common pathway for T lymphocyte activation involving both the CD3-Ti complex and CD2 sheep erythrocyte receptor determinants.* J Immunol, 1986. **137**(4): p. 1097-100.

81. Sayre, P.H. and E.L. Reinherz, *Structure and function of the erythrocyte receptor CD2 on human T lymphocytes: a review.* Scand J Rheumatol Suppl, 1988. **76**: p. 131-44.

82. Bromberg, J.S., *The biology of CD2: adhesion, transmembrane signal, and regulatory receptor of immunity.* J Surg Res, 1993. **54**(3): p. 258-67.

83. Sewell, W.A., et al., *Molecular cloning of the human T-lymphocyte surface CD2 (T11) antigen.* Proc Natl Acad Sci U S A, 1986. **83**(22): p. 8718-22.

84. Kamoun, M., et al., *Identification of a human T lymphocyte surface protein associated with the E-rosette receptor.* J Exp Med, 1981. **153**(1): p. 207-12.

85. Selvaraj, P., et al., *The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3.* Nature, 1987. **326**(6111): p. 400-3.

86. Jining, L., et al., *Design, structure and biological activity of beta-turn peptides of CD2 protein for inhibition of T-cell adhesion.* Eur J Biochem, 2004. **271**(14): p. 2873-86.

87. Takai, Y., et al., *Direct evidence for a receptor-ligand interaction between the T-cell surface antigen CD2 and lymphocyte-function-associated antigen 3.* Proc Natl Acad Sci U S A, 1987. **84**(19): p. 6864-8.

88. Bierer, B.E., et al., *Interaction of CD2 with its ligand, LFA-3, in human T cell proliferation.* J Immunol, 1988. **140**(10): p. 3358-63.

89. Endler-Jobst, B., et al., *Human T cell responses to IL-1 and IL-6 are dependent on signals mediated through CD2.* J Immunol, 1991. **146**(6): p. 1736-42.

90. Bierer, B.E., et al., *Synergistic T cell activation via the physiological ligands for CD2 and the T cell receptor.* J Exp Med, 1988. **168**(3): p. 1145-56.

91. Hahn, W.C., et al., *Interaction of CD2 with its ligand lymphocyte function-associated antigen-3 induces adenosine 3',5'-cyclic monophosphate production in T lymphocytes.* J Immunol, 1991. **147**(1): p. 14-21.

92. Hahn, W.C., S.J. Burakoff, and B.E. Bierer, *Signal transduction pathways involved in T cell receptor-induced regulation of CD2 avidity for CD58.* J Immunol, 1993. **150**(7): p. 2607-19.

93. Dengler, T.J., et al., *Structural and functional epitopes of the human adhesion receptor CD58 (LFA-3).* Eur J Immunol, 1992. **22**(11): p. 2809-17.

94. Wallich, R., et al., *Gene structure, promoter characterization, and basis for alternative mRNA splicing of the human CD58 gene.* J Immunol, 1998. **160**(6): p. 2862-71.

95. Xu, H., et al., *Human blood dendritic cells exhibit a distinct T-cell-stimulating mechanism and differentiation pattern.* Scand J Immunol, 1992. **36**(5): p. 689-96.

96. Hodge, J.W., et al., *Enhanced activation of T cells by dendritic cells engineered to hyperexpress a triad of costimulatory molecules.* J Natl Cancer Inst, 2000. **92**(15): p. 1228-39.

97. Kvale, D., P. Krajci, and P. Brandtzaeg, *Expression and regulation of adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) in human intestinal epithelial cell lines.* Scand J Immunol, 1992. **35**(6): p. 669-76.

98. Framson, P.E., et al., *Polarized expression and function of the costimulatory molecule CD58 on human intestinal epithelial cells.* Gastroenterology, 1999. **116**(5): p. 1054-62.

99. Ebert, E.C., A. Panja, and R. Praveen, *Human intestinal intraepithelial lymphocytes and epithelial cells coinduce interleukin-8 production through the CD2-CD58 interaction.* Am J Physiol Gastrointest Liver Physiol, 2009. **296**(3): p. G671-7.

100. Ebert, E.C., *CD2 activation of human lamina propria lymphocytes reduces CD3 responsiveness.* Immunology, 2006. **117**(1): p. 71-7.

101. Hoffmann, J.C., et al., *Reduced serum levels of a soluble form of the human adhesion receptor CD58 (LFA-3) in patients with inflammatory bowel disease.* Z Gastroenterol, 1996. **34**(9): p. 522-7.

102. Arijs, I., et al., *Mucosal gene expression of antimicrobial peptides in inflammatory bowel disease before and after first infliximab treatment.* PLoS One, 2009. **4**(11): p. e7984.

103. Abadia-Molina, A.C., et al., *CD48 controls T-cell and antigen-presenting cell functions in experimental colitis.* Gastroenterology, 2006. **130**(2): p. 424-34.

104. Koretz, K., P. Schlag, and P. Moller, *Sporadic loss of leucocyte-function-associated antigen-3 (LFA-3) in colorectal carcinomas.* Virchows Arch A Pathol Anat Histopathol, 1991. **419**(5): p. 389-94.

105. Moller, P., et al., *Frequency of abnormal expression of HLA-A,B,C and HLA-DR molecules, invariant chain, and LFA-3 (CD58) in colorectal carcinoma and its impact on tumor recurrence.* Int J Cancer Suppl, 1991. **6**: p. 155-62.

106. Smith, M.E., et al., *Loss of HLA-A,B,C allele products and lymphocyte function-associated antigen 3 in colorectal neoplasia.* Proc Natl Acad Sci U S A, 1989. **86**(14): p. 5557-61.

107. Lorenz, M.G., et al., *Induction of anti-tumor immunity elicited by tumor cells expressing a murine LFA-3 analog via a recombinant vaccinia virus.* Hum Gene Ther, 1999. **10**(4): p. 623-31.

108. Wang, P., et al., *CD58 expression of liver tissue in patients with chronic hepatitis B virus infection.* Chin Med J (Engl), 2008. **121**(6): p. 557-60.

109. Sheng, L., et al., *Investigation on correlation between expression of CD58 molecule and severity of hepatitis B.* World J Gastroenterol, 2006. **12**(26): p. 4237-40.

110. Mosnier, J.F., et al., *Expression of cytokine-dependent immune adhesion molecules by hepatocytes and bile duct cells in chronic hepatitis C.* Gastroenterology, 1994. **107**(5): p. 1457-68.

111. Garcia-Monzon, C., et al., *Down-regulation of intercellular adhesion molecule 1 on hepatocytes in viral chronic hepatitis treated with interferon alfa-2b.* Gastroenterology, 1993. **105**(2): p. 462-9.

112. Takahashi, H., et al., *Integrins and other adhesion molecules on lymphocytes from synovial fluid and peripheral blood of rheumatoid arthritis patients.* Eur J Immunol, 1992. **22**(11): p. 2879-85.

113. Raychaudhuri, S., et al., *Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk.* Nat Genet, 2009. **41**(12): p. 1313-8.

114. Satyanarayanajois, S.D., et al., *A peptide from the beta-strand region of CD2 protein that inhibits cell adhesion and suppresses arthritis in a mouse model.* Chem Biol Drug Des, 2010. **76**(3): p. 234-44.

115. De Jager, P.L., et al., *The role of the CD58 locus in multiple sclerosis.* Proc Natl Acad Sci U S A, 2009. **106**(13): p. 5264-9.

116. Brucklacher-Waldert, V., et al., *Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis.* Brain, 2009. **132**(Pt 12): p. 3329-41.

117. Horrocks, C., et al., *Adhesion molecule expression in psoriatic skin lesions and the influence of cyclosporin A.* Clin Exp Immunol, 1991. **84**(1): p. 157-62.

118. Prens, E., et al., *Adhesion molecules and IL-1 costimulate T lymphocytes in the autologous MECLR in psoriasis.* Arch Dermatol Res, 1996. **288**(2): p. 68-73.

119. Ellis, C.N. and G.G. Krueger, *Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes.* N Engl J Med, 2001. **345**(4): p. 248-55.

120. Miller, G.T., et al., *Specific interaction of lymphocyte function-associated antigen 3 with CD2 can inhibit T cell responses.* J Exp Med, 1993. **178**(1): p. 211-22.

121. Krueger, G.G., et al., *A randomized, double-blind, placebo-controlled phase III study evaluating efficacy and tolerability of 2 courses of alefacept in patients with chronic plaque psoriasis.* J Am Acad Dermatol, 2002. **47**(6): p. 821-33.

122. Stotler, C.J., et al., *Rapid resolution of GVHD after orthotopic liver transplantation in a patient treated with alefacept.* Blood, 2009. **113**(21): p. 5365-6.

123. Toor, A.A., et al., *Alefacept in corticosteroid refractory graft versus host disease: early results indicate promising activity.* J Dermatolog Treat, 2007. **18**(1): p. 13-8.

124. Shapira, M.Y., et al., *Rapid response to alefacept given to patients with steroid resistant or steroid dependent acute graft-versus-host disease: a preliminary report.* Bone Marrow Transplant, 2005. **36**(12): p. 1097-101.

125. Shapira, M.Y., et al., *Alefacept treatment for refractory chronic extensive GVHD.* Bone Marrow Transplant, 2009. **43**(4): p. 339-43.

