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MD (Res) Thesis

Therapeutic Strategies for Restoring Linear Growth in Children with Crohn's Disease

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Abstract

Linear growth retardation affects up to 40% of children with Crohn's disease (CD). It is caused by a combination of under-nutrition, and by a direct effect of cytokines on the axis linking human growth hormone (GH), insulin like growth factor-1 (IGF-1) and the growth plate of growing bones. In particular, the inflammation causes a functional insensitivity to GH, resulting in low circulating IGF-1. Current management is synonymous with the optimal management of childhood CD: to eliminate inflammation, and maintain remission. However, there is currently no consensus as to how to treat growth failure in patients whose inflammation remains intractable to treatment.

This thesis examined the hypothesis that successful treatment of inflammation would improve linear growth in children with CD. We performed a series of retrospective studies examining a cohort of children aged ≤17 years with CD treated at Bart's and The London Children Hospital. We determined the height standard deviation scores (SDS) of patients at diagnosis and investigated growth outcomes following treatments used to induce and maintain remission. These were: exclusive enteral nutrition (EEN), thiopurines and infliximab. Finally, as a potential new therapy, we considered the use of exogenously administered recombinant human IGF-1 (rhIGF-1) to restore plasma IGF-1 levels. We performed an open-labelled pharmacokinetic (PK) study of rhIGF-1 in 8 children with CD and growth failure.

9% of our patients had height SDS of -2 SDS at diagnosis; a four-fold increase compared to the normal age matched population. In addition, symptom duration negatively correlated with height SDS at diagnosis (r=-0.06; p=0.02). From 89

patients receiving EEN for primary induction of remission, 62.9% (56/89) of patients achieved complete remission. Over the course of 5 years, responders to EEN grew significantly better than non-responders (change in height SDS +0.18 [0.12] vs to -0.37 [0.13] respectively; p=0.005). This occurred despite no differences in duration of remission or treatment escalation between groups. 51% (26/51) patients treated with thiopurines were in remission at 12 months. These patients had improved growth (median change height SDS [IQR] 0.08 [-0.06 – 0.19] vs -0.24 [-0.61 - -0.07] in non-responders; p=0.001). 39.2% (20/51) of these patients started anti-TNF therapy. However, over 12 months, we found that this conferred no improvement in growth (median [IQR] at 0 months -0.38 [-1.73 to -0.10] as compared to -0.61 [-1.72 to -0.09] at 12 months; p=0.43), irrespective of response to treatment.

Subcutaneous treatment with 120 µg/kg twice daily rhIGF-1 restored plasma levels of IGF-1 in our patients, albeit it in some to high levels (median [range] concentration +2.09 [-1.27 to +5.21]). We were able to develop a PK mathematical model from these results to determine a more appropriate dosage. We found that in addition to patient's age and weight, PCDAI also needs to be taken into consideration when determining dose.

In summary, growth retardation is very common finding in paediatric CD. Response to EEN and thiopurines seems to result in beneficial effects upon growth. However, a number of children continued to have poor growth despite treatment. Our results show it is possible to increase circulating IGF-1 concentrations with exogenous injections, and develop a mathematical model to devise a dose to use in further trials.

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List of Abbreviations

5-ASA	5-aminosalycilates
6-MP	6-mercaptopurine
6-MMP	6-methylmercaptopurine
6-TGN	6 thioguanine
A1AT	Alpha-1-antitrypsin
ALS	Acid labile subunit
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Anti-TNF	Anti-tumour necrosis factor α
AZA	Azathioprine
BMI	Body mass index
CD	Crohn's disease
CI	Confidence interval
CRP	C-reactive protein
CS	Corticosteroids
ECCO	European Crohn’s and Colitis Organisation
ECG	Electrocardiogram
EEN	Exclusive enteral nutrition
EER	Estimated energy requirement
EIM	Extra-intestinal manifestation
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
GH	Growth hormone

GHIS	Growth hormone insensitivity syndrome
GI	Gastrointestinal
GLM	General linear model
HV	Height velocity
IBD	Inflammatory bowel disease
IFX	Infliximab
IGF-1	Insulin-like growth factor-1
IGFBPs	Insulin-like growth factor binding proteins
IL	Interleukin
IM	Immunomodulator
IQR	Interquartile range
MCV	Mean corpuscular volume
MP	Mercaptopurine
MPH	Mid-parental height
NG	Nasogastric
PCDAI	Pediatric Crohn's disease activity index
PIBD	Paediatric inflammatory bowel disease
PK	Pharmacokinetics
PLE	Protein-losing enteropathy
PPK	Population pharmacokinetics
QOL	Quality of life
RCT	Randomised controlled trial
rhGH	Recombinant human growth hormone
rhIGF-1	Recombinant human insulin-like growth factor-1
sc	Subcutaneous
SD	Standard deviation

SDS	Standard deviation score
SOCS	Suppressor of cytokine-inducible signalling
TNBS	Trinitrobenzenesulphonic acid
TPMT	Thiopurine-S-methyltransferase
UC	Ulcerative colitis
UK	United Kingdom
vs	Versus

Publications and Presentations

Papers

Rao A, Standing JF, Naik S, Savage MO, Sanderson IR. Mathematical modeling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study. BMJ Open. 2013 May 28;3(5).

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First Author Abstracts

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1 Introduction:

1.1 Crohn's disease

Crohn's disease (CD) is a form of inflammatory bowel disease (IBD); a group of chronic, idiopathic inflammatory disorders of the bowel which also include ulcerative colitis (UC), indeterminate colitis, and rare (mostly genetic) conditions of early infancy [1]. CD can occur in both adults and children, and can involve any part of the gastrointestinal tract from the oropharynx to the perianal area with discontinuous inflammation ('skip lesions'). It is characterised by transmural inflammation of the bowel wall. As a result, there is a tendency to form strictures and fistulae. The inflammation of CD is histologically identified as cryptitis with the formation of crypt abscesses and, in many patients, granulomas. CD is a chronic lifelong condition. It runs a relapsing and remitting course. There are several medical and surgical therapies available to manage the disease, but none are curative [1, 2].

1.1.1 Pathogenesis of Crohn's Disease

The cause of CD is unknown, but is likely to be multi-factorial and complex. Current theories suggest that the intestinal inflammation of CD is due to an abnormal and prolonged T-cell mediated immune response against a subset of commensal enteric bacteria in genetically susceptible individuals. The onset and reactivation of disease

is triggered by, as yet mostly undefined, environmental insults that transiently break the mucosal barrier, stimulate immune responses or alter the balance between enteric bacteria [3, 4]. Enteric microbiota can stimulate immune responses, either by functioning as adjuvants or antigens. As adjuvants they activate innate immune responses (including dendritic cells and other antigen presenting cells) and as antigens they stimulate the clonal expansion of T-cells that selectively recognise the antigen through their T-cell receptor. Therefore, patients with CD have enhanced recruitment and retention of effector macrophages, neutrophils and T cells into the inflamed intestine, where they are activated and release pro-inflammatory cytokines (Figure 1.1).

Having a sibling or parents with CD is a risk factor for its development. The relative risk of developing CD in siblings of affected patients is up to 35 times background population risk, and where both parents have CD up to 36% of offspring will develop the disease [5-7]. Disease location is also highly concordant between monozygotic twins with Crohn's disease. A Scandinavian study found that location was identical in 11 out of 17 monozygotic twins with Crohn's disease at diagnosis, and behaviour in 13/17 pairs [8]. This similarity was still seen after 10 years of disease. By contrast, monozygotic twins with UC were concordant for age of onset, but not for extent of disease at diagnosis or after 10 years.

The first IBD genetic susceptibility gene to be discovered was CARD-15 (caspase recruitment domain family member-15). This encodes for an intracellular receptor (nucleotide-binding oligomerisation domain containing protein 2; NOD2) for the bacterial proteoglycan fragment muramyl dipeptide [9, 10], thus demonstrating the

pivotal relationship between the gut immune system and intestinal microbiota at the heart of CD pathogenesis. Since then, several other susceptibility genes have been identified, including solute carrier family 22 members 4 and 5 (SLC22A4 and SLC22A5 (organic cation transporter) [11, 12] and discs large homolog 5 (DLG5; an epithelial scaffolding protein) [13]. The commonality between these implicated genes is that they all regulate several important biologic functions, including mucosal barrier integrity, immunoregulation and microbial clearance and / or homeostasis [3].

The involvement of the intestinal microbiota in the pathogenesis of CD has been well described. Animal models have demonstrated that colitis and immune activation fail to develop in the absence of commensal bacteria [14]. Furthermore, patients with CD exhibit altered intestinal microbiota, including reduced Firmicutes such as Clostridia cluster IV and *Faecalibacterium prausnitzii* and increased *Escherichia coli* [4, 15, 16]. However, sibling studies have shown that asymptomatic siblings of patients with CD share aspects of their intestinal dysbiosis without developing the disease itself [17]. The relatively low concordance of CD in twin studies [8] and the increased prevalence of CD in countries as they adapt a more 'Western' lifestyle [18] indicates that there is a strong environmental influence on IBD. Studies have implicated several environmental factors in the pathogenesis of CD, including smoking, stress, infections, diet and the use of antibiotics and anti-inflammatory drugs [3, 19-23].

In summary, chronic intestinal inflammation seems to develop via many different mechanisms, suggesting that CD is a heterogeneous disease, with the heterogeneity

occurring at genetic, immunologic, bacteriologic, phenotypic and therapeutic levels, all resulting in similar final common pathways. This theory could predict why no single treatment produces a universal therapeutic response in CD [3].

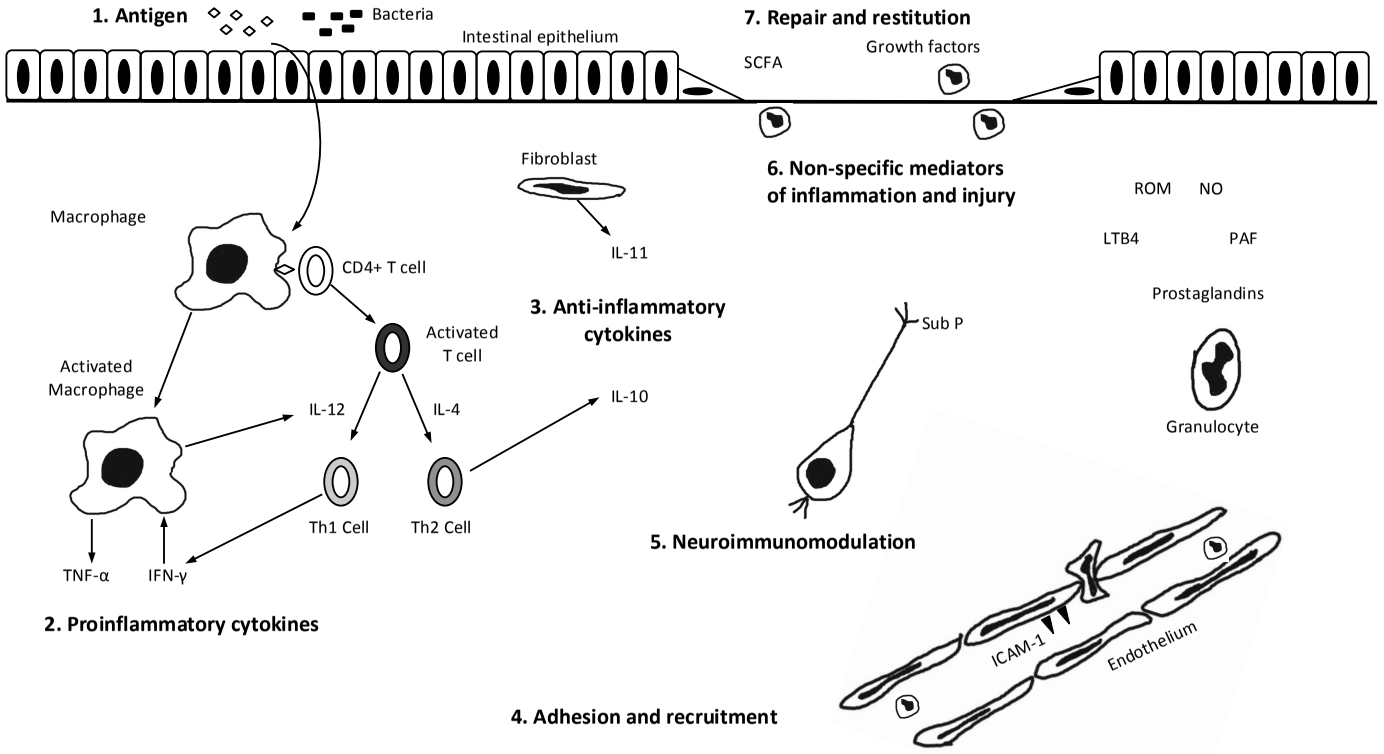

Figure 1.1 Proposed pathophysiology of chronic intestinal inflammation in CD.

Mucosal inflammation is triggered by antigen (1), likely bacterial in origin. Macrophages process and present this (HLA class II) to CD4+ T cells. Activated macrophages release pro-inflammatory cytokines (2) TNF-α and IL-12, inducing T-helper (Th1) responses. Release of IL-4 promotes T-helper 2 (Th2) differentiation and responses (3) e.g. IL-11 production. (4) Co-ordinated expression of integrins, chemokines, and adhesion molecules (e.g. ICAM-1) recruit peripheral cells. Neutropeptides, e.g. substance P (Sub P) modify local responses (5). Nonspecific mediators of inflammation and injury (6) may affect tissue destruction directly (e.g. reactive oxygen metabolites (ROM), nitric oxide (NO), leukotriene B4 (LTB4), platelet activating factor (PAF) and prostaglandins). Host responses induce mechanisms of restitution of the wound and repair (7). Short chain fatty acids (SCFAs) and growth factors contribute to this process.

1.2 Crohn's disease in childhood

Although the peak age of onset of CD is in young adulthood (20-30 years), up to a quarter of cases of CD now present in childhood or adolescence [24, 25]. Paediatric-onset CD presents its own unique problems not experienced by our adult colleagues. Disease is often more extensive at diagnosis than in adults, and follows an aggressive course [26, 27]. In addition, childhood is a time of significant physical changes and emotional maturation. There is also the likelihood of many years of treatment, and so the side-effects profile of medications is of great importance. All of this needs to be considered when managing these patients.

1.2.1 Epidemiology:

The incidence of CD is increasing worldwide [18]. The incidence remains highest in the Western world, with annual incidence in Europe of 12.7 per 100 000 person-years and in North America of 20.2 per 100 000 person-years [28]. However, the incidence and prevalence of CD are also increasing in previously low-incidence areas such as Asia and Eastern Europe [29, 30]. This increase is likely to be related to westernization of lifestyle, altered diet and changes in hygiene [31].

The incidence of paediatric CD is also increasing [24, 32-34]. A systemic review was performed describing the changing epidemiology of childhood onset IBD [24] . Articles published between 1950-2009 were searched and analysed. Of the 25 studies that calculated temporal trends in CD incidence, 15 (60%) reported significant increases. Rising rates were seen in both developed and developing countries. A recent national

cohort study in Scotland of incident cases of paediatric IBD showed the incidence of CD between 2003-2008 to be 4.75/100 000/year. This showed a significant increase from historical data from 1990-1995, which showed an incidence of 2.86/100 00/year [35]. A similar study using health administrative data performed in Ontario, Canada showed that the prevalence of paediatric CD has increased from 23.9/100,000 population in 1994 to 31.6/100 000 in 2005 [32]. By contrast, Malmborg et al [36] found that although the incidence rate of paediatric IBD (PIBD) in northern Stockholm was significantly higher in 2002-2007 than 1990-2001, the previous sharp increase in CD seems to have levelled out. However, it should be noted that the rate is still higher than reported from most other regions in the world ($13.9/10^5$ person-years).

The mean age of diagnosis in paediatric CD is in the teenage years. An epidemiological study of PIBD diagnosed in 2001-2007 in Wisconsin, USA found that the average age of onset of CD was 12.3 years, with the highest age-related incidence occurring at 14 years [37]. There is conflicting data as to whether the median age of onset of paediatric CD is also decreasing. The aforementioned Scottish study found a significant decrease in age at CD diagnosis from 13.2 to 12.1 years. By contrast, there was no significant change in either UC or non-Crohn's colitis (UC and IBDU combined) [35]. The Ontario study reported a significant increase the incidence of CD in patients aged 5-9 years old (8.7% per year, $p<0.0001$) [32]. However, retrospective data analysed from patients enrolled in the Swiss IBD Cohort Study showed that age of onset had increased between 1980-2010 [38]. In addition, a retrospective study of children diagnosed with IBD between 1991-2002 in Texas showed a significantly increased incidence of IBD in children aged 10-14yrs and 15-17years, with stable

incidence in children under 10. The highest incidence was in children ages 10-14yrs was among both patients with CD and those with UC [39].

In contrast to adults, in whom females are more commonly affected than males both in adult-onset and elderly-onset CD, there is a male predilection in the paediatric population [40]. The male: female ratio is commonly described as being around 1.5:1 [35, 41]. Although CD is more prevalent in Caucasians, it is present across all ethnic groups [28].

1.2.2 Presentation:

The classic triad of presenting symptoms is that of pain, weight loss and diarrhoea. However, a large population-based survey in the United Kingdom found that only 25% of patients presented with all three symptoms. A substantial minority of patients (44%) did not have diarrhoea. The commonest presenting symptom was abdominal pain, with 72% of patients reporting this. Other presenting features included fatigue, fever, poor growth and/or delayed puberty, anorexia, generalised systemic malaise, nausea or perianal disease [42].

1.2.3 Extra-intestinal manifestations (EIMs):

CD, as with all the other inflammatory bowel diseases, is also associated with extra-intestinal manifestations (EIMs). These can include cutaneous manifestations,

including erythema nodosum and pyoderma gangrenosum [43], arthralgia, ocular changes and osteopenia / osteoporosis. EIMs can be present at or before the time of diagnosis in some patients. A large American cohort study looking at 1649 paediatric IBD patients diagnosed between January 2000 and November 2003 found that 6% of patients reported EIMs prior to diagnosis. Overall, 24% of children developed at least 1 EIM by the end of follow-up. 33% of these were musculoskeletal [44]. Similar results have been found in other studies [45].

1.2.4 Diagnosis

The importance of having uniformity in the investigations and criteria used for diagnosis of paediatric IBD has been recognised. In 2005, the IBD Working Group of the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) published the Porto criteria for diagnosis [46]. These criteria have been revised recently (Figure 1.2). They state that accurate diagnosis of IBD should be based on a combination of history, physical and laboratory examination, esophagogastroduodenoscopy and ileocolonoscopy with histology, and imaging of the small bowel. Magnetic resonance enterography (MRE) is currently the imaging modality of choice [47]

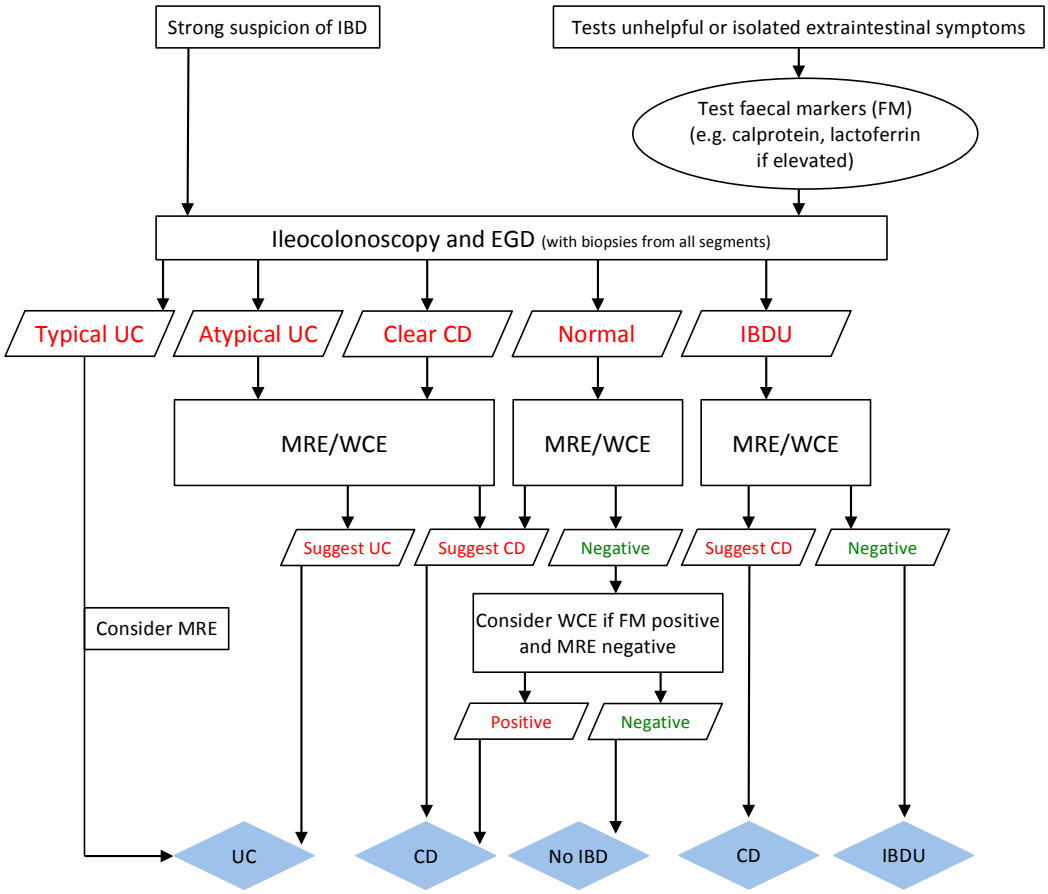

Figure 1.2 Evaluation of child/adolescent with intestinal or extraintestnal symptoms suggestive of IBD.

Atypical UC is a new IBD category and reflects a phenotype that should be treated as UC. IBD-U can be used as a tentative diagnosis after endocscopy, and can be used as a final diagnosis after imaging and a full endoscopic workup. UC is divided into typical UC and atypical UC, CD = Crohn's disease; EGD = oesophagogastroduodenoscopy; FM = faecal marker; IBD = inflammatory bowel disease; MRE = magnetic resonance enterography; UC = ulcerative colitis; WCE = wireless capsule endoscopy. [47]

1.2.5 Disease phenotype and classification:

The various phenotypes of CD in adults and children are defined by the Montreal classification [48] (with a recent update termed the Paris classification [49] – see below). This classifies disease by location and behaviour. The Montreal classification has also defined three separate age categories: A1 ≤16 years, A2 17-40 years, A3 >40 years [48]. Table 1.1

Table 1.1 Definition of Crohn's disease phenotype using the Montreal classification [48]

Age of onset	Location	Behaviour **
<16 years (A1)	Ileal (L1)	Non-stricturing, non-penetrating (B1)
16-40 years (A2)	Colonic (L2)	Stricturng (B2)
>40 years (A3)	Ileo-colonic (L3)	Penetrating (B3)
	*Isolated upper GI disease (L4)	+ 'p' if peri-anal disease

*L4 is a modifier that can be attached to L1-3 when concomitant Upper GI disease is present

** Patients who have the presence of both stricturing disease and penetrating disease will be classified as B3.

However, children are more likely to present with more extensive disease than adults [26]. Studies show that as many as 63% of children have ileocolonic disease at diagnosis, whereas in adults the most common site of disease at presentation is ileal. When followed up over a period of at least 2 years, approximately one third of patients will show disease extension. At the time of diagnosis, the majority of paediatric

patients will have the non-stricturing non-penetrating phenotype (B1). However, with time, a significant number of patients will show a change in disease behaviour, evolving from B1 phenotype to stricturing (B2) or penetrating (B3) disease [26, 27, 50, 51]. The Registre des maladies inflammatoires chroniques de l'intestin du Nord Ouest de la France (EPIMAD) registry records all incident cases of IBD since 1988 in northern France [34]. Follow up data on 404 patients diagnosed with CD between 1988-2002 aged <17 years at diagnosis were analysed. Over a period of ≥ 2 years follow up, the B2 and B3 phenotypes increased from 25% to 44% and 4% to 15%, respectively. By contrast, the B1 phenotype decreased from 71% at diagnosis to 41% at last follow up [27]

Because of the dynamic, evolving nature of paediatric CD, it has been suggested that the Montreal classification may not be sufficient in classifying phenotype in children. Therefore, the Paris classification has since been proposed. This has now further subdivided age at diagnosis as A1a (0 to <10 years) and A1b (10 to<17 years). In addition, disease above the distal ileum (L4) is now classified as L4a (proximal to the ligament of Treitz) and L4b (ligament of Treitz to above the distal ileum), and stenosing and penetrating disease can be classified in the same patient (B2B3). The presence of growth failure is also noted, with G1 indicating growth impairment in the patient at any time, and G0 no growth impairment [49, 52]. Table 1.2 outlines the differences between the Montreal and Paris classifications.

Table 1.2 Montreal and Paris Classifications for Crohn's Disease [48, 49]

	Montreal	**Paris**
Age at Diagnosis	A1: below 17 y	A1a: 0–<10y A1b: 10–<17 y
	A2: 17-40 y	A2: 17–40 y
	A3: Above 40 y	A3: >40 y
Location	L1: terminal ileal ± limited caecal disease	L1: distal 1/3 ileum ± limited caecal disease
	L2: colonic	L2: colonic
	L3: ileocolonic	L3: ileocolonic
	L4: Isolated upper disease*	L4a: upper disease proximal to Ligament of Treitz*
		L4b: upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum*
Behavior	B1: non-stricturing non-penetrating	B1: non-stricturing, non-penetrating
	B2: stricturing	B2: stricturing
	B3: penetrating p: perianal disease modifier	B3: penetrating
		B2B3: both penetrating and stricturing disease, either at the same or different times
		p: perianal disease modifier
Growth	n/a	G_0: No evidence of growth delay
		G_1: Growth delay

*In both the Montreal and Paris Classification systems L4 and L4a/L4b may coexist with L1, L2, L3, respectively. B1 – Non-stricturing, non-penetrating disease: uncomplicated inflammatory disease without evidence of stricturing or penetrating disease.

B2 - Stricturing disease: the occurrence of constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical examination combined with prestenotic dilation and/or obstructive signs or symptoms but without evidence of penetrating disease.

B3 - Penetrating disease: the occurrence of bowel perforation, intraabdo- minal fistulas, inflammatory masses and/or abscesses at any time in the course of the disease, and not secondary postoperative intra-abdominal complication (excludes isolated perianal or rectovaginal fistulae).

B2B3 – Stricturing and penetrating disease: the presence of both B2 and B3 phenotypes in the same patient, either at the same moment in time, or sepa- rately over a period of time

1.3 Growth Impairment in Paediatric CD

Impaired growth is one of the commonest extra-intestinal manifestations of paediatric Crohn's disease. Unlike in children with UC, in whom growth is a rarely a problem, 15-40% of children with CD experience linear growth retardation and delayed puberty [53, 54]. This is a challenge unique to paediatrics, and one that can affect the long-term outcome of patients and their quality of life (QOL). Indeed the new European Crohn's and Colitis Organisation (ECCO) and ESPGHAN guidelines on the medical management of paediatric CD have identified growth retardation as one of the factors that can be considered as potentially predictive for poor outcome [55]. Indeed, its importance in assessing disease activity in children with CD is demonstrated by the fact that it forms part of the assessment criteria included in the Pediatric Crohn's Disease Activity Index (PCDAI) [56], and its incorporation into the new Paris classification of paediatric IBD [49].

1.3.1 Normal growth

Normal growth is the progression of changes of height, weight and head circumference that are compatible with established standards for a given population [57]. A child's growth is dependent on both genes and the environment, and is principally mediated by a combination of hormones and nutrition [58]. The presence of normal growth is an indication of the child's overall good health and nutritional status.

Most healthy children grow in a predictable fashion, following a typical pattern of progression. This allows growth to be monitored in standardised charts for age and

gender in a population (see Appendix 1). Normal growth is pulsatile, with periods of rapid growth ("growth spurts") separated by periods of no measurable growth [59-61].

Normal linear growth in children occurs via activation of the growth hormone (GH) insulin-like growth factor-1 (IGF-1) axis while supported by adequate nutrition. GH is secreted by the anterior pituitary gland, and binds to GH receptors in many tissues, including hepatocytes. The binding of GH to its receptor activates JAK2, which leads to phosphorylation of STAT5. This is subsequently translocated into the nucleus where it is able to bind to regulatory elements of target genes, including IGF-1 [62]. The IGF-1 in turn stimulates the proliferation and hypertrophy of chondrocytes in the epiphyseal growth plate, resulting in long bone growth. The majority of IGF-1 circulates in ternary complexes with high-affinity binding proteins (IGFBPs) and the acid-labile subunit (ALS). IGF-1 first binds to an IGFBP, followed by the binding of ALS [63]. The formation of this ternary complex restricts IGF-1 to the circulation, prolonging its half-life, and modulating its bioavailability [64]. . There are currently 6 known IGFBPs. Most serum IGF-1 binds to IGFBP-3, and GH in turn regulates this [54, 64]. Of the other binding proteins, IGFBP-1 is regulated primarily by insulin levels, but expression is also increased in states of starvation and by corticosteroids (CS) in a dose-dependent manner. Factors that increase IGFBP-1 may decrease the availability of bioactive IGF-1 [54].

The fundamental importance of the GH/IGF-1 axis to normal linear growth is demonstrated in conditions where there is disruption to this pathway. Children with GH deficiency have a resultant short stature [65-67]. This is not only seen in children with primary GH deficiency, but also in conditions which result in low GH levels, such

as chronic renal disease [68-70], Turner's Syndrome [71] and Prader-Willi syndrome [72]. Treatment with recombinant human GH (rhGH) replacement therapy has been shown to improve growth in these patients [73-79].

The importance of IGF-1 has similarly been demonstrated. IGF-1 is the main mediator of GH action [80, 81]. First described by Laron in 1966 [82], growth hormone insensitivity syndrome (GHIS) encompasses a variety of genetic and acquired conditions in which the action of GH is absent or reduced. Affected children have very low circulating IGF-1, with normal or elevated GH, resulting in severe growth retardation. Untreated GHIS results in adult heights 4 to 12 standard deviations (SD) below the normal [83-85]. In addition, these children also have very low levels of IGFBP-3 [86]. Short for gestational age children also have lower IGF-1 and IGFBP-3 concentrations when compared to their healthy peers [64].

1.3.2 The definition of growth failure

There is still no universal agreement as to the definition of growth failure [87]. A commonly used definition is a height or height velocity (HV; change in length / time) ≤ -2 standard deviation scores (SDS) below the mean for age. A standard deviation score, or '*z*' score, expresses a value as a number of standard deviations above or below the reference mean or median value [88]. It is calculated using the following equation:

$$z = \frac{x-\mu}{\sigma}$$

μ = mean, x = score, σ = standard deviation.