126. Lindberg, E., G. Jarnerot, and B. Huitfeldt, *Smoking in Crohn's disease: effect on localisation and clinical course.* Gut, 1992. **33**(6): p. 779-82.

127. Kane, S.V., M. Flicker, and F. Katz-Nelson, *Tobacco use is associated with accelerated clinical recurrence of Crohn's disease after surgically induced remission.* J Clin Gastroenterol, 2005. **39**(1): p. 32-5.

128. Ryan, W.R., et al., *Crohn's disease patients who quit smoking have a reduced risk of reoperation for recurrence.* Am J Surg, 2004. **187**(2): p. 219-25.

129. Andersson, R.E., et al., *Appendectomy and protection against ulcerative colitis.* N Engl J Med, 2001. **344**(11): p. 808-14.

130. Koutroubakis, I.E. and I.G. Vlachonikolis, *Appendectomy and the development of ulcerative colitis: results of a metaanalysis of published case-control studies.* Am J Gastroenterol, 2000. **95**(1): p. 171-6.

131. Naganuma, M., et al., *Appendectomy protects against the development of ulcerative colitis and reduces its recurrence: results of a multicenter case-controlled study in Japan.* Am J Gastroenterol, 2001. **96**(4): p. 1123-6.

132. Cosnes, J., et al., *Effects of appendicectomy on the course of ulcerative colitis.* Gut, 2002. **51**(6): p. 803-7.

133. Hviid, A., H. Svanstrom, and M. Frisch, *Antibiotic use and inflammatory bowel diseases in childhood.* Gut, 2011. **60**(1): p. 49-54.

134. Shaw, S.Y., J.F. Blanchard, and C.N. Bernstein, *Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis.* Am J Gastroenterol, 2011. **106**(12): p. 2133-42.

135. Kronman, M.P., et al., *Antibiotic exposure and IBD development among children: a population-based cohort study.* Pediatrics, 2012. **130**(4): p. e794-803.

136. Mayberry, J.F., et al., *Diet in Crohn's disease two studies of current and previous habits in newly diagnosed patients.* Dig Dis Sci, 1981. **26**(5): p. 444-8.

137. Martini, G.A. and J.W. Brandes, *Increased consumption of refined carbohydrates in patients with Crohn's disease.* Klin Wochenschr, 1976. **54**(8): p. 367-71.

138. Jantchou, P., et al., *Animal protein intake and risk of inflammatory bowel disease: The E3N prospective study.* Am J Gastroenterol, 2010. **105**(10): p. 2195-201.

139. Tjonneland, A., et al., *Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study.* Gut, 2009. **58**(12): p. 1606-11.

140. Shoda, R., et al., *Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan.* Am J Clin Nutr, 1996. **63**(5): p. 741-5.

141. Shoda, R., et al., *Therapeutic efficacy of N-3 polyunsaturated fatty acid in experimental Crohn's disease.* J Gastroenterol, 1995. **30 Suppl 8**: p. 98-101.

142. Ashwin N. Ananthakrishnan, M.D., M.P.H Hamed Khalili, M.D Gauree G. Konijeti,, et al., *A Prospective Study of Long-term Intake of Dietary Fiber and Risk of Crohn's*

Disease and Ulcerative Colitis. Gastroenterology, 2013. **In press**.

143. Benjamin, J.L., et al., *Randomised, double-blind, placebo-controlled trial of fructo-oligosaccharides in active Crohn's disease.* Gut, 2011. **60**(7): p. 923-9.

144. Ventham, N.T., et al., *Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics.* Gastroenterology, 2013. **145**(2): p. 293-308.

145. Siedlinski, M., et al., *Association of cigarette smoking and CRP levels with DNA methylation in alpha-1 antitrypsin deficiency.* Epigenetics, 2012. **7**(7): p. 720-8.

146. Gross, T.J., et al., *A microRNA processing defect in smokers' macrophages is linked to SUMOylation of the endonuclease DICER.* J Biol Chem, 2014. **289**(18): p. 12823-34.

147. Spencer, S.J., *Perinatal nutrition programs neuroimmune function long-term: mechanisms and implications.* Front Neurosci, 2013. **7**: p. 144.

148. Haller, D., *Nutrigenomics and IBD: the intestinal microbiota at the cross-road between inflammation and metabolism.* J Clin Gastroenterol, 2010. **44 Suppl 1**: p. S6-9.

149. Ferguson, L.R., *Nutrigenetics, nutrigenomics and inflammatory bowel diseases.* Expert Rev Clin Immunol, 2013. **9**(8): p. 717-26.

150. Shenderov, B.A., *Gut indigenous microbiota and epigenetics.* Microb Ecol Health Dis, 2012. **23**.

151. Sales, N.M., P.B. Pelegrini, and M.C. Goersch, *Nutrigenomics: definitions and advances of this new science.* J Nutr Metab, 2014. **2014**: p. 202759.

152. Nimmo, E.R., et al., *Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway.* Inflamm Bowel Dis, 2012. **18**(5): p. 889-99.

153. Lin, Z., et al., *Identification of disease-associated DNA methylation in B cells from Crohn's disease and ulcerative colitis patients.* Dig Dis Sci, 2012. **57**(12): p. 3145-53.

154. Hasler, R., et al., *A functional methylome map of ulcerative colitis.* Genome Res, 2012. **22**(11): p. 2130-7.

155. Cooke, J., et al., *Mucosal genome-wide methylation changes in inflammatory bowel disease.* Inflamm Bowel Dis, 2012. **18**(11): p. 2128-37.

156. Glauben, R. and B. Siegmund, *Inhibition of histone deacetylases in inflammatory bowel diseases.* Mol Med, 2011. **17**(5-6): p. 426-33.

157. Takahashi, K., et al., *Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis.* J Immunol, 2009. **183**(10): p. 6522-9.

158. Truelove, S.C., *Ulcerative colitis provoked by milk.* Br Med J, 1961. **1**(5220): p. 154-60.

159. Dawson, A.M., *Nutritional disturbances in Crohn's disease.* Proc R Soc Med, 1971. **64**(2): p. 166-70.

160. Beeken, W.L., *Remediable defects in Crohn disease: a prospective study of 63 patients.* Arch Intern Med, 1975. **135**(5): p. 686-90.

161. Rigaud, D., et al., *Mechanisms of decreased food intake during weight loss in adult Crohn's disease patients without obvious malabsorption.* Am J Clin Nutr, 1994. **60**(5): p. 775-81.

162. Harries, A.D., et al., *Malnutrition in inflammatory bowel disease: an anthropometric study.* Hum Nutr Clin Nutr, 1982. **36**(4): p. 307-13.

163. Harries, A.D., et al., *Mid-arm circumference as simple means of identifying malnutrition in Crohn's disease.* Br Med J (Clin Res Ed), 1982. **285**(6351): p. 1317-8.

164. Lanfranchi, G.A., et al., *Assessment of nutritional status in Crohn's disease in remission or low activity.* Hepatogastroenterology, 1984. **31**(3): p. 129-32.

165. Rosenberg, I.H., J.M. Bengoa, and M.D. Sitrin, *Nutritional aspects of inflammatory bowel disease.* Annu Rev Nutr, 1985. **5**: p. 463-84.

166. Pons, R., et al., *Dietary intakes of children with Crohn's disease.* Br J Nutr, 2009. **102**(7): p. 1052-7.

167. Aghdassi, E., et al., *Adequacy of nutritional intake in a Canadian population of patients with Crohn's disease.* J Am Diet Assoc, 2007. **107**(9): p. 1575-80.

168. Gerasimidis, K., P. McGrogan, and C.A. Edwards, *The aetiology and impact of malnutrition in paediatric inflammatory bowel disease.* J Hum Nutr Diet, 2011. **24**(4): p. 313-26.

169. Nachum Vaisman, M.D.a., b,*, Iris Dotan, M.D.a,b, Aharon Halack, M.D.c, and and M.D. Eva Niv, *Malabsorption is a major contributor to underweight in Crohn's disease patients in remission.* Nutrition, 2006. **22**: p. 855 - 859

170. Shamir, R., M. Phillip, and A. Levine, *Growth retardation in pediatric Crohn's disease: pathogenesis and interventions.* Inflamm Bowel Dis, 2007. **13**(5): p. 620-8.

171. Nightingale, J.M., et al., *Colonic preservation reduces need for parenteral therapy, increases incidence of renal stones, but does not change high prevalence of gall stones in patients with a short bowel.* Gut, 1992. **33**(11): p. 1493-7.

172. Nightingale, J.M., et al., *Disturbed gastric emptying in the short bowel syndrome. Evidence for a 'colonic brake'.* Gut, 1993. **34**(9): p. 1171-6.

173. Nightingale, J.M., *The short-bowel syndrome.* Eur J Gastroenterol Hepatol, 1995. **7**(6): p. 514-20.

174. Richards, D.M. and M.H. Irving, *Assessing the quality of life of patients with intestinal failure on home parenteral nutrition.* Gut, 1997. **40**(2): p. 218-22.

175. Hoivik, M.L., et al., *Anaemia in inflammatory bowel disease: a population-based 10-year follow-up.* Aliment Pharmacol Ther, 2013.