In a normally distributed population, 68% of the population fall between -1 to +1SD of the mean, and 97.6% within -2 to +2 SD (Figure 1.3). Therefore a standard deviation score of <-2 refers to a value in the shortest 1.2% of the population

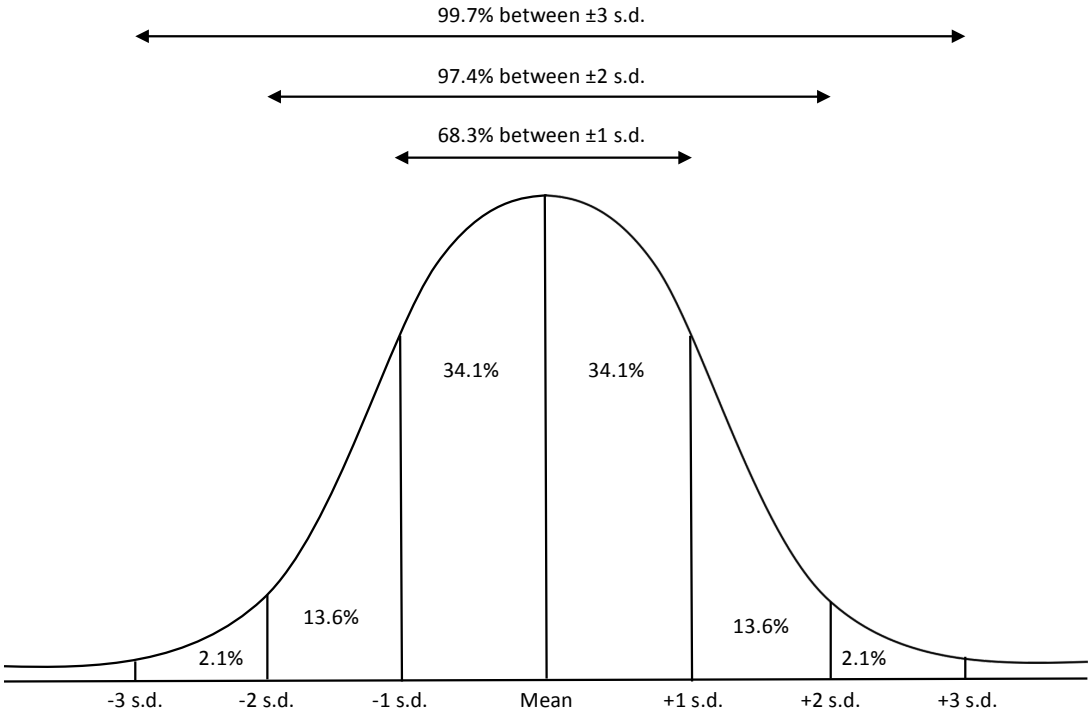

Figure 1.3 Standard deviation and population spread for a normal distribution.

Other definitions used for growth retardation include a combination of height SDS <-1 and HV SDS <-1, and a static height below the third percentile for age and gender (z-score <-2) [53, 89]. Where possible, it is of benefit to calculate the patient's target height, as derived from parental height. First described by Tanner in 1970 as a means of testing the effectiveness of growth promoting therapies, the target height is commonly determined by the corrected mid-parental height (adding or subtracting 6.5cm for boys and girls, respectively) [90]. This is an important measurement as family and twin studies in the general population have shown that the heritability of growth is high, ranging from 76-90% [91-93]. The same has been shown in IBD. An American study looking at the impact of parental height to final adult height in 108 children with paediatric-onset IBD found that the adult heights of these patients fell within 1 SD of their target heights [94]. However, this study included both children with CD and UC in its analysis. It has been well documented that children with UC have less growth impairment than children with CD. Therefore the inclusion of UC patients is likely to have had an impact upon the study findings. Nevertheless, it has demonstrated the influence of genetics, even in the presence of inflammation. However, a limiting factor of using target height as a measure of growth is that it, of course, relies on knowledge of parental height.

1.3.3 Prevalence of faltering growth at diagnosis

Poor growth is commonly present at diagnosis [42, 95]. The EPIMAD study found that from a French population of 261 patients with paediatric onset CD (<17 yrs) diagnosed between 1988 and 2004, 9.5% showed height SDS < -2 at diagnosis, with an overall mean score of -0.38 SD [96]. In some patients, growth failure may precede clinical evidence of bowel disease by a few years [97]. Kanof *et al* (1988) noted that 88% of patients had reduced height velocity prior to diagnosis, almost half of whom displayed this before developing any gastrointestinal problems [98]. The mean height SDS at diagnosis of populations of children with CD is generally described as ranging between -1.0 and -0.5 SDS [94, 99].

Patients with CD are commonly reported as having a longer time period from the onset of symptoms to the time of diagnosis than children with UC. This is likely to be due to the fact that patients with CD are more likely to present with non-specific symptoms, such as abdominal pain and weight loss, whereas children with colitis often present with bloody diarrhoea [100]. A study by Sawczenko *et al* looking at the presenting features of 739 children with IBD in Great Britain and Ireland found that the median delay from onset of symptoms to diagnosis was 5 months (range <1 month to 9 years), with children with CD having significantly longer delays than those with UC or IBDU ($p< 0.01$ and $p<0.001$ respectively). 18% had a delay of 1-3 years, and 9% a delay of more than 3 years. This is of fundamental importance as a delay in time to diagnosis correlates negatively with the degree of growth retardation [42, 101], which is in turn associated with reduced final adult height [94, 100, 101]. This highlights the

importance of prompt recognition and treatment of children with CD in order to improve their long-term growth outcomes.

Another factor that has been reported to correlate with growth failure is the site of disease location [99, 100]. Sawczenko *et al* found that the presence of active jejunal disease was associated with both reduced height and weight z scores when compared to patients with other disease locations (height mean *z* score -0.90 vs -0.5; p=0.041, weight mean *z* score -1.6 vs -1.1; p=0.02 respectively). [42]. Growth retardation can have a lasting effect. A study at Barts Health NHS Trust, examining final adult height in 123 patients diagnosed with CD in childhood, found that the presence of jejunal disease was not only associated with low height SDS scores at diagnosis, but that this persisted into adulthood. At final height, the patients with jejunal disease at diagnosis were shorter than those without jejunal disease (mean height SDS -0.70 vs -0.15; p=0.034) [101].

The data as to whether gender has an impact upon the amount of growth impairment present at diagnosis is conflicting. Generally, it is described as being greater in boys than girls, with the difference between the sexes increasing over time [41, 99]. However, in contrast, a cross-sectional study of 993 children with CS in the USA found no difference in growth between the genders [102]. There does not appear to be any correlation between age at diagnosis or ethnicity and height SDS [41, 42].

1.3.4 Mechanisms of growth failure in children with CD

Growth retardation in children with CD is multifactorial in origin, with undernutrition, the direct effects of inflammation, and the chronic use of corticosteroids (CS) important contributory factors. These factors are summarised in Table 1.3.

Table 1.3 Proposed factors contributing to growth impairment in children with CD [53, 54, 103, 104]

Factor	Reason
Decreased intake	Anorexia, nausea, vomiting, fear of worsening GI symptoms
Malabsorption	Loss of absorptive surface due to inflammation or resection of small bowel segments
Increased nutrient loss	Mucosal damage or resection leading to nutrient loss, protein-losing enteropathy (PLE)
Increased energy requirements	Increased energy requirements associated with active disease
Disease activity	Circulating pro-inflammatory cytokines impair linear growth
Corticosteroid treatment	Inhibition of IGF-1, and direct effects on the growth plate

1.3.4.1 Undernutrition

Reduced weight SDS are commonly seen in children with CD, and can be present in up to 85% of children at diagnosis [42, 105]. Chronic malnutrition has long been

recognised as a treatable cause of growth failure in children with CD [103, 106, 107]. There are several mechanisms that may precipitate this malnutrition (Table 1.3).

The nutritional deficiency in CD seems to be primarily related to inadequate intake. In children with CD and growth failure, calorie intake has been documented as being 43-82% less than the recommended amount [104, 108]. Thomas *et al* [109] reported that the calorie intake in 24 children with active CD was on average 400 kcal/day lower than that in healthy controls. Nutritional supplementation has been shown to improve growth velocity [110]. Aiges *et al* found that supplementing growth impaired CD patients with overnight nasogastric (NG) feeds significantly improved both their weight and height over a 12 month period. The mean weight gain of this cohort was 11.75kg, and height gain was 6.98cm. As a comparison, 6 control subjects, matched for age, pubertal status and degree of growth retardation who refused NG feeds showed no change in weight or height over this period [107]. Other factors which contribute to the malnutrition are malabsorption and increased nutrient loss [54]. Although it has been postulated that children with active CD may have increased energy requirements, this has so far not been demonstrated [111, 112]. However, children with CD have been shown to have decreased lean body mass similar to that seen in cachexia [113]. This combination of protein and calorie malnutrition results in decreased circulating levels of IGF-1 and IGFBPs [114, 115]. Grinspoon *et al* (1995) found that circulating IGF-1 levels decreased by approximately 50% after only 4 days of fasting in otherwise healthy adult women [116]. Starvation results in an acquired GH resistance. In animal models of starvation, the low circulating IGF-1 levels are coupled with decreased GH

receptor mRNA and decreased GH binding, suggesting GH receptor downregulation [117, 118].

1.3.4.2 Inflammation

Inflammation has a direct effect upon nutritional intake. Pro-inflammatory cytokines associated with active disease, such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), have been shown to interact directly at appetite centres, causing an anorexigenic effect [119, 120]. In rats with experimentally induced colitis, attenuating interleukin-1 (IL-1) activity by intracerebroventricular infusion of IL-1 receptor α significantly improved food intake [119]. Additionally, in patients with CD, increases in pro-inflammatory cytokines occur in association with alterations in hormones that control appetite, such as polypeptide YY [121] and ghrelin [122].

Therefore, it is very difficult to separate out the relative contributions of inflammation and undernutrition upon growth failure. However, animal studies, in which it is possible to control calorie intake, have assisted with this. Ballinger *et al* (2000) looked to elucidate the relative contributions of reduced calorie intake and inflammation to reduced linear growth in prepubertal rats with trinitrobenzenesulphonic acid (TNBS) induced colitis [123]. Linear growth was assessed in healthy free feeding controls, rats with TNBS induced colitis, and in a pair-fed group (i.e. healthy rats whose nutritional intake was restricted to match that of the colitic group). Changes in length over 5 days were measured. It was found that although the pair-fed rats grew 56% of the healthy free-feeding controls, the colitic group only grew 30%. In addition, even when the

colitic group were given supplemental feeds, although weight was restored to control values, the improvement in growth did not match that of control values. These rats remained shorter than the healthy controls. By contrast, nutritional supplementation caused a three-fold increase in length in the pair-fed group when compared to the colitic group ($p=0.007$). From this, the researchers concluded that 40% of the growth retardation in the TNBS colitic rats occurred as a direct result of the inflammatory process independent of the effects of undernutrition (Figure 1.4). IL-6 transgenic mouse lines bred to express high levels of circulating IL-6 from early after birth showed a reduced growth rate that ultimately led to mice 50-70% the size of non-transgenic littermates. This occurred despite comparable food intake per gram of body weight in both groups. Moreover, growth was improved in the transgenic mice by injecting the monoclonal antibody 15A7 from day 2 after birth, neutralizing the murine IL-6 receptor. This study suggests a direct causal effect of IL-6 upon growth in mice. This has also been shown in other animal studies [124, 125].

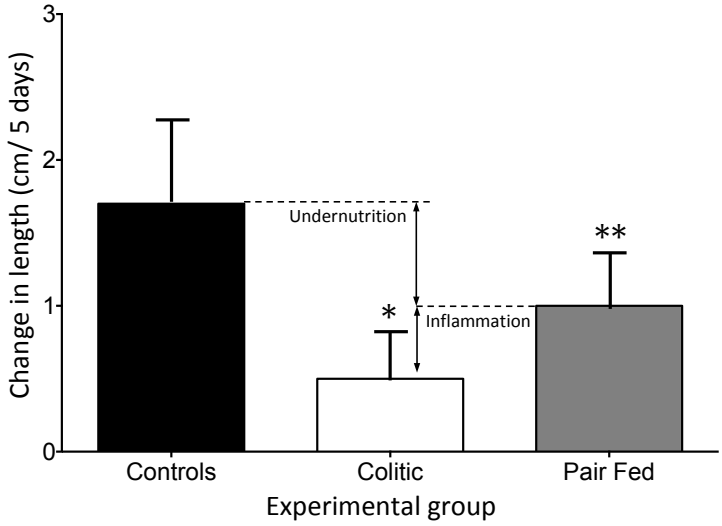

Figure 1.4 Effects of inflammation and undernutrition on linear growth retardation.
Linear growth retardation in rats with colitis compared with healthy free-feeding controls is due to both undernutrition and inflammation *p=0.02. In a healthy pair-fed group, food intake is restricted to that of the colitic group resulting in equal undernutrition, thereby separating the effects of inflammation from nutritional status. The difference in linear growth between the pair-fed rats and free-feeding controls is the contribution of undernutrition **p=0.002; the difference between the colitic group and the pair-fed group is the contribution of inflammation. Values are mean ± SD. Taken from Ballinger *et al* Gut 2000 [123]

The importance of IL-6 to growth in humans has also been demonstrated. IL-6 expression is increased in CD, and circulating levels parallel disease activity [126-128]. In humans, the IL6-174 G/C promoter polymorphism affects IL-6 transcription, with the GG genotype inducing the greatest levels over GC or CC genotypes [129, 130]. Sawczenko *et al* found that among 153 children of Northern European, Caucasian origin, at diagnosis those with the IL-6 GG genotype were more growth retarded than patients with other genotypes [125].

1.3.4.2 Cytokines and the GH/IGF-1 axis

Active inflammation is associated with low circulating IGF-1 levels. Rats with TNBS-induced colitis had lower circulating IGF-1 levels than their healthy counterparts [123]. The same has been demonstrated in children not only with CD, but also in other conditions of chronic inflammation [131-134]. Treatment of the inflammation in CD results in an increase in IGF-1 levels [133, 135]. For example, treatment with an enteral diet causes a reduction in cytokines such as IL-6, and an increase in IGF-1 within 3 days [136].

Cytokines have several points in the GH/IGF-1 pathway that they can act (see Figure 1.5): signal transduction of GH at the hepatocyte level, changes in IGFBP levels, or through direct effects on the growth plate. Although it is known that infusion of a cytokine antibody in animal models enhance growth, it is difficult to know at what level this is occurring [124]. Even *in vitro* growth plate chondrocyte experiments are difficult to interpret. Cells at the growth plate might express both cytokines and IGF-1 acting in an autocrine fashion, thus making it difficult to distinguish between the direct effects of cytokines from their actions on GH or IGF-1 signal transduction [53, 89].

There is evidence to demonstrate that inflammation causes GH insensitivity at the level of the hepatocyte. Levels of GH are normal in children with CD when measured in the urine [137], including after stimulation testing [138]. Suppressor of cytokine-inducible signalling protein (SOCS) are a group of proteins that contribute to the negative regulation of the growth promoting actions of GH. It has been shown *in vitro* that IL-6 induces SOC-3, inhibiting the signal transduction of the GH receptor, resulting

in deceased IGF-1 expression. TNF-α appears to lower IGF-1 by down regulating hepatic expression of GH receptor in mice by inhibiting Sp1 and Sp3 binding [139].

As previously discussed, IGF-1 binds to IGFBPs, which modulate its bioavailability. IGFBP-3, in combination with acid labile subunit (ALS), increases IGF-1 availability. These levels are decreased in active CD [133, 135], and increase in response to treatment as a patient enters remission [136]. IL-6 increases the proteolysis of IGFBP-3, and impairs the formation of the IGF-1/IGFBP-3/ALS complex, resulting in enhanced clearing of IGF-1 [140], and hence a shorter half-life [89, 141].

In vitro experiments investigating the effect of cytokines on the growth plate have produced conflicting results. In fetal rat metatarsal growth plates, IL-6 and IL-6 receptor-α decreased bone growth by 21% [142]. However this was not seen in postnatal mouse metatarsals [143].

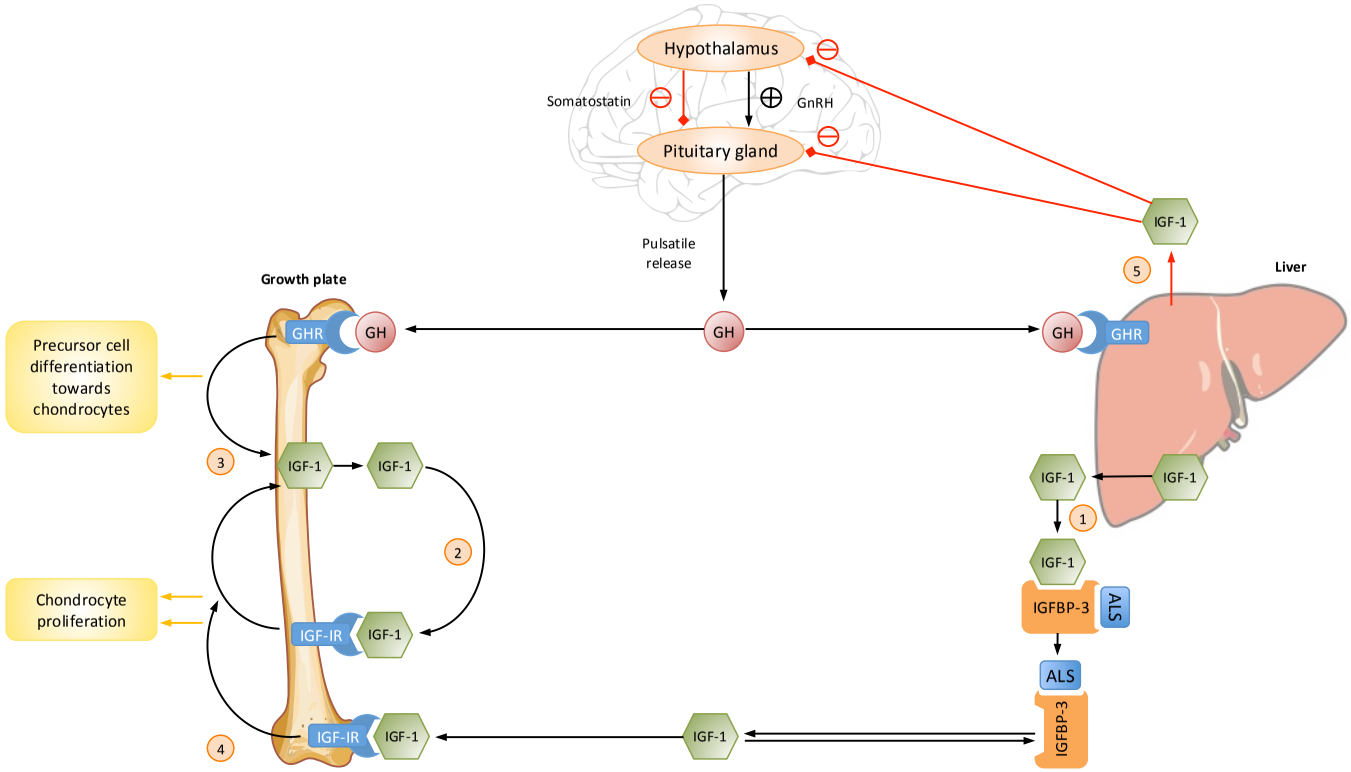

Figure 1.5 The GH/IGF-1 axis and its role in linear growth.

Hypothalamic release of GHrH stimulates the pulsatile release of GH from the pituitary gland. Somatostatin inhibits GHrH-stimulated release of GH. The GH receptor is expressed throughout the body, including within the liver and at the growth plate. GH binds to the extracellular domain of the GH receptor and induces upregulation of anabolic target genes, including IGF-1. The majority of circulating IGF-1 forms a ternary complex with IGFBP-3 and ALS. IGF-1 acts in an endocrine fashion (1) and an autocrine and paracrine fashion (2). GH also contributes directly to linear growth by inducing differentiation of precursor cells within the growth plate towards chondrocytes (3). IGF-1 stimulates mitosis of epiphyseal chondrocytes (4) and also mediates negative feedback of GH (5).

1.3.4.2 Delayed puberty

Puberty is the period of time during which adolescents reach sexual maturity and become capable of reproduction. It is associated with widespread physical changes, including the development of pubic and axillary hair, increase in fat and muscle tissue, and in females, menarche and breast development [144, 145]. One of the hallmarks of puberty is the adolescent growth spurt, during which there is a sudden acceleration of height velocity. Girls will on average reach their peak height velocity at 12 years of age. They average a peak velocity of 9cm / year, with a total gain of 25cm during the pubertal growth period. Boys, on the other hand, have their pubertal growth spurt at around age 14 years, and attain a peak height velocity of 10.3cm/yr, resulting in a gain of 28cm [146]. A delay in the onset of puberty will therefore delay the pubertal growth spurt. Pubertal delay, which is defined as the absence of breast development in girls or testicular enlargement in boys that is 2.0 or 2.5 SD later than the population mean [147], is very common in children with CD. It is especially prevalent in those in whom a remission has never been achieved or who have had frequent relapses during the prepubertal period [148]. Delayed puberty is more likely to occur in boys with CD [149], but is also commonly seen in girls. An American study by Gupta *et al* [150] compared the age at menarche of 34 girls with CD to that of 545 controls. They found that the median age of menarche in girls with CD was 13.9 years, as compared to 12.0 years in the control population. They also reported that this delay in menarche was closely related to the bone age of the patient.

The aetiology of delayed puberty in CD is multi-factorial. Undernutrition is a major contributing factor. It is well known that a low body mass index (BMI) results in pubertal delay [151, 152]. As has been mentioned previously, children with CD are often underweight for their age and gender [42], with reduced calorie intake [107]. However, some children with persistently active disease do not enter puberty even when nutritionally supplemented, suggesting that other factors apart from undernutrition contribute to the pubertal delay [148]. Animal and human studies have demonstrated a range of abnormalities is the hypothalamic/pituitary / gonadal / end-organ axis, including abnormalities of sex steroid synthesis [153], hypogonadotrophic hypogonadism [149] and abnormalities of sex steroid action [154]. It is likely that pro-inflammatory cytokines have an active role in this [89]. However, as it is still not known what triggers the onset of puberty in healthy children [147], this makes elucidating the reasons for delayed puberty in CD very difficult to ascertain [53].

1.4 Treatment of growth retardation in children with CD

CD is a chronic lifelong condition. It runs a relapsing and remitting course. There are several medical and surgical therapies available to manage the disease, but none are curative. The aim of management in all patients is to induce remission and then maintain the remission for as long as possible, while minimising side effects as a result of treatment [155]. As has been discussed, growth failure in active CD is associated with malnutrition, increased pro-inflammatory cytokines and decreased circulating IGF-1. All of these show improvement as disease goes into remission [54, 135, 136].

Therefore, the first principle of treatment for growth restrictive patients is synonymous with the optimal management of childhood CD [156].

It is now felt that the resolution of symptoms alone is not adequate in disease management. The importance of trying to achieve mucosal healing, or endoscopic remission to improve disease outcomes and progression has been demonstrated [157], and is now recognised as a fundamental aim to achieve.

1.4.1 Exclusive enteral nutrition:

Exclusive enteral nutrition (EEN) refers to the use of an enteral formula (either elemental or polymeric), which is given exclusively instead of a normal diet for a prescribed period of time to patients with active CD in order to induce remission. A benefit of EEN over other induction agents is that in addition to its proven efficacy, it has an excellent safety profile. It has also been shown to improve patient quality of life [158]. Indeed, the recent ECCO/ESPGHAN guidelines have recommended EEN as the induction therapy of choice [55].

Nutritional treatment with an elemental diet was initially included in the management of CD in adults to provide pre-operative nutritional support [159]. However, it was noticed that these patients showed an improvement in their symptoms [160]. Total parenteral nutrition had been previously used as form of therapy on the basis that it provided 'bowel rest' [161]. Following these observations, several clinical trials then proceeded to demonstrate the efficacy of EEN as a treatment for active CD in adults [162, 163]. In one such study, 27 patients with CD suffering from 32 exacerbations were treated for 4 weeks with an elemental diet [162]. By the end of the 4 weeks of

treatment, 29 of the exacerbations had entered remission both clinically and biochemically. Following from this, further studies have demonstrated its efficacy in children [164], and it is now well established as a treatment to induce remission in active paediatric CD. Although initial studies used elemental formula, further studies have since shown that the more palatable polymeric diet is just as efficacious [165] [166] [159]. Despite this, the pattern of EEN use differs considerably worldwide. EEN is the most commonly therapy to induce remission in newly diagnosed children with CD in the UK, with CS treatment given in cases of non-response [167]. The same is seen in many other European countries, including Sweden [168] and the Netherlands [169]. However, an international survey of 167 paediatric gastroenterologists worldwide found that only 4% of North American gastroenterologists used nutritional therapy frequently, in comparison to 62% of Western European gastroenterologists. This can be explained by differences in perceived efficacy of EEN between countries (78.7% of Western European practitioners felt that EEN was effective compared to only 45.6% of Americans), and the commonly held belief that patients will struggle to comply with treatment [170-172].

1.4.1.1 Efficacy of EEN in inducing remission:

Studies have shown that EEN induces remission in up to 85% of children with newly diagnosed CD [173-176]. One of the first studies to show the efficacy of EEN treatment in children was performed by Sanderson *et al* in London in 1987 [164]. 17 children with active small bowel CD were randomised to receive either a treatment with a

normal diet and high dose corticosteroids (CS) (2IU/kg/day intra-muscular adrenocorticotrophic hormone for 5 days followed by prednisolone 2g/kg/day (maximum 30mg/day) with a reducing regimen started after 3 weeks), or EEN with an elemental diet for 6 weeks followed by a 6 week food reintroduction programme. This study showed that the disease activity of the children in both groups significantly improved, with no difference being seen between groups in disease activity index (as assessed using the Lloyd-Still disease activity index score [177]), ESR, CRP or albumin concentrations. This demonstrated that elemental diet was as effective as CS in inducing remission in patients with active CD of the small bowel. Further studies have also confirmed these findings [178-180].

Although EEN has been shown to be efficacious in the treatment of CD, there is a question as to whether it should be used as the preferred primary therapy in the induction of remission. In a Cochrane review comparing efficacy of induction between EEN and CS in both adults and children, meta-analysis of 6 trials containing 352 patients in total (192 of whom received EEN and 160 CS, respectively) yielded an odds ratio of 0.33 (95% confidence interval [CI] 0.21%-0.53%) in favour of CS therapy [159]. However, many paediatric studies were excluded from this study due to questions about the methodology used; a decision that has since been questioned [181]. Two meta-analyses looking specifically at enteral nutrition therapy to induce remission in children with CD have found no difference in remission rates between treatment with CS and treatment with EEN [182, 183]. One by Heuschkel *et al* [182] examined the pooled results of 5 randomised controlled trials (RCTs) and two non-randomised trials, including 194 children in total. This found that enteral nutrition was as effective at

inducing remission as CS, with a relative risk (RR) of 0.98 (95% confidence interval (CI) 0.73 – 1.33) . The other meta-analysis contained 7 RCTs comparing EEN to CS use for induction of remission, comprising of a total of 204 patients; 100 treated by CS and 104 by EEN [183]. No difference was found in remission rates between those treated with EEN or CS (risk ratio 0.96 (95% CI 0.6 to 1.14) v 0.97 (95% CI 0.68 to 1.4), although the authors note that there were numerous important differences in trial design, including duration of treatment (3-10 weeks), disease location, disease duration (newly diagnosed v chronic), additional treatment, method of administration (oral v NGT) and type of formula used. This makes interpretation of results difficult. The authors conclude that a large study is needed to provide a definitive answer as to whether EEN is superior to CS treatment for the induction of remission, although they state that the sample size needed to conclusively answer this question (80% power at 5% significance level) would be 3000 children per group [183]!

It was initially thought that EEN is more effective in the treatment of ileal disease than colonic disease [184, 185]. A study by Afzal *et al* comparing response to EEN in 65 children with CD at different disease locations found a significantly lower remission rate in the colitic patients as compared to patients with ileocolonic or ileal disease (50% in the colitic group compared to 82.1% in the ileocolonic and 91.7% in the ileal groups respectively; p=0.021) [186]. This finding was confirmed by endoscopy and biopsy results where the colitic group did not show the same post-treatment improvements seen in the ileocolonic group**.** One possible explanation for this is that aetio-pathogenic mechanisms may differ depending on disease location, resulting in variation in responsiveness to treatment [172]. However, different findings are

reported in other studies. An Australian cohort study found no relationship between disease location and response in 24 children with CD treated with EEN [173]. In addition, a recent study from England found no significant difference between remission rates achieved by children with isolated small bowel disease as compared to those with isolated colonic disease (10/13 (77%) v 15/19 (79%) respectively, p=0.88) [187]. In fact, the lowest remission rate was noted in the isolated terminal ileal disease group (1/4, 25%), but as this group only contained very small numbers, this result needs to be interpreted with caution.

1.4.1.2 Direct anti-inflammatory action

Treatment with EEN has been shown to suppress inflammatory markers and increase levels of anti-inflammatory cytokines and growth-stimulating factors. A study looking at the anti-inflammatory effects of EEN in 12 children treated with a polymeric diet found that the ESR and IL-6 levels started to fall as early as day 3 into treatment, with the PCDAI and CRP decreasing by day 7 [136].

It has also been shown that EEN results in mucosal healing and a decrease in mucosal pro-inflammatory cytokine production [188, 189]. Therefore, it is likely that it also exerts direct anti-inflammatory effects. This is especially likely given that the epithelial surface interacts directly with luminal contents [105] and contributes to innate immune responses, leading to activation of other immune responses as required. It may be that EEN works by radically altering the luminal environment to such an extent

that it varies the signal from the intestinal epithelium to the mucosal immune system [190]

1.4.1.3 Effect of EEN on growth and long term outcomes

The immediate beneficial effects of EEN to growth have been well documented. Indeed, the impact of EEN has been described as occurring only a few days into treatment [136]. Sanderson *et al* [164] found that the children treated with an elemental diet had a significantly better HV standard deviation score (SDS) at 6 months post-treatment compared to children treated with CS (+0.3 (SD 2.03) v -2.8 (2.5), $p<0.05$) (Figure 1.6). This occurred despite similar remission rates being achieved in both groups. Similarly, a further study in England compared height velocity in 24 children randomised to receive either a normal diet and prednisolone 2mg/kg/day (max 60mg) for 2 weeks followed by dose tapering or EEN with elemental feed for 4 weeks [109]. It was found that the mean HV SDS of patients receiving EEN at 6 months was +0.32 (SD 3.32) compared with -3.1 (SD 2.8) in the CS group ($p<0.05$). A Cochrane review of interventions for Crohn's disease related growth failure and two other paediatric meta-analyses of EEN have all concluded that EEN is superior to CS with regards to promoting growth in the first year after diagnosis [156, 183, 191, 192].

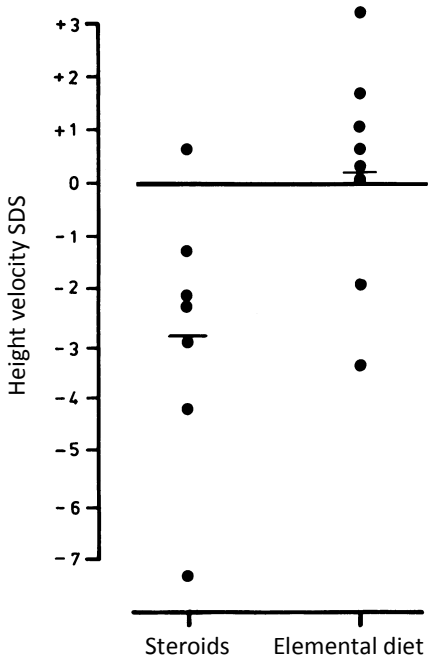

Figure 1.6 The effects of corticosteroids as compared to an elemental diet on height velocity over a 6 month period.
17 children with active small bowel CD were randomised to receive either a treatment with a normal diet and high dose CS (2IU/kg/day intra-muscular adrenocorticotrophic hormone for 5 days followed by prednisolone 2g/kg/day (maximum 30mg/day) with a reducing regimen started after 3 weeks) or EEN with an elemental diet for 6 weeks followed by a 6 week food reintroduction programme. After 6 months, the children treated in the CS group had significantly lower HV SDS than those in the elemental diet group (mean -2.8 vs +0.3; $p<0.05$). Taken from Sanderson *et al* 1987 Arch Dis Child [164].