176. Goodhand, J.R., et al., *Prevalence and management of anemia in children, adolescents, and adults with inflammatory bowel disease.* Inflamm Bowel Dis, 2012. **18**(3): p. 513-9.

177. Gasche, C., et al., *Iron, anaemia, and inflammatory bowel diseases.* Gut, 2004. **53**(8): p. 1190-7.

178. Kulnigg, S. and C. Gasche, *Systematic review: managing anaemia in Crohn's disease.* Aliment Pharmacol Ther, 2006. **24**(11-12): p. 1507-23.

179. Hodges, P., et al., *Vitamin and iron intake in patients with Crohn's disease.* J Am Diet Assoc, 1984. **84**(1): p. 52-8.

180. Lomer, M.C., et al., *Intake of dietary iron is low in patients with Crohn's disease: a case-control study.* Br J Nutr, 2004. **91**(1): p. 141-8.

181. Ripoli, J., et al., *Nutritional follow-up of patients with ulcerative colitis during periods of intestinal inflammatory activity and remission.* Arq Gastroenterol, 2010. **47**(1): p. 49-55.

182. Semrin, G., et al., *Impaired intestinal iron absorption in Crohn's disease correlates with disease activity and markers of inflammation.* Inflamm Bowel Dis, 2006. **12**(12): p. 1101-6.

183. Park, C.H., et al., *Hepcidin, a urinary antimicrobial peptide synthesized in the liver.* J Biol Chem, 2001. **276**(11): p. 7806-10.

184. Bridle, K.R., et al., *Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis.* Lancet, 2003. **361**(9358): p. 669-73.

185. Nemeth, E., et al., *IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin.* J Clin Invest, 2004. **113**(9): p. 1271-6.

186. Lee, P., et al., *The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes.* Proc Natl Acad Sci U S A, 2004. **101**(25): p. 9263-5.

187. Yamaji, S., et al., *Inhibition of iron transport across human intestinal epithelial cells by hepcidin.* Blood, 2004. **104**(7): p. 2178-80.

188. Oustamanolakis, P., et al., *Serum hepcidin and prohepcidin concentrations in inflammatory bowel disease.* Eur J Gastroenterol Hepatol, 2011. **23**(3): p. 262-8.

189. Wang, J. and K. Pantopoulos, *Regulation of cellular iron metabolism.* Biochem J, 2011. **434**(3): p. 365-81.

190. Harries, A.D. and R.V. Heatley, *Nutritional disturbances in Crohn's disease.* Postgrad Med J, 1983. **59**(697): p. 690-7.

191. Main, A.N., et al., *Mg deficiency in chronic inflammatory bowel disease and requirements during intravenous nutrition.* JPEN J Parenter Enteral Nutr, 1981. **5**(1): p. 15-9.

192. Nishi, Y., et al., *Zinc status and its relation to growth retardation in children with chronic inflammatory bowel disease.* Am J Clin Nutr, 1980. **33**(12): p. 2613-21.

193. Bousvaros, A., et al., *Vitamins A and E serum levels in children and young adults with inflammatory bowel disease: effect of disease activity.* J Pediatr Gastroenterol Nutr, 1998. **26**(2): p. 129-35.

194. Main, A.N., et al., *Vitamin A deficiency in Crohn's disease.* Gut, 1983. **24**(12): p. 1169-75.

195. Janczewska, I., et al., *Metabolism of vitamin A in inflammatory bowel disease.* Hepatogastroenterology, 1991. **38**(5): p. 391-5.

196. Wright, J.P., et al., *Vitamin A therapy in patients with Crohn's disease.* Gastroenterology, 1985. **88**(2): p. 512-4.

197. Bai, A., et al., *All-trans retinoic acid ameliorates trinitrobenzene sulfonic acid-induced colitis by shifting Th1 to Th2 profile.* J Interferon Cytokine Res, 2010. **30**(6): p. 399-406.

198. Hengstermann, S., et al., *Altered status of antioxidant vitamins and fatty acids in patients with inactive inflammatory bowel disease.* Clin Nutr, 2008. **27**(4): p. 571-8.

199. Buffinton, G.D. and W.F. Doe, *Altered ascorbic acid status in the mucosa from inflammatory bowel disease patients.* Free Radic Res, 1995. **22**(2): p. 131-43.

200. Tsujikawa, T., et al., *Supplement of a chitosan and ascorbic acid mixture for Crohn's disease: a pilot study.* Nutrition, 2003. **19**(2): p. 137-9.

201. Garg, M., et al., *Review article: vitamin D and inflammatory bowel disease--established concepts and future directions.* Aliment Pharmacol Ther, 2012. **36**(4): p. 324-44.

202. Dimeloe, S., et al., *Regulatory T cells, inflammation and the allergic response-The role of glucocorticoids and Vitamin D.* J Steroid Biochem Mol Biol, 2010. **120**(2-3): p. 86-95.

203. Compston, J.E., et al., *Osteomalacia after small-intestinal resection.* Lancet, 1978. **1**(8054): p. 9-12.

204. Driscoll, R.H., Jr., et al., *Vitamin D deficiency and bone disease in patients with Crohn's disease.* Gastroenterology, 1982. **83**(6): p. 1252-8.

205. Ulitsky, A., et al., *Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life.* JPEN J Parenter Enteral Nutr, 2011. **35**(3): p. 308-16.

206. Lemire, J.M., et al., *Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions.* J Nutr, 1995. **125**(6 Suppl): p. 1704S-1708S.

207. Tokuda, N., et al., *1,25-Dihydroxyvitamin D3 down-regulation of HLA-DR on human peripheral blood monocytes.* Immunology, 1992. **75**(2): p. 349-54.

208. Penna, G. and L. Adorini, *1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation.* J Immunol, 2000. **164**(5): p. 2405-11.

209. Sadeghi, K., et al., *Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns.* Eur J Immunol, 2006. **36**(2): p. 361-70.

210. Cohen-Lahav, M., et al., *Vitamin D decreases NFkappaB activity by increasing IkappaBalpha levels.* Nephrol Dial Transplant, 2006. **21**(4): p. 889-97.

211. Wu, S., et al., *Vitamin D receptor deletion leads to reduced level of IkappaBalpha protein through protein translation, protein-protein interaction, and post-translational modification.* Int J Biochem Cell Biol, 2010. **42**(2): p. 329-36.

212. Simmons, J.D., et al., *Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility.* Gut, 2000. **47**(2): p. 211-4.

213. Penna, G., et al., *Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3.* Blood, 2005. **106**(10): p. 3490-7.

214. Morales-Tirado, V., et al., *1alpha,25-dihydroxyvitamin D3 (vitamin D3) catalyzes suppressive activity on human natural regulatory T cells, uniquely modulates cell cycle progression, and augments FOXP3.* Clin Immunol, 2011. **138**(2): p. 212-21.

215. Kang, S.W., et al., *1,25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region.* J Immunol, 2012. **188**(11): p. 5276-82.

216. Khoo, A.L., et al., *Seasonal variation in vitamin D(3) levels is paralleled by changes in the peripheral blood human T cell compartment.* PLoS One, 2012. **7**(1): p. e29250.

217. Mora, J.R., M. Iwata, and U.H. von Andrian, *Vitamin effects on the immune system: vitamins A and D take centre stage.* Nat Rev Immunol, 2008. **8**(9): p. 685-98.

218. Palmer, M.T., et al., *Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells.* J Biol Chem, 2011. **286**(2): p. 997-1004.

219. Na, S., et al., *A Nonsecosteroidal Vitamin D Receptor Modulator Ameliorates Experimental Autoimmune Encephalomyelitis without Causing Hypercalcemia.* Autoimmune Dis, 2011. **2011**: p. 132958.

220. Colin, E.M., et al., *1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis.* Arthritis Rheum, 2010. **62**(1): p. 132-42.

221. van Hamburg, J.P., et al., *TNF blockade requires 1,25(OH)2D3 to control human Th17-mediated synovial inflammation.* Ann Rheum Dis, 2012. **71**(4): p. 606-12.

222. Zold, E., et al., *Impaired regulatory T-cell homeostasis due to vitamin D deficiency in undifferentiated connective tissue disease.* Scand J Rheumatol, 2010. **39**(6): p. 490-7.

223. Terrier, B., et al., *Restoration of regulatory and effector T cell balance and B cell homeostasis in systemic lupus erythematosus patients through vitamin D supplementation.* Arthritis Res Ther, 2012. **14**(5): p. R221.

224. Verlinden, L., et al., *The vitamin D analog TX527 ameliorates disease symptoms in a chemically induced model of inflammatory bowel disease.* J Steroid Biochem Mol Biol, 2012.

225. Jussila, A., et al., *High and increasing prevalence of inflammatory bowel disease in Finland with a clear North-South difference.* J Crohns Colitis, 2012.

226. Carr, I. and J.F. Mayberry, *The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second- generation South Asians in Leicester (1991-1994).* Am J Gastroenterol, 1999. **94**(10): p. 2918-22.

227. Khalili, H., et al., *Geographical variation and incidence of inflammatory bowel disease among US women.* Gut, 2012. **61**(12): p. 1686-92.

228. Ananthakrishnan, A.N., et al., *Higher predicted vitamin D status is associated with reduced risk of Crohn's disease.* Gastroenterology, 2012. **142**(3): p. 482-9.

229. Naderi, N., et al., *Association of vitamin D receptor gene polymorphisms in Iranian patients with inflammatory bowel disease.* J Gastroenterol Hepatol, 2008. **23**(12): p. 1816-22.

230. Hughes, D.J., et al., *Common variation in the vitamin D receptor gene and risk of inflammatory bowel disease in an Irish case-control study.* Eur J Gastroenterol Hepatol, 2011. **23**(9): p. 807-12.

231. Bentley, R.W., et al., *Vitamin D receptor gene polymorphism associated with inflammatory bowel disease in New Zealand males.* Aliment Pharmacol Ther, 2011. **33**(7): p. 855-6.

232. Dresner-Pollak, R., et al., *The BsmI vitamin D receptor gene polymorphism is associated with ulcerative colitis in Jewish Ashkenazi patients.* Genet Test, 2004. **8**(4): p. 417-20.

233. Eloranta, J.J., et al., *Association of a common vitamin D-binding protein polymorphism with inflammatory bowel disease.* Pharmacogenet Genomics, 2011. **21**(9): p. 559-64.

234. Xue, L.N., et al., *Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn's disease: a meta-analysis.* Inflamm Bowel Dis, 2013. **19**(1): p. 54-60.

235. Froicu, M., et al., *A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases.* Mol Endocrinol, 2003. **17**(12): p. 2386-92.

236. Liu, W., et al., *Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis.* J Clin Invest, 2013. **123**(9): p. 3983-96.

237. Ooi, J.H., et al., *Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis.* J Nutr, 2013. **143**(10): p. 1679-86.

238. Assa, A., et al., *Vitamin D Deficiency Promotes Epithelial Barrier Dysfunction and Intestinal Inflammation.* J Infect Dis, 2014.

239. Zhu, Y., et al., *Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease.* Eur J Immunol, 2005. **35**(1): p. 217-24.

240. Lagishetty, V., et al., *Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis.* Endocrinology, 2010. **151**(6): p. 2423-32.

241. Strauch, U.G., et al., *Calcitriol analog ZK191784 ameliorates acute and chronic dextran sodium sulfate-induced colitis by modulation of intestinal dendritic cell numbers and phenotype.* World J Gastroenterol, 2007. **13**(48): p. 6529-37.

242. Kong, J., et al., *Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier.* Am J Physiol Gastrointest Liver Physiol, 2008. **294**(1): p. G208-16.

243. Jorgensen, S.P., et al., *Clinical trial: vitamin D3 treatment in Crohn's disease - a randomized double-blind placebo-controlled study.* Aliment Pharmacol Ther, 2010. **32**(3): p. 377-83.

244. Bendix-Struve, M., et al., *Vitamin D3 treatment of Crohn's disease patients increases stimulated T cell IL-6 production and proliferation.* Aliment Pharmacol Ther, 2010. **32**(11-12): p. 1364-72.

245. Samson, C.M., et al., *Improved outcomes with quality improvement interventions in pediatric inflammatory bowel disease.* J Pediatr Gastroenterol Nutr, 2012. **55**(6): p. 679-88.

246. Johnson, L.A., et al., *CARD-024, a vitamin D analog, attenuates the pro-fibrotic response to substrate stiffness in colonic myofibroblasts.* Exp Mol Pathol, 2012. **93**(1): p. 91-8.

247. Yang, K., et al., *Dietary induction of colonic tumors in a mouse model of sporadic colon cancer.* Cancer Res, 2008. **68**(19): p. 7803-10.

248. Kallay, E., et al., *Characterization of a vitamin D receptor knockout mouse as a model of colorectal hyperproliferation and DNA damage.* Carcinogenesis, 2001. **22**(9): p. 1429-35.

249. Jenab, M., et al., *Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations:a nested case-control study.* Bmj, 2010. **340**: p. b5500.

250. Fedirko, V., et al., *Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European ppulations.* Cancer Epidemiol Biomarkers Prev, 2012. **21**(4): p. 582-93.

251. Weinstein, S.J., et al., *Serum 25-hydroxyvitamin D and risks of colon and rectal cancer in Finnish men.* Am J Epidemiol, 2011. **173**(5): p. 499-508.

252. Wactawski-Wende, J., et al., *Calcium plus vitamin D supplementation and the risk of colorectal cancer.* N Engl J Med, 2006. **354**(7): p. 684-96.

253. Sandborn, W.J., et al., *Delayed-release oral mesalamine 4.8 g/day (800-mg tablet) is effective for patients with moderately active ulcerative colitis.* Gastroenterology, 2009. **137**(6): p. 1934-43 e1-3.

254. Flourie, B., et al., *Randomised clinical trial: once- vs. twice-daily prolonged-release mesalazine for active ulcerative colitis.* Aliment Pharmacol Ther, 2013. **37**(8): p. 767-75.

255. Romkens, T.E., et al., *High mucosal healing rates in 5-ASA-treated ulcerative colitis patients: results of a meta-analysis of clinical trials.* Inflamm Bowel Dis, 2012. **18**(11): p. 2190-8.

256. Hanauer, S.B., et al., *An Oral Preparation of Mesalamine as Long-Term Maintenance Therapy for Ulcerative ColitisA Randomized, Placebo-Controlled Trial.* Annals of Internal Medicine, 1996. **124**(2): p. 204-211.

257. Miner, P., et al., *Safety and efficacy of controlled-release mesalamine for maintenance of remission in ulcerative colitis. Pentasa UC Maintenance Study Group.* Dig Dis Sci, 1995. **40**(2): p. 296-304.

258. Sutherland, L. and J.K. Macdonald, *Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis.* Cochrane Database Syst Rev, 2006(2): p. CD000544.

259. Rasmussen, S.N., et al., *5-Aminosalicylic acid in the treatment of Crohn's disease. A 16-week double-blind, placebo-controlled, multicentre study with Pentasa.* Scand J Gastroenterol, 1987. **22**(7): p. 877-83.

260. Mahida, Y.R. and D.P. Jewell, *Slow-release 5-amino-salicylic acid (Pentasa) for the treatment of active Crohn's disease.* Digestion, 1990. **45**(2): p. 88-92.

261. Singleton, J.W., et al., *Mesalamine capsules for the treatment of active Crohn's disease: results of a 16-week trial. Pentasa Crohn's Disease Study Group.* Gastroenterology, 1993. **104**(5): p. 1293-301.

262. Hanauer, S.B. and U. Stromberg, *Oral Pentasa in the treatment of active Crohn's disease: A meta-analysis of double-blind, placebo-controlled trials.* Clin Gastroenterol Hepatol, 2004. **2**(5): p. 379-88.

263. Malchow, H., et al., *European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment.* Gastroenterology, 1984. **86**(2): p. 249-66.

264. Akobeng, A.K. and E. Gardener, *Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's Disease.* Cochrane Database Syst Rev, 2005(1): p. CD003715.

265. Truelove, S.C. and L.J. Witts, *Cortisone in ulcerative colitis; preliminary report on a therapeutic trial.* Br Med J, 1954. **2**(4884): p. 375-8.

266. Lennard-Jones, J.E., et al., *An assessment of prednisone, salazopyrin, and topical hydrocortisone hemisuccinate used as out-patient treatment for ulcerative colitis.* Gut, 1960. **1**: p. 217-22.

267. Franchimont, D., et al., *Glucocorticoids and inflammation revisited: the state of the art. NIH clinical staff conference.* Neuroimmunomodulation, 2002. **10**(5): p. 247-60.

268. Franchimont, D., *Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies.* Ann N Y Acad Sci, 2004. **1024**: p. 124-37.

269. Lennard-Jones, J.E., et al., *Prednisone as Maintenance Treatment for Ulcerative Colitis in Remission.* Lancet, 1965. **1**(7378): p. 188-9.

270. Summers, R.W., et al., *National Cooperative Crohn's Disease Study: results of drug treatment.* Gastroenterology, 1979. **77**(4 Pt 2): p. 847-69.

271. Singleton, J.W., et al., *A trial of sulfasalazine as adjunctive therapy in Crohn's disease.* Gastroenterology, 1979. **77**(4 Pt 2): p. 887-97.

272. Salomon, P., et al., *How effective are current drugs for Crohn's disease? A meta-analysis.* J Clin Gastroenterol, 1992. **14**(3): p. 211-5.

273. Peter Irving, D.R., Fergus Shanahan, *Clinical dilemmas in inflammatory bowel disease*. 2006. 28-29.

274. Rutgeerts, P.J., *Review article: the limitations of corticosteroid therapy in Crohn's disease.* Aliment Pharmacol Ther, 2001. **15**(10): p. 1515-25.

275. Greenberg, G.R., et al., *Oral budesonide as maintenance treatment for Crohn's disease: a placebo-controlled, dose-ranging study. Canadian Inflammatory Bowel Disease Study Group.* Gastroenterology, 1996. **110**(1): p. 45-51.

276. Motil, K.J., et al., *Growth failure in children with inflammatory bowel disease: a prospective study.* Gastroenterology, 1993. **105**(3): p. 681-91.

277. Griffiths, A.M., et al., *Growth and clinical course of children with Crohn's disease.* Gut, 1993. **34**(7): p. 939-43.