However, less well documented are the long-term outcomes following induction therapy with EEN. There have been few studies published on this, and even fewer on the impact on growth. A study from England investigated the long-term outcomes of a cohort of paediatric patients with CD whose first treatment was EEN [193]. 79 patients were followed up for a maximum of 7 years (median 3 years, range 1-7 years) post-diagnosis. Out of 40 patients who responded to initial treatment with EEN, 62% of patients relapsed, with a median duration of remission of 54.5 weeks (range 4-312),

meaning that 25/40 (38%) of patients did not relapse during this time period. In addition, 21/44 (47%) of children started on EEN did not receive any CS during the study period. In those patients who did eventually require CS treatment, their use was postponed for a median 68 weeks (range 6-190) post-diagnosis. The investigators found that the patients who responded to EEN had improved weight *z* scores from presentation to 12 months, but a non-significant rise in *z* score for height. However, there is no longer-term height data reported. A recent retrospective Scottish study looked at outcomes in 109 patients over two years following induction therapy with EEN [194]. 58% (63/109) of patients relapsed during the follow-up period, with a median time to relapse of 6.5 months (Inter Quartile Range (IRQ): 7 months). As has been reported previously, patients who responded to EEN showed significantly better HV at 6 months than those who did not. However, this difference was not maintained over the 2 year follow-up period. Height *z* score showed no significant improvement from -0.6 at baseline (SD 1.1) to -0.8 (1.0) at 24 months. However, these are the only studies that have reported growth outcomes over a longer period than a year in children with newly diagnosed CD treated for induction of remission with EEN.

1.4.2 Thioprines

Azathiorpine (AZA) has been used as maintenance therapy in CD for over 40 years [195-197]. AZA is a pro-drug. It is converted to mercaptopurine (MP) via glutathione-S-transferase (GST). The MP is subsequently metabolised via three competing pathways (Figure 1.7) [198, 199]. One of these pathways leads to degradation by aldehyde oxidase (AOX) and xanthine oxidase (XO), resulting in the inactive metabolite 6-thiouric

acid, which is excreted in urine. A second pathway results in methylation by thiopurine-S-methyltransferase (TPMT) to form 6-methylmercaptopurine (MMP). The immunosuppressive properties of AZA/6-MP are mediated via the third metabolic pathway: activation through the purine salvage pathway to thioguanine nucleotides (TGNs). These result in apoptosis and inactivation of T-lymphocytes

The gene for TPMT is located on chromosome *6p22.*3 [200]. Inheritance of a combination of alleles either for high or low activity determines activity levels [199]. Low TPMT activity has been observed in up to 11% of the Caucasian population, with 0.3% having negligible activity [201]. Interracial variability is also seen, with on average the North American Black population having lower TPMT activity than Caucasians [202]. The importance of knowing a patient's TPMT activity level before starting thiopurine treatment is because deficiency causes preferential metabolism towards TGNs, which can result in severe myelotoxicity. However, importantly, there are also a cohort of patients who are hypermethylators ('shunters') of thiopurines, resulting in low TGN concentrations accompanied by increased MMP [203]. This carries an increased risk of hepatotoxicity [199]. Another major side effect associated with thiopurines is pancreatitis, which is unrelated to TPMT activity [204, 205]. Patients may also discontinue its use due to other side effects, including GI disturbance and rashes. However, children are reported to have lower rates of intolerance and subsequent discontinuation because of adverse effects than adults [50].

Although a 2010 Cochrane review concluded that AZA / 6-MP are effective therapy for inducing remission in adult patients with active CD [206], in children, thiopurines alone are not recommended as induction therapy. However, they are recommended by

ECCO / ESPGHAN as an option for maintenance of CS free remission in children at risk of poor disease outcome [207]

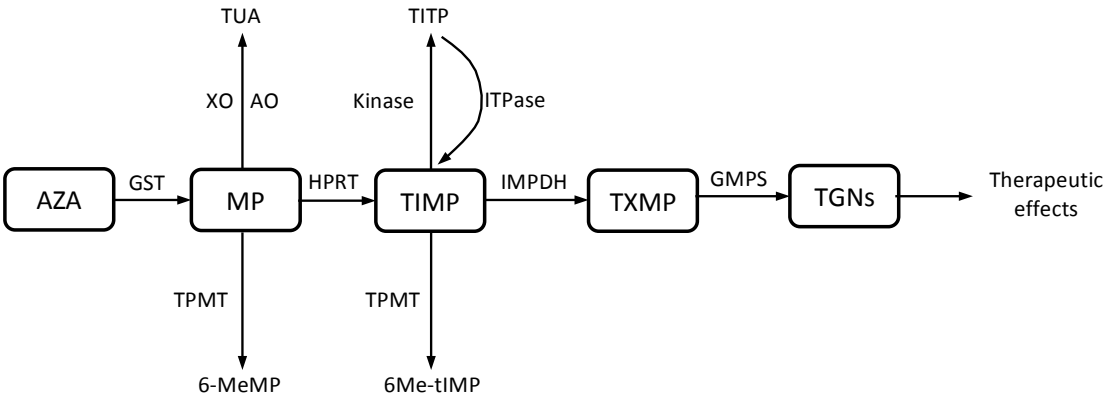

Figure 1.7 The metabolic pathway of thiopurines.
6-MeMP: 6-methylmercaptopurine; 6Me-tIMP: 6-methyl thioinosine monophosphate; AO: Aldehyde oxidase; AZA: Azathioprine; GMPS: Guanosine monophosphate synthetase; GST: Glutathione-S-transferase; HPRT: Hypoxanthine guanine phosphoribosyltransferase; IMPDH: Inosine monophosphate dehydrogenase; MP: Mercaptopurine; TGN: thioguanine nucleotide; TIMP: Thioguanine monophosphate; TITP: Thioinsine triphosphate; TPMT: Thiopurine S-methyltransferase, TUA: Thiouric acid, TXMP: Thioxanthosine monophosphate; XO: Xanthine oxidase [199, 208]

1.4.2.1 Efficacy of thiopurines

Markowitz *et al* (2000) conducted a prospective, double blind, placebo-controlled multicentre randomised controlled trial (RCT) whose primary objective was to determine whether 6-MP decreased the need for CS in children with newly diagnosed moderate-to-severe CD [209]. The patients randomised to the 6-MP treatment arm had lower cumulative CS use over 18 months than the control arm. In addition, the relapse rate in the remitters in the 6-MP arm was only 9%, as compared to 47% of

controls (p=0.007) after induction of remission with CS. Other studies have also demonstrated the efficacy of thiopurines in children with CD. An Italian retrospective study of 123 children with CD reported a 70% efficacy rate of azathioprine [210]. In adults, the 2009 Cochrane review concluded that thiopurines had a positive effect on maintaining remission. 8 trials, comprising 550 patients in total were included in this meta-analysis, 208 of whom were on AZA. The overall remission rate at 1 year in patients treated with AZA was 71% (95% confidence interval (CI) 64-77%), as compared to 55% (95% CI 49-61%) for placebo [211].

However, recently the efficacy of thiopurines has been called into question [212], especially as evidence demonstrating the beneficial effects of anti-TNF-α medication, such as infliximab (IFX) is increasing [213, 214]. The much anticipated SONIC trial [215] in adults found that in 508 treatment-naive adults with moderate-to-severe CD, only 30% of patients treated with AZA alone went into CS-free clinical remission at week 26, in comparison to 44.4% of patients receiving IFX alone and 56.8% of patients receiving combination therapy (p=0.006 and p<0.001 in comparison with patients receiving IFX and combination therapy, respectively). Despite the suggestion that in the case of moderate-to-severe CD, earlier introduction of thiopurines further enhances their efficacy [216-218], similar findings have also been seen in children. The recent RISK study [219] looked to compare the effectiveness of early treatment with anti-TNF, as compared to early immunomodulator (IM) use in children with CD. 552 children diagnosed between 2008 and 2012 at 28 North American gastroenterology centres were included. Three groups were identified: early anti-TNF, early IM, or no early immunotherapy, and outcomes were measured at the end of 1 year. The results

showed that early anti-TNF treatment was more efficacious than early IM treatment (85.3% vs 60.3% in remission, p=0.0017). Interestingly, this study also found that there was no statistically significant difference in remission rates of those treated with early IM as compared to no immunotherapy use at all (60.3% vs 54.4% in remission, p=0.49).

1.4.2.2 Impact of thiopurine therapy on growth in children with CD

Very few studies have investigated the effects of AZA or 6-MP upon growth, and fewer still have demonstrated a beneficial effect [156, 220]. Even Markowitz's RCT did not find any difference in linear growth between the 6-MP and placebo group after 18 months [209]. The one study that has demonstrated either maintained or improved height *z* scores in response to AZA has used a much higher dose of treatment (3mg/kg) than that usually recommended (2mg/kg) [221]. By contrast, multiple studies have demonstrated the beneficial effects of anti-TNF treatment upon growth, both using IFX [222-224] and adalimumab [225, 226]. Indeed, the RISK study found that only the patients treated with IFX showed a marked increment in height SDS 1 year after therapy. No such improvement was seen in the thiopurine group [219]. One possible explanation for this difference is the impact of mucosal healing. Anti-TNFs have been shown to result in mucosal healing [222, 227, 228]. However, this is not seen with thiopruines. In the SONIC trial, only 16.5% (18/109) patients in the AZA alone arm showed mucosal healing at week 26, as compared to 30.1% (28/93) patients receiving IFX alone (p=0.02). However, it has to be noted that the combination treatment arm of the trial had the best mucosal healing rates (43.9%; 47/107 patients; p<0.001) [215].

This raises the question as to whether thiopurines would be more effective if used in combination with a treatment that was known to promote mucosal healing, such as EEN, as opposed to usage alone or in conjunction with CS, which are known to produce clinical remission, but not mucosal healing [174]. However, no such studies have been performed as of yet.

1.5 Current outcomes and future directions

Even with more therapeutic options and the recognition that children with growth failure require more aggressive management [55], growth failure remains a significant problem in the management of paediatric CD. Sawczenko *et al* found that although height SDS improved in children with CD from diagnosis, the mean final height deficit compared with target height was -2.4 cm (95% CI -3.6 to - 1.2), with 19% of patients achieving a final height less than -8.0cm below target [101], An American multicentre study investigated height at diagnosis, at 1 year and 2 years in 176 children diagnosed with CD. Mean (SD) height z score at diagnosis was -0.49 (1.2), and this remained at -5.0 (1.2) at 1 year post-diagnosis, and -0.46 (1.1) at two years. Interestingly, this study described no sustained benefits in terms of growth with either immunomodulator or IFX use [220].

1.5.1 Intervention on the GH/IGF-1 axis

Improving growth by controlling inflammation is the absolute aim of treating CD. However, despite the best efforts of clinicians, there are some children whose disease

remains intractable to treatment. There is only a very limited time period in which we can intervene to effect growth, and so it would be of considerable benefit if we could provide an adjuvant therapy specifically focused on growth.

1.5.1.1 Recombinant human GH (rhGH)

rhGH therapy is already widely used with excellent efficacy in children with growth impairment due to pathology other than GH deficiency, including Turner's syndrome and chronic renal failure [76, 77]. As has been discussed above, inflammatory states are associated with a functional GH resistance. However, rhGH has also been used to good effect in growth retarded children with conditions of active inflammation, such as cystic fibrosis and juvenile idiopathic arthritis [79, 229]. The use of GH was first described in children with CD in 1974 [230]. Since then, there have been several trials, both open labelled and RCT, investigating the impact of rhGH treatment upon growth in children with CD. The results have been mixed, but overall have indicated potential efficacy [231-233]. In a study from Cincinnati, USA [234], 20 children were randomly allocated to receive either GH and CS or CS alone for 12 weeks, with an extension phase of 52 weeks for the children who responded well to GH. HV significantly improved in the group treated with GH, with mean (95% CI) height z scores increasing from -1.1 (-1.6 to -0.6) to -0.4 (-1 to +0.2) during the 52 week extension period ($p=0.004$). However, it should be noted that the inclusion criteria for the GH treatment extension phase was that the patient should either have achieved remission (PCDAI<10) or have mild disease activity (PCDAI<30), thus excluding patients in whom

inflammation-related GH resistance might be expected. The same was seen in a Scottish RCT comprising of 22 children with CD and growth failure (height SDS < -1 with HV SDS <-1). Although the group treated with GH showed a significant increase in HV after 6 months ($p<0.001$), the mean PCDAI at entry was only 12.5 [235]. No trials have been performed looking at the efficacy of GH in patients with active inflammation only.

1.5.1.2 Recombinant human IGF-1 (rhIGF-1)

Another potential treatment for growth failure in paediatric CD, especially when a degree of GH resistance exists, is rhIGF-1. Patients with GH insensitivity syndrome (GHIS) have been successfully managed with rhIGF-1 therapy. Chernausek *et al* published a report examining the long-term efficacy and safety of rhIGF-1 therapy for short children with GH insensitivity syndrome. Entry criteria included age > 2 years, height and circulating IGF-1 SDS <-2 for age and gender. RhIGF-I was administered subcutaneously in doses between 60-120 mg/kg twice daily. The results were very encouraging. Height velocity increased from 2.8 cm/yr on average at baseline to 8.0 cm/year during the first year of treatment ($P<0.0001$). Although HV was lower in subsequent years, it still remained above baseline for up to 8 years [236]. The main adverse effect seen with treatment was hypoglycaemia, followed by injection site lipohypertrophy. However, although adverse effects were common, they were very rarely of sufficient severity to interrupt or modify treatment [236, 237]

There have been no studies on the effect of rhIGF-1 treatment in children with CD. However, it has been used in an animal study. Rats with TNBS-induced colitis grow poorly. This is associated with increased circulating IL-6 levels and low IGF-1. Exogenous IGF-1 given to these rats significantly enhanced their growth ($p<0.0001$) [123]. This did not occur as a result of a reduction in the severity of intestinal inflammation or improvement in nutritional status in the treated group. However, it must be noted that these rats still did not grow as well as the healthy controls.

Restoring IGF-1 levels in children with CD who may have some endogenous production is not straightforward, because high levels of IGF-1 might be harmful. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have double the incidence of colon cancer [238, 239]. Although the exact pathogenesis of this is unknown, it is thought to be directly related to this sustained increase in GH-IGF-1, either by facilitating the growth of pre-existing tumours, or by initiating their development [240, 241]. This is an important consideration in children with CD, as inflammation is also a risk factor for intestinal cancer. Therefore, for rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess.

1.5.1.3 Mathematical modelling

Mathematical and simulation modelling have long been used in industries such as aeronautics to design and develop products more efficiently [242]. They are now being increasingly used in the pharmaceutical industry in the process of drug

development [243-246]. Population pharmacokinetic (PPK) models can be used at each stage of drug development [247]. They enable drug-dosing regimens to be individualised to a patient based upon specific patient parameters (e.g. disease activity) [248-250]. This is of particular use in drugs that may be harmful in concentrations above the therapeutic range. It is also very useful in patient populations in whom the number of samples to be collected per patient is limited due to medical and ethical reasons, such as critical care patients and oncology patients [248, 251, 252]

Within paediatrics, PPK models are being increasingly used, most commonly in the area of oncology [253-256]. For example, an American PPK study has been used to recommend a piperacillin/tazobactam dosing regimen in children with febrile neutropenia [253]. In addition, PPK models are being increasingly used in other areas of paediatrics, such as HIV medicine [257], and determining medication dosages post-solid organ transplants [258, 259] or post- cardiac surgery [260]. Amongst other things, a PPK model has been used to investigate the clearance of morphine in children with sickle cell disease [261]. PPK has been used to recommend a dosing regimen of ϵ-aminocaproic acid in children undergoing posterior spinal fusion surgery [262].