278. Boot, A.M., et al., *Bone mineral density and nutritional status in children with chronic inflammatory bowel disease.* Gut, 1998. **42**(2): p. 188-94.

279. Wales, J.K. and R.D. Milner, *Variation in lower leg growth with alternate day steroid treatment.* Arch Dis Child, 1988. **63**(8): p. 981-3.

280. Stephens, R.V. and H.T. Randall, *Use of concentrated, balanced, liquid elemental diet for nutritional management of catabolic states.* Ann Surg, 1969. **170**(4): p. 642-68.

281. Voitk, A.J., et al., *Experience with elemental diet in the treatment of inflammatory bowel disease. Is this primary therapy?* Arch Surg, 1973. **107**(2): p. 329-33.

282. Morin, C.L., et al., *Continuous elemental enteral alimentation in children with Crohn's disease and growth failure.* Gastroenterology, 1980. **79**(6): p. 1205-10.

283. Logan, R.F., et al., *Reduction of gastrointestinal protein loss by elemental diet in Crohn's disease of the small bowel.* Gut, 1981. **22**(5): p. 383-7.

284. Navarro, J., et al., *Prolonged constant rate elemental enteral nutrition in Crohn's disease.* J Pediatr Gastroenterol Nutr, 1982. **1**(4): p. 541-6.

285. Morin, C.L., et al., *Continuous elemental enteral alimentation in the treatment of children and adolescents with Crohn's disease.* JPEN J Parenter Enteral Nutr, 1982. **6**(3): p. 194-9.

286. Sanderson, I.R., et al., *Remission induced by an elemental diet in small bowel Crohn's disease.* Arch Dis Child, 1987. **62**(2): p. 123-7.

287. Ruuska, T., et al., *Exclusive whole protein enteral diet versus prednisolone in the treatment of acute Crohn's disease in children.* J Pediatr Gastroenterol Nutr, 1994. **19**(2): p. 175-80.

288. Zoli, G., et al., *A randomized controlled study comparing elemental diet and steroid treatment in Crohn's disease.* Aliment Pharmacol Ther, 1997. **11**(4): p. 735-40.

289. Soo, J., et al., *Use of exclusive enteral nutrition is just as effective as corticosteroids in newly diagnosed pediatric Crohn's disease.* Dig Dis Sci, 2013. **58**(12): p. 3584-91.

290. Afzal, N.A., et al., *Colonic Crohn's disease in children does not respond well to treatment with enteral nutrition if the ileum is not involved.* Dig Dis Sci, 2005. **50**(8): p. 1471-5.

291. Buchanan, E., et al., *The use of exclusive enteral nutrition for induction of remission in children with Crohn's disease demonstrates that disease phenotype does not influence clinical remission.* Aliment Pharmacol Ther, 2009. **30**(5): p. 501-7.

292. Giaffer, M.H., G. North, and C.D. Holdsworth, *Controlled trial of polymeric versus elemental diet in treatment of active Crohn's disease.* Lancet, 1990. **335**(8693): p. 816-9.

293. Gonzalez-Huix, F., et al., *Polymeric enteral diets as primary treatment of active Crohn's disease: a prospective steroid controlled trial.* Gut, 1993. **34**(6): p. 778-82.

294. Royall, D., et al., *Comparison of amino acid v peptide based enteral diets in active Crohn's disease: clinical and nutritional outcome.* Gut, 1994. **35**(6): p. 783-7.

295. Raouf, A.H., et al., *Enteral feeding as sole treatment for Crohn's disease: controlled trial of whole protein v amino acid based feed and a case study of dietary challenge.* Gut, 1991. **32**(6): p. 702-7.

296. Ludvigsson, J.F., et al., *Elemental versus polymeric enteral nutrition in paediatric Crohn's disease: a multicentre randomized controlled trial.* Acta Paediatr, 2004. **93**(3): p. 327-35.

297. Grogan, J.L., et al., *Enteral feeding therapy for newly diagnosed pediatric Crohn's disease: a double-blind randomized controlled trial with two years follow-up.* Inflamm Bowel Dis, 2012. **18**(2): p. 246-53.

298. Rigaud, D., et al., *Controlled trial comparing two types of enteral nutrition in treatment of active Crohn's disease: elemental versus polymeric diet.* Gut, 1991. **32**(12): p. 1492-7.

299. Zachos, M., M. Tondeur, and A.M. Griffiths, *Enteral nutritional therapy for induction of remission in Crohn's disease.* Cochrane Database Syst Rev, 2007(1): p. CD000542.

300. O'Morain, C., A.W. Segal, and A.J. Levi, *Elemental diet as primary treatment of acute Crohn's disease: a controlled trial.* Br Med J (Clin Res Ed), 1984. **288**(6434): p. 1859-62.

301. Gorard, D.A., et al., *Initial response and subsequent course of Crohn's disease treated with elemental diet or prednisolone.* Gut, 1993. **34**(9): p. 1198-202.

302. Riordan, A.M., et al., *Treatment of active Crohn's disease by exclusion diet: East Anglian multicentre controlled trial.* Lancet, 1993. **342**(8880): p. 1131-4.

303. Griffiths, A.M., et al., *Meta-analysis of enteral nutrition as a primary treatment of active Crohn's disease.* Gastroenterology, 1995. **108**(4): p. 1056-67.

304. Schwab, D., M. Raithel, and E.G. Hahn, *[Enteral nutrition in acute Crohn disease].* Z Gastroenterol, 1998. **36**(11): p. 983-95.

305. Heuschkel, R.B., et al., *Enteral nutrition and corticosteroids in the treatment of acute Crohn's disease in children.* J Pediatr Gastroenterol Nutr, 2000. **31**(1): p. 8-15.

306. Dziechciarz, P., et al., *Meta-analysis: enteral nutrition in active Crohn's disease in children.* Aliment Pharmacol Ther, 2007. **26**(6): p. 795-806.

307. Wilschanski, M., et al., *Supplementary enteral nutrition maintains remission in paediatric Crohn's disease.* Gut, 1996. **38**(4): p. 543-8.

308. Verma, S., C.D. Holdsworth, and M.H. Giaffer, *Does adjuvant nutritional support diminish steroid dependency in Crohn disease?* Scand J Gastroenterol, 2001. **36**(4): p. 383-8.

309. Takagi, S., et al., *Effectiveness of an 'half elemental diet' as maintenance therapy for Crohn's disease: A randomized-controlled trial.* Aliment Pharmacol Ther, 2006. **24**(9): p. 1333-40.

310. Akobeng, A.K. and A.G. Thomas, *Enteral nutrition for maintenance of remission in Crohn's disease.* Cochrane Database Syst Rev, 2007(3): p. CD005984.

311. Watanabe, O., et al., *Enteral nutrition decreases hospitalization rate in patients with Crohn's disease.* J Gastroenterol Hepatol, 2010. **25 Suppl 1**: p. S134-7.

312. Esaki, M., et al., *Factors affecting recurrence in patients with Crohn's disease under nutritional therapy.* Dis Colon Rectum, 2006. **49**(10 Suppl): p. S68-74.

313. Yamamoto, T., et al., *Prospective clinical trial: enteral nutrition during maintenance infliximab in Crohn's disease.* J Gastroenterol, 2010. **45**(1): p. 24-9.

314. Hirai, F., et al., *Effectiveness of concomitant enteral nutrition therapy and infliximab for maintenance treatment of Crohn's disease in adults.* Dig Dis Sci, 2013. **58**(5): p. 1329-34.

315. Sazuka, S., et al., *Concomitant use of enteral nutrition therapy is associated with sustained response to infliximab in patients with Crohn's disease.* Eur J Clin Nutr, 2012. **66**(11): p. 1219-23.

316. Yamamoto, T., et al., *Impact of long-term enteral nutrition on clinical and endoscopic recurrence after resection for Crohn's disease: A prospective, non-randomized, parallel, controlled study.* Aliment Pharmacol Ther, 2007. **25**(1): p. 67-72.

317. Yamamoto, T., et al., *Enteral nutrition to suppress postoperative Crohn's disease recurrence: a five-year prospective cohort study.* Int J Colorectal Dis, 2013. **28**(3): p. 335-40.

318. Esaki, M., et al., *Preventive effect of nutritional therapy against postoperative recurrence of Crohn disease, with reference to findings determined by intra-operative enteroscopy.* Scand J Gastroenterol, 2005. **40**(12): p. 1431-7.

319. Day, A.S., et al., *Systematic review: nutritional therapy in paediatric Crohn's disease.* Aliment Pharmacol Ther, 2008. **27**(4): p. 293-307.

320. Fell, J.M., et al., *Mucosal healing and a fall in mucosal pro-inflammatory cytokine mRNA induced by a specific oral polymeric diet in paediatric Crohn's disease.* Aliment Pharmacol Ther, 2000. **14**(3): p. 281-9.

321. Berni Canani, R., et al., *Short- and long-term therapeutic efficacy of nutritional therapy and corticosteroids in paediatric Crohn's disease.* Dig Liver Dis, 2006. **38**(6): p. 381-7.

322. Borrelli, O., et al., *Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: a randomized controlled open-label trial.* Clin Gastroenterol Hepatol, 2006. **4**(6): p. 744-53.