1.6 Conclusion

The incidence of paediatric CD is increasing worldwide, and is a significant healthcare concern. As we have no cure for CD, the aim of management is to induce and maintain remission for as long as possible, while minimising side effects. This is especially the

case in our paediatric patients, who potentially have decades of treatment ahead of them.

The importance of promoting growth in children with CD is well recognised, as demonstrated by its inclusion both in the Paediatric Crohn's Disease Activity Index, and the new Paris disease classification. In the new ECCO / ESPGHAN guidelines for the management of paediatric IBD, poor growth has been clearly stated as a poor prognostic factor.

The recent years, there has been considerable progress in our knowledge of the underlying mechanisms causing this growth impairment. Whereas growth failure was Initially thought to be purely a result of undernutrition, we now understand that the interplay between pro-inflammatory cytokines and the GH/IGF-1 axis also has an impact. The importance of mucosal healing both for disease progression and growth is now appreciated.

However, despite these advances in our knowledge, and increases in our treatment repertoire, linear growth retardation remains a significant problem for children with CD, with many children still not achieving their growth potential. This introduces two important questions into our management of children with CD and growth failure: First; are we currently utilising the best possible treatment options available to promote growth? Secondly; are there any new potential treatment options available to us that have not previously been considered?

1.7 Hypotheses and aims

My over-arching hypothesis was that in children with CD, when inflammation is successfully treated, then this will result in enhanced linear growth. This was understood in a series of multiple hypotheses.

1. First, in section 3.0 ('The prevalence of growth retardation at diagnosis in children with CD'), I hypothesised that despite advances in the awareness of paediatric CD and its increasing prevalence worldwide, more than 50% of children with CD will experience symptoms for ≥6 months at the time of diagnosis, and that height SDS at diagnosis will correlate with symptom duration.

   To study this, I retrospectively reviewed data collected from children with CD diagnosed at our centre between 2006-2011, with the following aims:

   Aim 1: To determine height SDS at the time of diagnosis.

   Aim 2: To determine the time to diagnosis in patients, and whether this is affected by presenting symptoms, disease phenotype or activity, age or gender.

   Aim 3: To assess whether any of the above factors has an impact on height SDS at the time of diagnosis.

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2. Secondly, in section 4.0 ('Long term clinical and growth outcomes following initial induction of remission with exclusive enteral nutrition'), I hypothesised that children who entered complete remission following treatment with EEN would show better growth over a 5 year follow-up period than non-responders, due to the presence of a sustained CS free clinical remission and better clinical outcomes.

To study this, we identified a cohort of children newly diagnosed with CD at our centre between 01/01/2003-31/12/2008 that received EEN as induction of remission therapy, and had 60 months of follow-up data available. Data was compared between responders to EEN and non-responders at the following time points: 0, 1 and 5 years. To complete this study, I first examined the tolerance, compliance and efficacy of EEN before studying clinical outcomes and growth. I had the following aims:

Aim 1: To assess tolerance and compliance to EEN.

Aim 2: To assess the efficacy of EEN in the induction of remission in these children.

Aim 3: To compare the clinical course and outcomes between patients who entered remission following EEN to those who did not.

Aim 4: To compare growth over 5 years between patients in whom remission was induced with EEN to those who did not enter remission with EEN.

Aim 5: To determine the effects of CS treatment upon growth in these children.

3. Third, in section 5.0 ('Response of Crohn's disease activity to thiopurines and its impact upon linear growth'), I hypothesised that thiopurine and/or Infliximab therapy in children with CD would result in increased height SDS over a 12 month period, due to a decrease in disease activity and CS use.

   To study this, we identified a cohort of children (<17 yrs of age) with CD who were started on a thiopurine treatment between 01/01/2007-31/12/2010 at our centre, and had 12 months of follow-up data available. Data was recorded at 0,3,6 and 12 months, with the following aims:

   Aim 1: To assess the tolerance of children to thiopurines.

   Aim 2: To determine the response of CD activity to thiopurine therapy.

   Aim 3: To assess the impact of thiopurine therapy on growth in these patients.

   Aim 4: To assess the impact of anti-TNF therapy on growth in these patients.

4. Finally, because growth retardation is still a problem in paediatric CD, even with the development of more effective anti-inflammatory medications, in section 6.0 ('Mathematical modeling to restore circulating IGF-1 concentrations in children with CD-induced growth failure: a pharmacokinetic study'), I hypothesised that children with active CD and growth retardation would have low circulating IGF-1

levels, and that these levels could be restored and maintained within the normal range by exogenous administration of recombinant human IGF-1.

To study this, I performed an open label pharmacokinetic study in 8 children aged > 10 years with known CD and growth retardation, investigating the effects of once daily and twice daily subcutaneous injections of rhIGF-1. The results of this study were used to develop a mathematical model to determine a dosing regimen that would result in IGF-1 concentrations no higher than +2.5 SDS for each patient's age and gender. I undertook the following aims:

Aim 1: To assess baseline IGF-1 and IGFBP-3 levels in children with active CD and faltering growth.

Aim 2: To assess the tolerance of children with active CD to rhIGF-1.

Aim 3: To determine whether exogenous administration of subcutaneous rhIGF-1 can restore IGF-1 levels to the normal range in children with CD.

Aim 4: To develop a new mathematical model to determine an individualised dosing regimen to restore circulating IGF-1 concentrations to within the normal range for age.

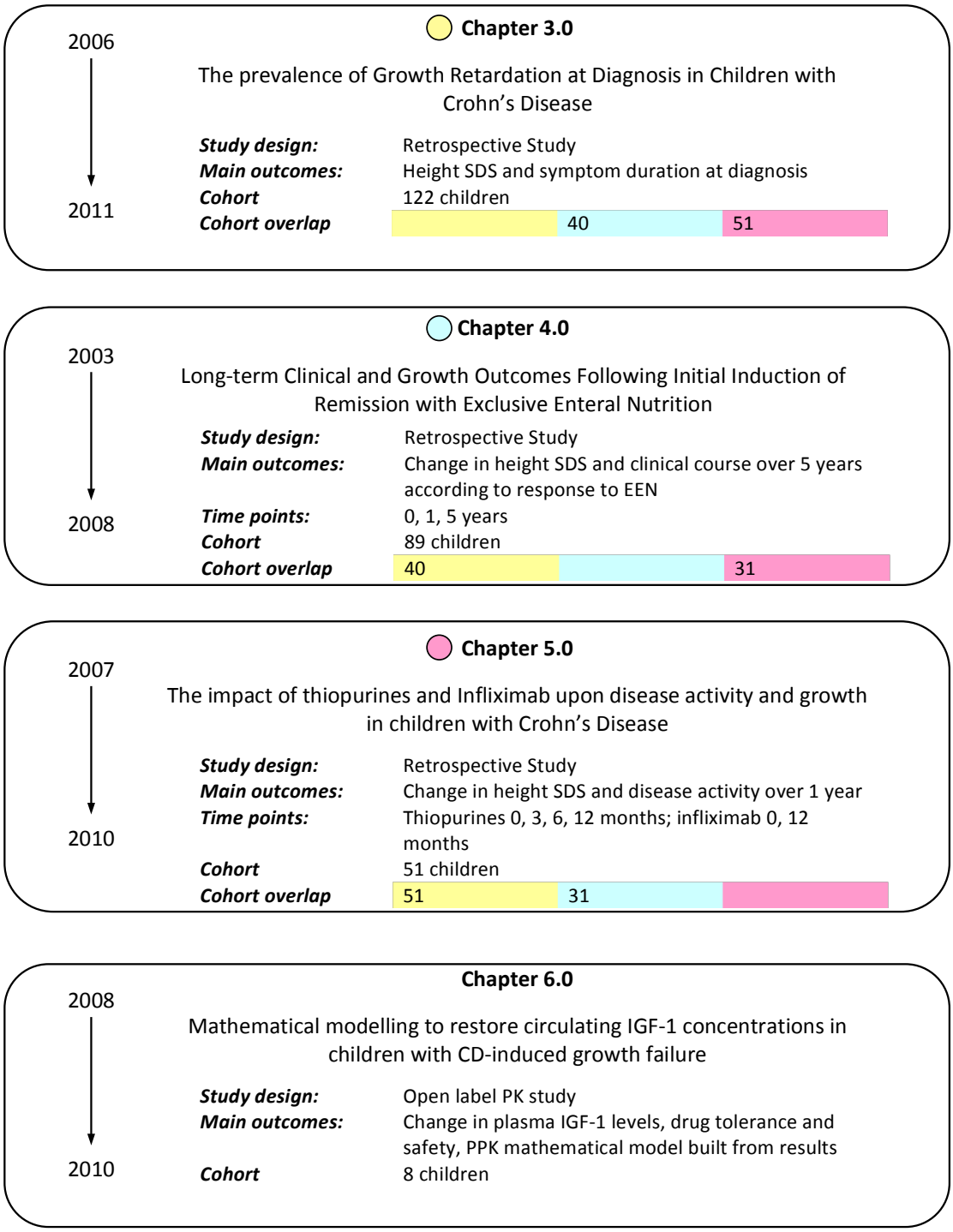

Figure 1.8 Summary of results chapters.
Cohort numbers and overlapping patients between studies colour coded according to chapters.

2 Materials and methods

2.1 The prevalence of growth retardation at diagnosis in children with CD diagnosed between 2006-2011 at Barts Health NHS Trust

2.1.1 Study design

Data at diagnosis from children diagnosed with CD between 2006 and 2011 were retrospectively collected. All children were diagnosed at Barts and The London Children's Hospital, even if they initially presented at another hospital. This hospital serves as a tertiary referral centre for East London and the surrounding area, including Essex and parts of Kent. Children who received their initial diagnosis elsewhere before being transferred to our centre for subsequent management were excluded. The 2005 Porto criteria were used for diagnosis [46]. Data were collected from The Royal London Hospital Inflammatory Bowel Disease database (InfoFlex), electronic patient records (EPR) and patient notes. The InfoFlex IBD Patient Management System is an information-processing tool that was specifically designed for the modelling of patient care pathways. Data were inputted in to InfoFlex by clinicians at the time of a patient's hospital visit. A later version was developed in collaboration with the IBD Registry Board in order to collect data for the UK IBD Registry. This is available to all trusts and boards across the UK. This allows the data collected for IBD to be shared with any other clinical specialities within the trust or board already using InfoFlex (e.g. endoscopy or cancer services). The application provides clinicians with a disease-specific electronic patient record, work list generation and local reporting tools, as well

as enabling automated uploading of anonymised data to the national IBD Registry [263, 264].

All data were arranged and processed for handling using Microsoft™ Office Excel in compliance with the Barts Health NHS Trust information governance regulations.

The demographic data collected for this component of the thesis included: age at diagnosis, ethnicity and gender. Disease location was defined using the Montreal classification [48] as the Paris classification had not been published when the research was begun [49]. Details of systemic markers of disease activity (Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), platelet (PLT) count and serum albumin (Alb)) were also collected. Each patient had his or her Pediatric Crohn's Disease Activity Index (PCDAI) calculated. The PCDAI is a validated index of a child's disease activity which incorporates symptoms, examination findings and laboratory results to form an overall assessment of the patient [56] (see Appendix 2).

2.1.2 Auxology data

The term 'auxology' derives from the Greek ' let it grow'. It refers to the science of somatic growth and development [265]. In our study, height and weight data were taken from the patient's first visit to the hospital, either in the outpatient department or, in the case of admission, as an inpatient on the ward. They were assessed using standard anthropometric techniques on calibrated scales. Weight was measured without shoes on and wearing light clothing. Height measurements were taken using a stadiometer. Children's shoes and bulky clothing were removed, as were any hair

ornaments if they interfered with the measurement. Patients were instructed to stand with their feet flat, together and against the wall, looking straight ahead with their chin parallel to the floor, arms by the sides of their trunks with palms flat on thighs, and shoulders levels. The back of the head, scapulae, buttocks and heels were against the vertical backboard of the stadiometer (see Figure 2.1). Height and weight data was then converted to SDS using an auxology SDS calculator (http://www.phsim.man.ac.uk/SDScalculator), which converts values to SDS according to Standardised UK Growth charts reference data.

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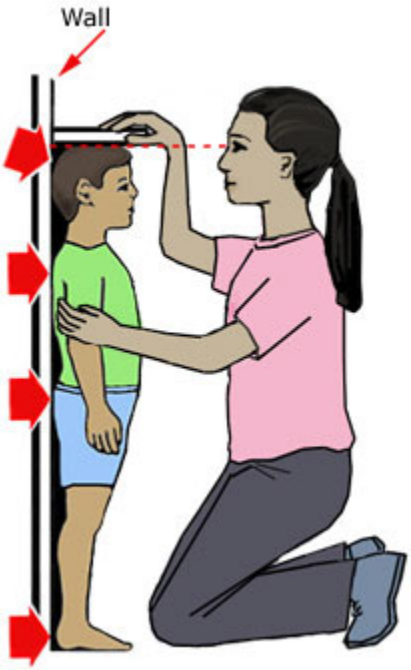

Figure 2.1 Accurate measurement of a patient's height using a stadiometer.
Shoes should be off, and feet together with a child's heels, buttocks, scapulae and back of head touching the backboard. The chin should be parallel to the floor. Taken from the Centres for Disease Control and Prevention **[266]**

2.1.3 Statistical analysis

Statistical analysis was performed using SPSS (version 20; SPSS Inc, Chicago, IL) and Prism (version 5; GraphPad, San Diego, CA) software. Categorical data was compared between groups using the chi-squared test, unless more than 25% of cells contained counts of 5 or less, in which case the Fisher's Exact test was used.

Continuous data are presented as means ± SD or medians and interquartile ranges (IQR) or range as appropriate for the distribution normality. The following were investigated to check whether data was normally distributed: skewness and kurtosis *z*-values (should be between -1.96 to +1.96 if data is normally distributed), the Shapiro-Wilk test p-value (>0.05 in normally distributed data). In addition, histograms, normal Q-Q plots and box plots were used to visually indicate that the data was approximately normally distributed. Continuous parametric data were compared between groups using a Student's t-test. Non-parametric data were analysed using Mann Whitney U test. For multiple group comparisons ANOVA was used to simultaneously compare group means with continuous parametric data, and Kruskal-Wallis with non-parametric data.

For linear regression models the dependent variable was either normally distributed or appropriately transformed prior to model inclusion. A General Linear Model (GLM), specifically analysis of covariance (ANCOVA), was used for multivariate analysis in order to describe associations between a dependent variable, such as time to diagnosis or height SDS at diagnosis, and covariates which included both categorical and continuous data e.g. age, gender, ethnicity, disease location and behaviour, presenting

symptoms and disease activity. If continuous data was non-parametric then it was appropriately transformed (e.g. Log10) and the distribution tested for normality using the previously described methods before inclusion in the multivariate model.

Linear regression investigates a linear relationship between two variables: x (predictor, independent) and y (dependent, outcome). Simple linear regression was used with parametric data. Alternatively a non-linear regression model was used with non-parametric data.

Correlation analysis is concerned with measuring the degree of association between two variables, x and y. The Pearson correlation coefficient was used in the case of parametric data, and Spearman's rank correlation with non-parametric data.

Statistical significance was always two-tailed and defined as a p value of less than 0.05.

2.1.4 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 UK National Research Ethics Service (NRES) guidelines [267, 268].