323. Grover, Z., R. Muir, and P. Lewindon, *Exclusive enteral nutrition induces early clinical, mucosal and transmural remission in paediatric Crohn's disease.* J Gastroenterol, 2013.

324. Munkholm Larsen, P., et al., *Elemental diet: a therapeutic approach in chronic inflammatory bowel disease.* J Intern Med, 1989. **225**(5): p. 325-31.

325. Knight, C., et al., *Long-term outcome of nutritional therapy in paediatric Crohn's disease.* Clin Nutr, 2005. **24**(5): p. 775-9.

326. Cameron, F.L., et al., *Clinical progress in the two years following a course of exclusive enteral nutrition in 109 paediatric patients with Crohn's disease.* Aliment Pharmacol Ther, 2013. **37**(6): p. 622-9.

327. Frivolt, K., et al., *Repeated exclusive enteral nutrition in the treatment of paediatric Crohn's disease: predictors of efficacy and outcome.* Aliment Pharmacol Ther, 2014. **39**(12): p. 1398-407.

328. Leach, S.T., et al., *Sustained modulation of intestinal bacteria by exclusive enteral nutrition used to treat children with Crohn's disease.* Aliment Pharmacol Ther, 2008. **28**(6): p. 724-33.

329. Lionetti, P., et al., *Enteral nutrition and microflora in pediatric Crohn's disease.* JPEN J Parenter Enteral Nutr, 2005. **29**(4 Suppl): p. S173-5; discussion S175-8, S184-8.

330. Schneider, S.M., et al., *Effects of total enteral nutrition supplemented with a multi-fibre mix on faecal short-chain fatty acids and microbiota.* Clin Nutr, 2006. **25**(1): p. 82-90.

331. Whelan, K., et al., *Enteral feeding: the effect on faecal output, the faecal microflora and SCFA concentrations.* Proc Nutr Soc, 2004. **63**(1): p. 105-13.

332. Tjellstrom, B., et al., *Effect of exclusive enteral nutrition on gut microflora function in children with Crohn's disease.* Scand J Gastroenterol, 2012. **47**(12): p. 1454-9.

333. Sanderson, I.R., *Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium.* J Nutr, 2004. **134**(9): p. 2450S-2454S.

334. Nahidi, L., et al., *Differential effects of nutritional and non-nutritional therapies on intestinal barrier function in an in vitro model.* J Gastroenterol, 2012. **47**(2): p. 107-17.

335. Meister, D., et al., *Anti-inflammatory effects of enteral diet components on Crohn's disease-affected tissues in vitro.* Dig Liver Dis, 2002. **34**(6): p. 430-8.

336. de Jong, N.S., S.T. Leach, and A.S. Day, *Polymeric formula has direct anti-inflammatory effects on enterocytes in an in vitro model of intestinal inflammation.* Dig Dis Sci, 2007. **52**(9): p. 2029-36.

337. Breese, E.J., et al., *The effect of treatment on lymphokine-secreting cells in the intestinal mucosa of children with Crohn's disease.* Aliment Pharmacol Ther, 1995. **9**(5): p. 547-52.

338. Ardizzone, S., et al., *Randomised controlled trial of azathioprine and 5-aminosalicylic acid for treatment of steroid dependent ulcerative colitis.* Gut, 2006. **55**(1): p. 47-53.

339. Gisbert, J.P., et al., *Comparative effectiveness of azathioprine in Crohn's disease and ulcerative colitis: prospective, long-term, follow-up study of 394 patients.* Aliment Pharmacol Ther, 2008. **28**(2): p. 228-38.

340. Prefontaine, E., et al., *Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease.* Cochrane Database Syst Rev, 2009(1): p. CD000067.

341. Kamperidis, N., et al., *Factors associated with nonadherence to thiopurines in adolescent and adult patients with inflammatory bowel disease.* J Pediatr Gastroenterol Nutr, 2012. **54**(5): p. 685-9.

342. Bohon, J. and C.R. de los Santos, *Structural effect of the anticancer agent 6-thioguanine on duplex DNA.* Nucleic Acids Res, 2003. **31**(4): p. 1331-8.

343. Thomas, C.W., et al., *Selective inhibition of inflammatory gene expression in activated T lymphocytes: a mechanism of immune suppression by thiopurines.* J Pharmacol Exp Ther, 2005. **312**(2): p. 537-45.

344. Tiede, I., et al., *CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes.* J Clin Invest, 2003. **111**(8): p. 1133-45.

345. Elliott, M.J., et al., *Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis.* Lancet, 1994. **344**(8930): p. 1105-10.

346. van Dullemen, H.M., et al., *Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2).* Gastroenterology, 1995. **109**(1): p. 129-35.

347. Ricart, E., et al., *Infliximab for Crohn's disease in clinical practice at the Mayo Clinic: the first 100 patients.* Am J Gastroenterol, 2001. **96**(3): p. 722-9.

348. Targan, S.R., 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**(15): p. 1029-35.

349. Hanauer, S.B., et al., *Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial.* Lancet, 2002. **359**(9317): p. 1541-9.

350. Sands, B.E., et al., *Infliximab maintenance therapy for fistulizing Crohn's disease.* N Engl J Med, 2004. **350**(9): p. 876-85.

351. Hanauer, S.B., et al., *Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial.* Gastroenterology, 2006. **130**(2): p. 323-33; quiz 591.

352. Sandborn, W.J., et al., *Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial.* Ann Intern Med, 2007. **146**(12): p. 829-38.

353. Lofberg, R., et al., *Adalimumab produces clinical remission and reduces extraintestinal manifestations in Crohn's disease: results from CARE.* Inflamm Bowel Dis, 2012. **18**(1): p. 1-9.

354. Russell, R.K., et al., *A British Society of Paediatric Gastroenterology, Hepatology and Nutrition survey of the effectiveness and safety of adalimumab in children with inflammatory bowel disease.* Aliment Pharmacol Ther, 2011. **33**(8): p. 946-53.

355. Sandborn, W.J., et al., *Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial.* Gut, 2007. **56**(9): p. 1232-9.

356. Colombel, J.F., et al., *Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial.* Gastroenterology, 2007. **132**(1): p. 52-65.

357. Rutgeerts, P., et al., *Infliximab for induction and maintenance therapy for ulcerative colitis.* N Engl J Med, 2005. **353**(23): p. 2462-76.

358. Sandborn, W.J., et al., *Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis.* Gastroenterology, 2012. **142**(2): p. 257-65 e1-3.

359. Sandborn, W.J., et al., *Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial.* Gastroenterology, 2001. **121**(5): p. 1088-94.

360. ten Hove, T., et al., *Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease.* Gut, 2002. **50**(2): p. 206-11.

361. Van den Brande, J.M., et al., *Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease.* Gastroenterology, 2003. **124**(7): p. 1774-85.

362. Liu, C., et al., *Anti-TNF therapy enhances mucosal healing through downregulation of IL-21 expression and Th17 cell infiltration in Crohn's disease.* Clin Exp Immunol, 2013.

363. Di Sabatino, A., et al., *Peripheral regulatory T cells and serum transforming growth factor-beta: relationship with clinical response to infliximab in Crohn's disease.* Inflamm Bowel Dis, 2010. **16**(11): p. 1891-7.

364. Biancheri, P., et al., *Effect of tumor necrosis factor-alpha blockade on mucosal addressin cell-adhesion molecule-1 in Crohn's disease.* Inflamm Bowel Dis, 2013. **19**(2): p. 259-64.

365. Arijs, I., et al., *Mucosal gene expression of cell adhesion molecules, chemokines, and chemokine receptors in patients with inflammatory bowel disease before and after infliximab treatment.* Am J Gastroenterol, 2011. **106**(4): p. 748-61.

366. Ibd Working Group of the European Society for Paediatric Gastroenterology, H. and Nutrition, *Inflammatory bowel disease in children and adolescents: recommendations for diagnosis--the Porto criteria.* J Pediatr Gastroenterol Nutr, 2005. **41**(1): p. 1-7.

367. B K Sandhu, J.M.E.F., R M Beattie, S G Mitton, *Guidelines for the management of Inflammatory Bowel Disease (IBD) in children in the United Kingdom.* 2008.

368. Hyams, J.S., et al., *Development and validation of a pediatric Crohn's disease activity index.* J Pediatr Gastroenterol Nutr, 1991. **12**(4): p. 439-47.

369. Silverberg, M.S., et al., *Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology.* Can J Gastroenterol, 2005. **19 Suppl A**: p. 5-36.

370. Bischoff-Ferrari, H.A., et al., *Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes.* Am J Clin Nutr, 2006. **84**(1): p. 18-28.

371. Pearce, S.H. and T.D. Cheetham, *Diagnosis and management of vitamin D deficiency.* BMJ, 2010. **340**: p. b5664.

372. council, G.c., *Population and Households by Ethnicity in Glasgow - Estimates of Changes 2001-2010 for Community Planning Partnership areas and Neighbourhoods.* 2012.

373. Ng, S.C., et al., *Relationship between human intestinal dendritic cells, gut microbiota, and disease activity in Crohn's disease.* Inflamm Bowel Dis, 2011. **17**(10): p. 2027-37.

374. Lee, Y.S., et al., *Efficacy of Early Infliximab Treatment for Pediatric Crohn's Disease: A Three-year Follow-up.* Pediatr Gastroenterol Hepatol Nutr, 2012. **15**(4): p. 243-9.

375. Punati, J., et al., *Effect of early immunomodulator use in moderate to severe pediatric Crohn disease.* Inflamm Bowel Dis, 2008. **14**(7): p. 949-54.

376. Aomatsu, T., et al., *Fecal calprotectin is a useful marker for disease activity in pediatric patients with inflammatory bowel disease.* Dig Dis Sci, 2011. **56**(8): p. 2372-7.

377. Jorgensen, S.P., et al., *Active Crohn's disease is associated with low vitamin D levels.* J Crohns Colitis, 2013.

378. Ananthakrishnan, A.N., et al., *Normalization of Plasma 25-Hydroxy Vitamin D Is Associated with Reduced Risk of Surgery in Crohn's Disease.* Inflamm Bowel Dis, 2013. **19**(9): p. 1921-1927.

379. El-Matary, W., S. Sikora, and D. Spady, *Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease.* Dig Dis Sci, 2011. **56**(3): p. 825-9.

380. Guihot, G., et al., *Enteral nutrition modifies gut-associated lymphoid tissue in rat regardless of the molecular form of nitrogen supply.* J Pediatr Gastroenterol Nutr, 1997. **24**(2): p. 153-61.

381. Sanderson, I.R., et al., *Age and diet act through distinct isoforms of the class II transactivator gene in mouse intestinal epithelium.* Gastroenterology, 2004. **127**(1): p. 203-12.

382. Di Sabatino, A., et al., *Intraepithelial and lamina propria lymphocytes show distinct patterns of apoptosis whereas both populations are active in Fas based cytotoxicity in coeliac disease.* Gut, 2001. **49**(3): p. 380-6.

383. Ng, S.C., et al., *Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis.* Inflamm Bowel Dis, 2010. **16**(8): p. 1286-98.

384. Van Assche, G., et al., *The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis.* J Crohns Colitis, 2010. **4**(1): p. 7-27.

385. NRES, *Differentiating audit, service evaluation and research.* http://www.nres.npsa.nhs.uk/applications/guidance/research-guidance/?entryid62=66988, 2007.

386. group, D.m.a.a., *GLA 2008 Round Ethnic Group Population Projections*, 2009, Greater London Authority.

387. Walker, D.G., et al., *Differences in Inflammatory Bowel Disease Phenotype between South Asians and Northern Europeans Living in North West London, UK.* Am J Gastroenterol, 2011. **106**(7): p. 1281-9.

388. Fu, Y.T., et al., *Hypovitaminosis D in adults with inflammatory bowel disease: potential role of ethnicity.* Dig Dis Sci, 2012. **57**(8): p. 2144-8.

389. *ACORN. www.caci.co.uk/acorn-classification.aspx Last accessed Feb, 2011.*

390. WHO, UNICEF, and UNU, *Iron deficiency anaemia: Assessment, prevention and control. Report of a joint WHO/UNICEF/UNU consultation.*, 1998, World Health Organization: Geneva.

391. Gasche, C., et al., *Guidelines on the diagnosis and management of iron deficiency and anemia in inflammatory bowel diseases.* Inflamm Bowel Dis, 2007. **13**(12): p. 1545-53.

392. Siebert, U., et al., *Predictors of temporary and permanent work disability in patients with inflammatory bowel disease: results of the swiss inflammatory bowel disease cohort study.* Inflamm Bowel Dis, 2013. **19**(4): p. 847-55.

393. Probert, C.S., et al., *Epidemiological study of ulcerative proctocolitis in Indian migrants and the indigenous population of Leicestershire.* Gut, 1992. **33**(5): p. 687-93.

394. Karlsen, S., D. Millward, and A. Sandford, *Investigating ethnic differences in current cigarette smoking over time using the health surveys for England.* Eur J Public Health, 2012. **22**(2): p. 254-6.

395. Cosnes, J., et al., *Long-term evolution of disease behavior of Crohn's disease.* Inflamm Bowel Dis, 2002. **8**(4): p. 244-50.

396. Rashid, S.T., et al., *Inflammatory bowel disease in the South Asian population of Northwest England.* Am J Gastroenterol, 2008. **103**(1): p. 242-3; author reply 243-4.

397. Waller, J., et al., *Awareness of cancer symptoms and anticipated help seeking among ethnic minority groups in England.* Br J Cancer, 2009. **101 Suppl 2**: p. S24-30.

398. Cross, R.K., et al., *Racial differences in disease phenotypes in patients with Crohn's disease.* Inflamm Bowel Dis, 2006. **12**(3): p. 192-8.

399. Boyd, C.A. and J.K. Limdi, *Vitamin D deficiency and disease outcomes in South Asian patients with IBD.* Dig Dis Sci, 2013. **58**(7): p. 2124-5.

400. Kift, R., et al., *Lifestyle factors including less cutaneous sun exposure contribute to starkly lower vitamin D status in UK South Asians compared to the white Caucasian population.* Br J Dermatol, 2013.

401. Blanck, S. and F. Aberra, *Vitamin d deficiency is associated with ulcerative colitis disease activity.* Dig Dis Sci, 2013. **58**(6): p. 1698-702.

402. Kasahara, A.K., R.J. Singh, and A. Noymer, *Vitamin D (25OHD) Serum Seasonality in the United States.* PLoS One, 2013. **8**(6): p. e65785.

403. Kotsopoulos, J., et al., *Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies.* Cancer Epidemiol Biomarkers Prev, 2010. **19**(4): p. 938-46.

404. Hart, A.L., *Vitamin D and inflammatory bowel disease: chicken or egg?* Inflamm Bowel Dis, 2013. **19**(2): p. 459-60.

405. Pariente, B., et al., *Development of the Lemann index to assess digestive tract damage in patients with Crohn's disease.* Gastroenterology, 2015. **148**(1): p. 52-63 e3.

406. Fiorino, G., et al., *Bowel Damage as Assessed by the Lemann Index is Reversible on Anti-TNF Therapy for Crohn's Disease.* J Crohns Colitis, 2015. **9**(8): p. 633-9.

APPENDIX I

NHS
National Research Ethics Service
East London Research Ethics Committee 1
Room 24, 2nd Floor Burdett House, Mile End Hospital, London E1 4DG
Telephone Number: 020 8 223 8602
Email Address: Sandra.Burke@thpct.nhs.uk

Dr Nick Croft
Honorary Consultant in Paediatric Gastroenterology
Barts and the London NHS Trust
Honorary Consultant in Paediatric Gastroenterology
Dept Paediatric Gastroenterology
David Hughes Building
Whitechapel Road
E1 2BB

04 February 2011

Dear Dr Croft

Study title: **Inflammation and immunity in diseases involving the gastrointestinal tract in children and adults**
REC reference: **P/01/023**
Amendment date: **07 January 2011**

The above amendment was reviewed on 28 January 2011 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Patient/Carer Informaiton Sheet [revised version]		07 January 2011
Notice of Substantial Amendment (non-CTIMPs)		07 January 2011
Covering Letter		07 January 2011

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

P/01/023:	Please quote this number on all correspondence

Yours sincerely

Miss Sandra Burke
Research Ethics Committee (REC) Co-ordinator
East London Research Ethics Committee 1
(Formerly known as East London and The City REC 1)

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *BLT/QMUL Joint R&D Dept*

APPENDIX II

Variables collected for the long term follow up of children with Crohn's disease who were treated with EEN as primary therapy.

Age

Sex

Ethnicity

Montreal Classification (Disease location, disease behaviour, presence of perianal or upper GI disease)

Tolerance to EEN

Method of EEN administration (NG vs oral)

CRP, ESR, Albumin on diagnosis

Remission achieved with EEN (Yes/No)

CRP, ESR, ALbumin 6 weeks after initiation of treatment with EEN

Time to clinical relapse

Steroids during course of follow up (yes/no)

Number of steroids courses during follow up

Time to first course of steroids during follow up

Thiopurines required during follow up (yes/no)

Time to first use of thiopurines

Anti-TNF required during follow up (yes/no)

Time to first use of anti-TNF

Surgery required during follow up (yes/no)

Time to first operation

Vitamin D checked during follow up (yes/no)

Date vitamin D checked

Vitamin D level

Vitamin D deficiency (yes/no)

Vitamin D insufficiency (yes/no)

Change of Montreal classification during follow up (yes/no)

Date / time to change of Montreal Classification

Final Montreal Classification (Location, Behavior, L4, perianal)

Location expansion (yes/no)

New L4 (yes/no)

New perianal disease (yes/no)

New stricture (yes/no)

New penetration (yes/no)

Final Montreal classification

APPENDIX III

Modulen Protocol for newly diagnosed children with CD at Barts' Health

The whole Modulen program is 10.5 weeks (3 to 4 days in hospital on Modulen only, 6 weeks Modulen only and 4 weeks of Modulen and food)

Building up on the Modulen in hospital

- **Usually stay in hospital for 3'4 days to allow for gradual build up of Modulen e.g.:**
    - **Day 0: 'The last supper' and the taste test: If they've just been told of the diagnosis that day, it's a good idea for them to have something tasty to eat before the program begins. If they come in especially to start Modulen then this isn't necessary. The taste test involves the nurses making up one cup of Modulen, dividing it into three cups and then adding a different flavour of Nesquik to each. The patient should then decide which flavour they like best.**
    - **Day 1: 1/3 total volume e.g. 3 cups**
    - **Day 2: 2/3 total volume e.g. 6 cups**
    - **Day 3: Total volume e.g. 9cups, then home that night or the following morning**
- **If unable to drink Modulen then insert NG.**

6 weeks of Modulen only

- **Add ½ ' 1 teaspoon of Nesquick to each cup. It seems to help compliance to stick to 1 Nesquick flavour (Can also use Crusha syrup or the flavour modules from Nestle' although we don't usually do this)**
- **Nothing else except for water and sugar'free gum/mint (No sugar'free sweets, no flavoured water/ squash etc etc etc)**
- **Tell family to avoid cooking/eating in front of child during first couple of weeks to help with compliance**
- **It's good if the families buy their own flasks/ bottles to put Modulen in as the shaker cups aren't that sturdy**

4 weeks of Modulen and food re?introduction

- **Introduce a new food every 2 days: follow information sheet guidance**
- **On week 1, give full volume Modulen, then reduce each week**

e.g. week 1: 9 cups, week 2: 6 cups, week 3: 4 cups, week 4: 2 cups

Requirements

Usually aim for 120% EAR for newly diagnosed Crohn's patients who have lost weight.