2.2 Long term clinical and growth outcomes following initial induction of remission therapy with exclusive enteral nutrition

2.2.1 Study design

This was a retrospective study conducted to determine the short and long term outcomes of using exclusive enteral nutrition (EEN) to induce remission in children with newly diagnosed CD, and to examine the resultant effects upon linear growth. Data were collected at the following time points: at diagnosis (i.e. baseline), at the time of completion of the course of EEN (6 weeks), and then at 1 year and 5 years post-diagnosis. All patients had 60 months of follow-up data available. This makes our study unique, as previously published data looking at long term outcomes either had a variable length of follow-up [193], or data for 2 years post-treatment only [194]

Data was collected retrospectively from The Royal London Hospital Inflammatory Bowel Disease database (InfoFlex see 2.1.1), electronic patient records (EPR), clinic letters and inpatient notes. The data collected for this study included demographic details, disease location and behaviour, serial measurements of height and weight, initial therapy used to induce remission and concomitant medical therapy and systemic markers of disease activity: ESR, CRP and platelet (PLT). If the data were available to do so, the PCDAI was also calculated (see Appendix 2). Details of relapses and treatment escalations were recorded. As the patients included in this study were diagnosed before the publication of the Paris classification [49], disease phenotype and location was stratified according to the Montreal classification [48]. Height and weight were assessed using standard anthropometric techniques (see above: Auxology, 2.12), and

were recorded on the same calibrated scales upon each child's visit to the outpatient department. Height and weight data were then converted to standard deviation (SDS) scores using an auxology SDS calculator (see section 2.1.2). In the case of the patients who progressed to the transition clinic (Young Adult IBD clinic) during the study period, their height at 5 years was taken from their last visit to the paediatric clinic as one of the criteria for transition to the young adult/transitional clinic is that patients have reached their final adult height before the time of transfer. The study end point was defined as the follow-up visit at the end of 5 years from the time of diagnosis.

All data were arranged and processed for handling using Microsoft™ Office Excel in compliance with the Barts Health NHS Trust information governance.

2.2.1.1 Patient selection

The study population consisted of paediatric patients (aged <16yrs) who were newly diagnosed with CD between 1st Jan 2003 - 31st Dec 2008 and treated at Barts and The London Hospital for Children, London, as these dates allowed for a full 5-year follow-up period. All children were diagnosed using standard endoscopic, radiological and histological diagnostic criteria. As all of the children were diagnosed between 2003-2008, the 2005 Porto criteria as recommended by the IBD Working Group of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) were used for diagnosis [46].

2.2.1.2 Inclusion criteria:

The children included in the study were those who received either EEN or CS at Bart's and London Hospital to induce remission after diagnosis and had follow-up data for 5 years at Barts and The London Hospital for children. This included patients who were transitioned from the paediatric clinic to the adult or transition clinics at The Royal London Hospital.

2.2.1.3 Exclusion criteria:

Children who were started on treatment to induce remission prior to transfer of management to Barts and The London Hospital for Children were excluded from the study. Other exclusion criteria were: use of medications to induce remission at diagnosis other than EEN or CS (e.g. antibiotics or 5-aminosalicylates alone), the presence of co-morbidities affecting growth (for example Turner's syndrome, chronic renal failure), use of immunosuppressive agents for other co-morbidities, and transfer of follow-up during the 5 years from Barts and The London Hospital for Children to other units or countries.

2.2.2 Treatment regime

EEN is the preferred primary induction therapy for newly diagnosed CD at this centre. The EEN regime and subsequent food reintroduction follows a standard protocol

(Appendix 3) [164]. However, the decision to use other therapies with consideration to intolerance or non-response to EEN was that of the treating physician. It was also the treating physician's decision to escalate treatments to immunosuppressive drugs should it be felt the patient required this.

The EEN prescribed was Modulen® (polymeric formula, Nestle, UK, see Appendix 4) given exclusively as the sole nutritional source for a period of 6 weeks. Up to a teaspoon of Nesquik® (Nestle, UK) was added to each cup to improve palatability. Other than this, water, sugar free chewing gum or mints were the only other things allowed. We encouraged patients to take EEN orally, but if there were concerns regarding inadequate intake, nasogastric (NG) feeding was instigated early. Daily volumes of formula were prescribed based upon the child's estimated energy requirement (EER), aiming for 120% EER in a newly diagnosed patient [55]. The EER for our patient sample was between 1800 - 3300 Kilocalories, depending upon the child's age and gender. The standard concentration of Modulen is 1kcal /ml, therefore the volume required was 1.8 – 3.3 L/day, according to the child's requirements. The Modulen® program at The Royal London lasts 10.5 weeks in total: 3-4 days in hospital for establishment of EEN feeding, 6 weeks of exclusive Modulen® and then a four week weaning period with food reintroduction. A normal diet was gradually reintroduced with one type of food reintroduced every two days and concomitant slow reduction of formula volumes. The formula was reduced at a rate of 250-500ml/week. A paediatric dietician, who would meet with the patient at regular intervals, oversaw this whole process. Following discharge from hospital, patients were also regularly seen in the outpatient department.

2.2.3 Indices to evaluate response to EEN

The response to EEN was assessed using Physician's Global Assessment (PGA) [56]. The PGA is the scale (inactive, mild-moderate, moderate-severe) based on the clinical assessment of the physician [56, 269]. At the end or shortly after the cessation of EEN, patients were defined as responders or non-responders. Those who were classed as having inactive disease at this time were defined as responders, whereas those with mild, moderate or severe disease were categorised as non-responders to EEN. Paediatric Crohn's Disease Activity Index (PCDAI) was also calculated where all the information to do so was available. There is a strong correlation between PGA and PCDAI [56]. Relapse was defined as an increase in disease activity, as determined by PGA (from inactive disease to mild-moderate, or moderate-severe disease), with changes in symptoms and/or necessitating an escalation in the on-going therapy.

2.2.4 Statistical analysis

Statistical analysis was performed using SPSS (version 20; SPSS Inc., Chicago, IL) and Prism (version 5; GraphPad, San Diego, CA) software. Data are presented as means ± SD or medians and IQR or ranges as appropriate for the distribution normality and range. Categorical data was compared between groups using the chi-squared test, unless more than 25% of cells contained counts of 5 or less, in which case the Fischer's Exact test was used. Continuous data was tested for distribution normality (see section 2.1.3). Continuous data were compared between groups using a t-test or Mann

Whitney U test if the data was parametric or non-parametric respectively. ANOVA or Kruskal-Wallis was used for simultaneously comparing means in several groups if the data was parametric or non-parametric respectively. Paired data was analysed using a paired t-test for continuous parametric data and Wilcoxon signed-rank test if the data was non-parametric. Repeated measures were analysed using a one-way repeated measures ANOVA or Friedman test, depending upon data distribution normality Multivariate regression analysis (see section 2.1.3) was used to find any associations between measures of response and adjusted for covariates (including age, ethnicity, gender, disease location). If the continuous dependent variable was non-parametric in distribution then it was log10 transformed before inclusion in the multiple regression analysis.

A Linear regression model or correlation analysis was used where appropriate and as described in section 2.1.3

Statistical significance was two –tailed and defined as a p value less than 0.05.

2.2.5 Ethical approvals

This study was designed as an audit for Barts and The London Health NHS Trust and did not require formal ethical approval according to UK National Research Ethics Service (NRES) guidelines [267]. The Clinical Effectiveness Unit audit number for this project is 992

2.3 Response of Crohn's disease activity to thiopurines and its impact upon linear growth

2.3.1 Study design

This was a retrospective study evaluating the response to thiopurines in children with known CD commenced on thiopurine therapy between 2007-2010, and its effect on linear growth. Data was collected at the following time points: at baseline (i.e. the time at which the patient was stared on thiopurine) then subsequently at 3 months, 6 months and 12 months. Data was collected retrospectively from The Royal London Hospital Inflammatory Bowel Disease database (InfoFlex see 2.1.1), electronic patient records (EPR), clinic letters and inpatient notes.

The data collected for this study included demographic details, disease location and behaviour, serial measurements of height and weight, disease duration at initiation of thiopurine, dose per kg of azathioprine used, and any concomitant medical therapy. Systemic markers of disease activity (ESR, CRP and PLT) was also included, as were FBC, and ALT. MCV was also collected as it has been reported that increases in MCV can be used as an indicator of 6-TGN levels [270]. If the data was available to do so, the PCDAI was calculated (Appendix 1).

Thiopurine methyltransferase (TPMT) levels were collected for each patient. This is due to the fact that once AZA is converted to 6-MP, it can then go down 2 separate pathways, either being inactivated by TPMT, or activated to cytotoxic 6-thioguanine nucleotides (6-TGN) by a multi-enzymatic process. Approximately 11% of the

population has a reduced TPMT activity (10-26 units/ml/RBC), and 0.3% of the population has a true deficiency (<10 units/ml/RBC). Deficiency or reduced activity of TPMT results in an increased level of conversation to 6-TGN, and a higher risk of toxicity [271]. Therefore it is important to know a patient's TPMT level before starting treatment with a thiopurine, as this will affect the dosage given.

It is now possible to measure levels of thiopurine metabolites. The measurement of 6-TGN allows clinicians to determine whether lack of response to treatment is due to suboptimal dosing (including as a result of lack of compliance) or non-responsiveness to medication. The normal therapeutic range for 6-TGN levels is 200-400 pmol/RBC x 10^8, with lower levels indicating either non-compliance or under dosing [208, 272, 273]. However, the ability to measure 6-TGN levels at our unit was not available at the time of this study.

CS usage, both at baseline and over the 12-month study period, was also recorded. Disease phenotype and location was stratified according to the Montreal classification [48]. The primary outcomes were the patients' clinical response to thiopurine, and its subsequent impact upon height SDS. Height was assessed using standard anthropometric techniques, and was measured on the same calibrated scales upon each child's visit to the outpatient department (see auxology: 2.1.2). Height data was converted to standard deviation (SDS) scores using an auxology SDS calculator.Secondary outcomes recorded were: primary intolerance requiring cessation of thiopurine, including the prevalence of leucopenia, hepatitis and pancreatitis, and any escalation to infliximab or surgery within the 12 months.

In our centre, no patient is started on anti-TNF therapy without also being on a thiopurine. Therefore, it was not possible to study the effects of anti-TNF use independently. In patients who also received anti-TNF therapy, height, weight, inflammatory markers and PGA was collected at the start of anti-TNF treatment, and then again at 12 months. Where the information to do so was available, the PCDAI was also calculated at 0 and 12 months.

All data were arranged and processed for handling using Microsoft™ Office Excel in compliance with the Barts Health NHS Trust information governance.

2.3.1.1 Patient selection

The study population consisted of paediatric patients with known CD attending the Children' IBD clinic at Barts and The London Children Hospital. All children were diagnosed at <16 yrs using standard endoscopic, radiological and histological diagnostic criteria, as stated by the Porto Criteria [46].

2.3.1.2 Inclusion criteria:

The children included in the study were those who were started on either AZA or 6-MP for management of their paediatric CD at Barts and the London Hospital for Children, and had 12 months of follow-up data available post-initiation of the thiopurine.

2.3.1.3 Exclusion criteria:

Children who were started on thiopurine prior to transfer of management to Barts and The London Hospital for Children were excluded from the study. Other exclusion criteria were: initiation of thiopurine or other immunosuppressive agents for other non-CD illnesses, the presence of co-morbidities affecting growth (for example Turner's syndrome, chronic renal failure), and transfer of care over the 1 year study period from Barts and The London Hospital for Children to another centre or country.

2.3.2 Treatment regime

The thiopurine was started at the treating clinician's discretion. Bloods were check at the following time points: 0, 4, 6, 8 and 10 weeks, 2 months, 3 months and then 3 monthly afterwards, unless the treating physician decided that they were needed more frequently (for example due to increased disease activity). It was also the treating physician's decision to change any therapies for each individual patient.

Anti-TNF therapy was started at the treating clinician's discretion. The starting dose of IFX administered was 5mg/kg with 3 induction doses at weeks 0, 2 and 6, followed by maintenance therapy of 5mg/kg every 8 weeks. Higher doses up to 10 mg/kg and/or shorter intervals between treatments were decided on at the treating clinician's discretion.

2.3.3 Indices to evaluate response to thiopurine

The response to thiopurine was assessed using Physician's Global Assessment (PGA) [56].. The PGA was assessed at baseline, and then again at 3,6 and 12 months. Response was defined as CS free PGA of inactive disease. Where the information was available, the PCDAI was also calculated at these time points. There is a strong correlation between PGA and PCDAI [56]. Height SDS were recorded at 0, 3, 6 and 12 months.

2.3.4 Statistical analysis

Statistical analysis was performed using SPSS (version 20; SPSS Inc, Chicago, IL) and Prism (version 5; GraphPad, San Diego, CA) software. P values <0.05 were considered significant. Data are presented as means ± SD or medians as appropriate for the distribution normality. Continuous data were compared between groups using an Student's t-test for parametric data, and Mann Whitney U tests for non-parametric data. CRP, ESR, PCDAI and height SDS were analysed using a one-way repeated measures ANOVA or Friedman test, depending upon data distribution normality. Paired data was analysed using a paired t-test or Wilcoxon signed rank. Categorical data were compared between groups using chi-squared test or Fishers Exact test. Multivariate analysis was used to find any associations between factors (including age, ethnicity, gender, disease location) and response. Linear regression and correlation were used as described in section 2.1.3

2.3.5 Ethical approvals

Because we were evaluating the outcome as a service evaluation of thiopurine usage in our paediatric services, we did not require formal ethical approval, according to the guidelines of the UK National Research Ethics Service (NRES) [268]

2.4 Mathematical modelling to restore circulating IGF-1 concentrations in children with CD-induced growth failure: a pharmacokinetic study

2.4.1 Study design

This study was a pharmacokinetic, open-label study using once per day, and twice per day doses of rhIGF-1 (120µg/kg per dose) given by subcutaneous injection. These results were used to design a mathematical model that would define dosing regimes that return circulating IGF-1 concentrations into the normal range, without reaching levels that risk cancer.

2.4.1.1 Patient selection

8 children with known CD as diagnosed using standard endoscopic, radiological and histological diagnostic criteria [46] were recruited from the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. The height velocity (HV) standard deviation scores (SDS) of all children aged above 10 with CD attending

the clinic were measured. Height velocity refers to the change in a child's height in cm measured over a period of time. Normal HV changes at different points of childhood. Height was measured in each child at two intervals, at least 6 months apart, and using the auxology software Growth XP (www.auxology.com), this value was converted into a SDS, according to each child's age and gender,). Children were screened to identify those with HV measured over at least a 6 month period of <-2 SDS. The children were further screened according to the below inclusion criteria.

2.4.1.2 Inclusion criteria

Inclusion criteria were: age ≥ 10 years, a HV measured over at least a 6 month period of -2 SDS according to the patient's age and gender, and evidence of active inflammation as demonstrated by either an erythrocyte sedimentation rate (ESR) >25 mm/hr and/or a C-reactive protein (CRP) > 10 mg/l (Table 2.1). PCDAI was not chosen as a criterion because it is a composite of other entry criteria, including growth [56]. However, the index was calculated on each child.

Table 2.1 **Inclusion criteria for the study.**

Age	> 10 years
HV measured over > 6 months	< -2 SDS
ESR	> 25 mm/hr
And / or CRP	> 10 mg/l

Inclusion criteria were based upon age, height velocity and inflammatory markers

2.4.1.3 Exclusion criteria

Exclusion criteria were: corticosteroid use in the preceding 3 months, active or suspected neoplasia, known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK), and the presence of closed epiphyses, as determined by bone age x-ray.

2.4.1.4 Calculation of sample size

The sample size was derived from the number of children needed to show a significant increase in IGF-1, based on the increases in IGF-1 seen in a cohort of paediatric endocrinology children with no GI disease treated with rhIGF-1 for growth hormone insensitivity syndrome (GHIS) (industry data). This study showed that in 18 children, the mean concentration of IGF-1 before the drug was 330 ng/ml (SEM 20 ng/ml) and the mean concentration 535 ng/ml (SEM 20 ng/ml) 4 h after treatment. Therefore, to detect a difference in concentration of 205 (535-330) before drug and after 4 h and a SD of 84.85 (calculated from SEM with sample size of 18), with 80% power at the 5% level of significance, four patients are required. Because Crohn's disease is a cause of protein-losing enteropathy (PLE), an additional theoretical variable existed in our patients: that they may lose drug by PLE. We therefore doubled the sample size to 8 to account for this.