Often need to concentrate the feed because the fluid requirement will be lower than the energy requirement

Making Modulen

20% = 1kcal/ml (standard concentration)

Add 6 scoops to the shaker cup and then add 210ml cool, boiled water

This gives a final volume of 250ml

25% = 1.25kcal/ml

Add 8 scoops to the shaker cup and then add 180ml cool, boiled water

This gives a final volume of 250ml

30%= 1.5kcal/ml

Add 10 scoops to the shaker cup and then add 160ml cool, boiled water

This gives a final volume of 250ml

Follow up

Patients are followed up by the gastro team in the IBD clinic on a Wednesday. The gastro team will bleep if they feel a patient needs to be seen.

REINTRODUCTION OF FOODS FOLLOWING EEN

One food at a time should be re-introduced into the diet. The portion size to be taken each day should be 1-1,5 times the normal amount.

If after re-introducing a certain food, symptoms reoccur or worsen, then this food should be removed from patient's diet and can be attempted to be re-introduced again at a later date. No further food should be re-introduced until symptoms have cleared or improved.

Once a food has been tested and found satisfactory, it can be included in any subsequent meal.

Date	Reactions if any (with date)
Potatoes	
Lamb	
Pear	
Chicken	
Yeast (Take 2 brewers yeast tablets or use Marmite)	
Milk	
Wheat (Try white spaghetti and if this is negative, then 100% wholemeal wheat flour may be used	
Butter	

Bread (Use only 100% wholemeal bread. Only try if test to yeast and wheat was negative)

Egg

Cheese

Cabbage

After re-introducing the above foods in their stated order, the following should be introduced in the same way, as discussed with the dietician

Beef

Pork

Carrots

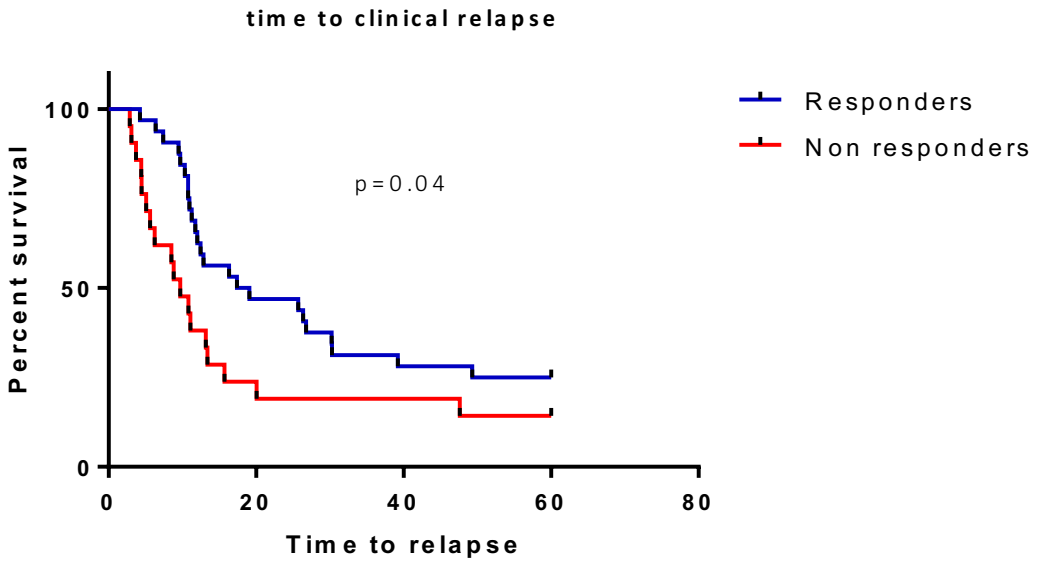
Tomatoes

Onions

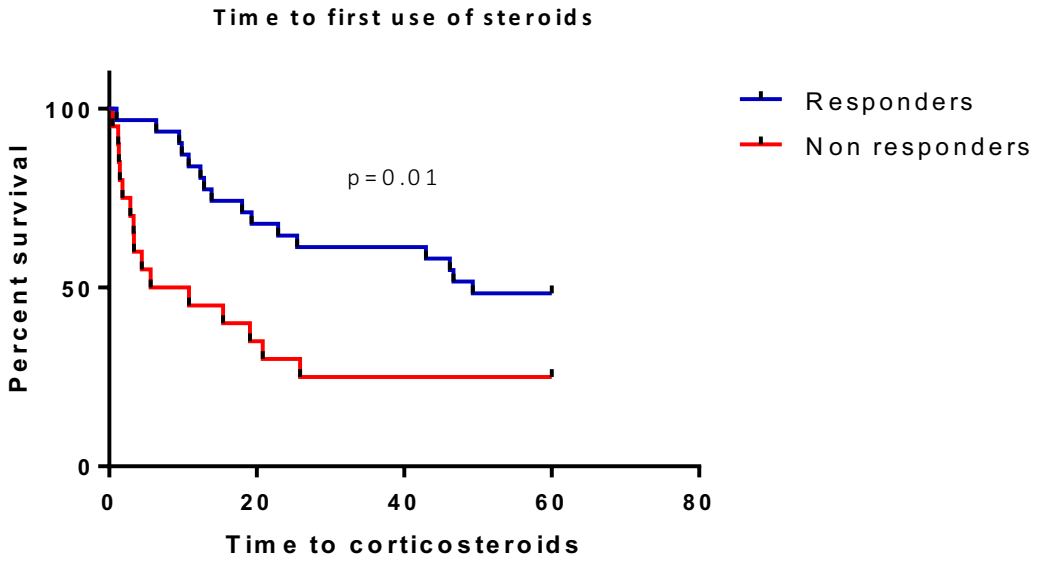
Peas

Barley (try pearl barley)

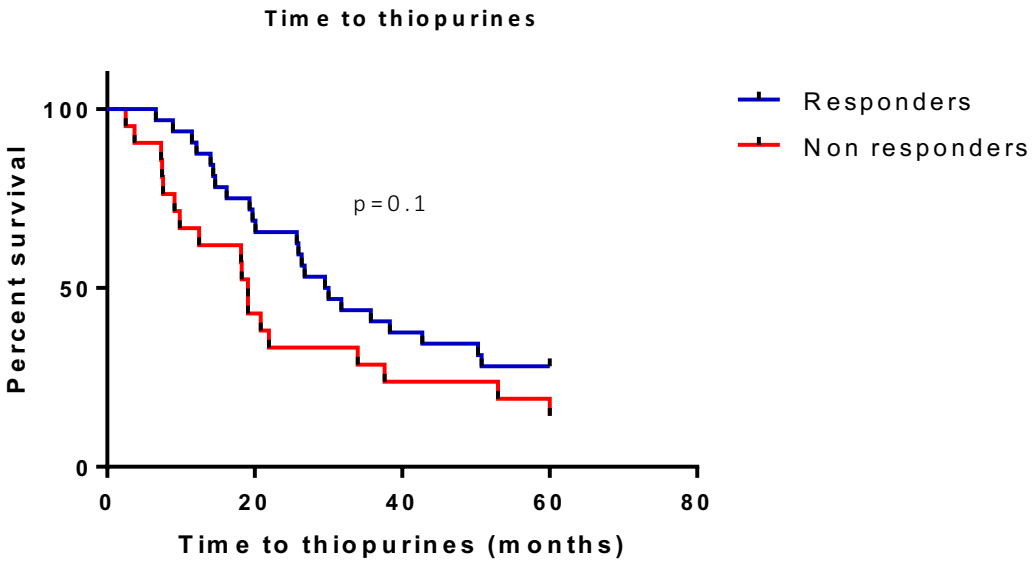
APPENDIX IV



Survival analysis of time to clinical relapse between responders and non responders to EEN after excluding the patients who did not tolerate EEN



Survival analysis of time to first use of corticosteroids among responders and non responders to EEN after excluding the patients who did not tolerate EEN.



Survival analysis of time to first use of thiopurines among responders and non responders to EEN after excluding patients that did not tolerate EEN.