2.4.2 Study baseline investigations

Prior to entry into the study, all patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status, including a bone age. In addition, they also had an electrocardiogram (ECG) and faecal α-1-antitrypsin levels (AIAT) (mg/g) measured to detect the presence of PLE; control values described as 1.0 -1.15 mg/g [274-276].

2.4.3 Auxology measurements

Height measurements were measured on every child at each attendance to the outpatients department (see auxology 2.1.2). Height velocity for each patient was calculated from height measurements taken at least 6 months apart. These values were converted to SDS using auxology software.

2.4.4 Study drug

Ipsen UK® supplied the rh-IGF-1. The formulated protein was aseptically filled under good medical practice conditions. The trial medication was only supplied in response to a prescription that was labelled by the pharmacist as part of the normal dispensing process. A dose of 120µg/kg BD was chosen because it was successfully used in a recent trial for the treatment of primary IGF-1 deficiency in children (industry data).

2.4.5 Study protocol

Exogenous rh-IGF-1 was administered by subcutaneous injections at doses of 120µg/kg over two admissions. Admission 1 (see figure 2.2A) investigated the effects of a single dose of rhIGF-1 over a 24 h in-patient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1 and blood glucose measurement. Due to their importance in the regulation of the bioavailability of IGF-1, and low levels seen in conditions resulting in reduced IGF-1 levels, IGFBP-3 and ALS were also measured [54, 82, 277].

Stool samples were collected to measure faecal alpha-1-antitrypsin (A1AT) concentrations in order to assess the presence and degree of protein-losing enteropathy [278]. The PCDAI was also calculated.

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120µg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 h. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed non - CS treatment for CD.

Following a wash out period of at least 3 months, the participants were readmitted for admission 2 (Figure 2.2.B), investigating the effects of repeated doses of rh-IGF-1. On this occasion, six doses of rhIGF-1 were administered over a 5-day trial period. Doses were given at 0, 12, 72, 84, 96 and 108 h. The injection sites were rotated according to the patient's wishes. Serial blood samples for further IGF-1, IGFBP-3, ALS and blood sugar were collected at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 h. Additional

samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes and inflammatory markers were measured daily. Patients were kept in as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the 2 days in which they received no injection. Patients were discharged home on day 6.

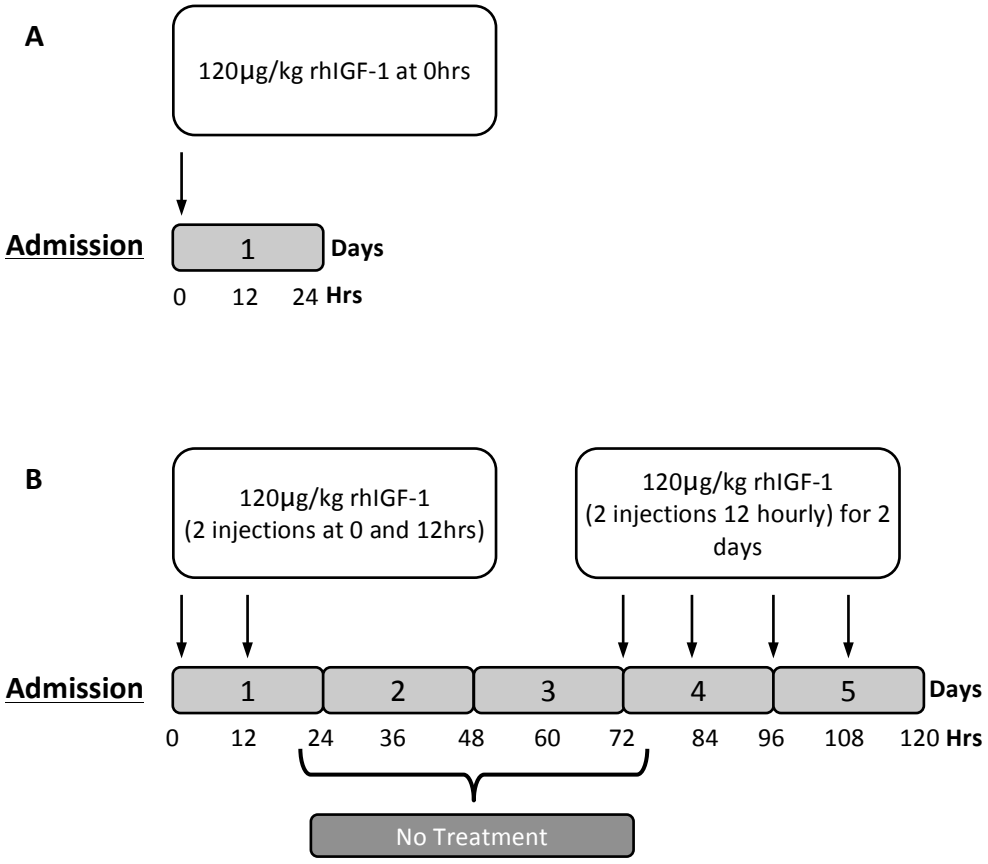

Figure 2.2 rhIGF-1 injection regimen in the study patients.
A) During admission 1, patients were given a single dose of rhIGF-1 (120µg/kg) at 0 hours. Serial blood samples were taken before discharge home after 24 hrs. B) Admission 2: this followed a wash out period of at least 3 months. On this occasion, six doses of rhIGF-1 were administered over a 5-day trial period. Doses were given at 0, 12, 72, 84, 96 and 108 h. Serial blood samples were taken over this period. Patients were admitted as in-patients on the days that they received injections due to possible side effect of hypoglycaemia.

2.4.6 Assays and samples

2.4.6.1 Blood glucose, FBC, ESR and electrolytes

Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, California, USA). FBC, ESR, electrolyte and CRP levels were analysed at the Barts and The London NHS Trust CPA accredited laboratory.

2.4.6.2 IGF-1 and IGFBP-3

Serum samples for measurement of IGF-1 and IGFBP-3 levels were frozen within 2 hrs of collection, and stored at -20°C until analysis.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc. Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3 mg/l and the intra-assay coefficient of variation averaged 13%. IGF-1 and IGFBP-3 levels were converted to SDS according to the patient's age and gender (Appendix 5).

2.4.6.3 Acid Labile Subunit

Serum samples for measurement ALS levels were frozen within 2 hrs of collection, and stored at -20°C until analysis. ALS levels were measured by radioimmunoassay (ALS-

RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8% to 17.4%. ALS levels were converted to SDS for patients' age and gender (see Appendix 5).

2.4.6.4 Faecal α-1-antitrypsin levels

Stool samples were frozen within 1 hr of collection, and stored at -20°C until analysis. The samples were analysed at St George's University Hospital CPA accredited laboratory.

2.4.7 Mathematical model development

A mathematical model for the administration of rhIGF-1, based on detailed pharmacokinetics, was formed to allow for a dosing regimen to be determined which would return circulating IGF-1 levels back to within the normal range without concentrations rising above this. This dosing regimen can thus be applied to all children with CD.

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEN V7.1

A model was built using the following parameters (Table 2.2 and Figure 2.3)

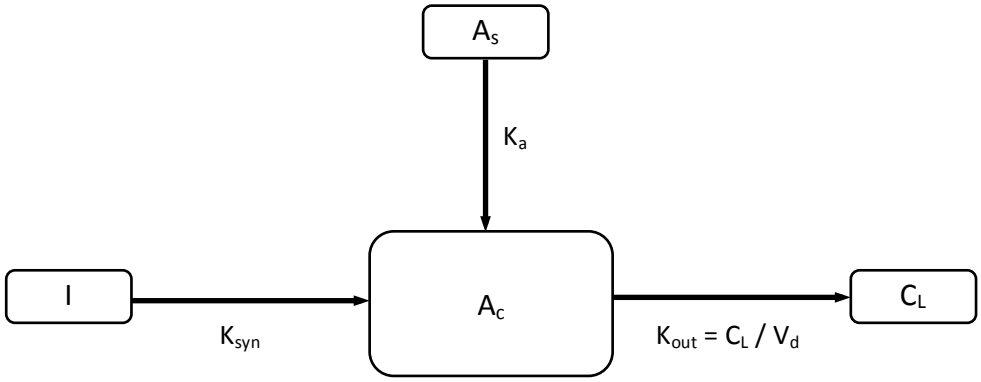

Figure 2.3 Diagram depicting the model formula

Table 2.2 Summary key of formula components

Symbol	Result
I	Endogenous IGF-1
K_{syn}	A zero order production rate of endogenous IGF-1 (µg/h)
A_s	The exogenous subcutaneous dose administered
K_a	A first order absorption rate of the exogenous subcutaneous IGF-1
A_c	The amount of circulating IGF-1 at time t
K_{out}	A first order elimination rate constant of IGF-1 in h^{-1}
C_L	IGF-1 clearance
V_D	Distribution volume

The concentration of IGF-1 in the circulating compartment (Ac) is determined by the rate of IGF-1 synthesis (K_{syn}) and rate of clearance (K_{out}). Under steady state conditions, which we assume is occurring at baseline, both values will be equivalent. However, by taking into account the biological influences affecting the volume of distribution (V_D) and clearance (C_L), which determine K_{out}, and adding exogenous IGF-1,

this model becomes more applicable to our clinical situation. Therefore this translates to a turnover model according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \times A_c + K_a \times A_s$$

$$\frac{dA_s}{dt} = - K_a \times A_s$$

K_{syn} was further scaled with linear weight due to its affecting the volume of distribution. Other covariates, including CRP, ESR, PCDAI and age, were tested for their impact on K_{syn}.

To check if the model's predicted increases in IGF-1 accurately compared with those actually observed in our patients following exogenous dosing, a series of mathematical assessments were carried out in addition to basic goodness-of-fit plots. Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [279] and model simulations was undertaken. The OFV is proportional to −2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with 1 degree of freedom, determined using a chi-squared distribution, gives a significantly improved fit with a probability $p<0.05$. The model was optimised to achieve an average concentration of IGF-1. This formed the basis of a utility function and was used to predict the average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was

defined as C_{ave} of +0.50 SDS with linear penalty for deviations above and below this target.

$$\text{Target} = SDS_i \times \varepsilon_i$$

$$\text{Utility} = \sum_{i=1}^{n} (\text{target} - SDS_i)^2$$

By taking into account individual variability from the target, the utility function could be made more accurate. Thus our target is the SDS aim (in this case +0.50); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; and ε_i denotes the individual deviations from the target. Utility minimised ε_i by least squares regression.

The model was extrapolated to see how it would fair under the constraints of a larger clinically relevant population with individual variability. This was achieved by using demographic details for 384 children aged 8–14 years from the clinical database. Each patient was assigned a random PCDAI score (from a uniform distribution 0–48) and also individual model parameters. With this extrapolation, the utility function (a predicted output that resembles the likely target value) could be further maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

2.4.8 Safety discussion

The safety data for rhIGF-1 comes from its wide use in children with Growth Hormone Insensitivity Syndromes (GHIS) [236]. The main adverse event seen is hypoglycaemia. For this reason, the protocol of this study required hospital admission when the rhIGF-1 was given.

2.4.9 Ethical approvals

Ethical approval was obtained from the East London & City Research Ethics Committee (reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (Eudract number: 2007-004269-16). The study was conducted in accordance with the Declaration of Helsinki. All participants and their parents gave written informed consent before participation in the study (see Appendix 6).

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3 Prevalence of growth retardation at diagnosis in children with Crohn's disease

3.1 Introduction

The incidence of paediatric CD is increasing across all ethnicities [24, 280]. Some studies have also reported a reduction in the mean age at diagnosis [35]. Childhood is a time of significant physical changes and emotional maturation. Therefore, it is important that children with CD are recognised and diagnosed promptly to try and modulate the impact that inflammation has on their growth and overall development.

Children with CD often experience a considerable duration of time between the onset of symptoms and diagnosis. A UK study looking at presenting features of paediatric IBD reported that 18% of children with CD had a symptom duration of 1-3 years, and 9% symptoms for more than 9 years[42]. This is important as there is a negative correlation between the duration of symptoms and height SDS at diagnosis [42, 101]. Poor growth is recognised as a poor prognostic factor in CD [55]. In addition, although some children do catch up with this growth deficit following treatment, a significant number of children do not [220], and fail to achieve their predicted target height [101].

3.4 Results

There were 122 incident cases of paediatric CD diagnosed between 1st January 2006 to 31st December 2011 at Barts Health NHS Trust. 74 patients (60.7%) were male and 48 (39.3%) female. 46.7% (57/122) of patients were Caucasian, 31.1% (38/122) Asian, 12.3% (15/122) Afro-Caribbean and 9.9% (12/122) of other ethnicities (Table 3.1). The median (range) age at diagnosis was 12.46 (4.79 - 16.68) years (Figure 3.1)

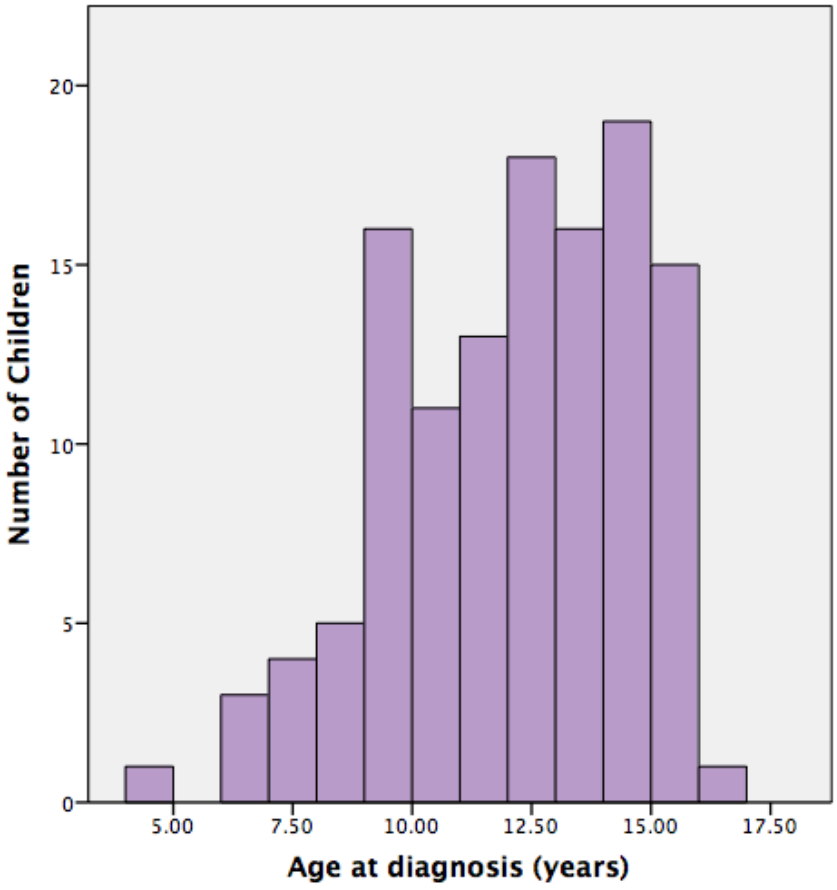

Figure 3.1 The age at diagnosis of 122 children with CD diagnosed in the paediatric IBD clinic at Barts and The London Hospital for Children between 2006-2011.
The median age was 12.46 years, with a range of 4.79 to 16.68 years.

3.4.1 Aim 1: To assess height SDS at diagnosis

Height at diagnosis was available in 102 of the patients. The mean [SD] height SDS at diagnosis was negative at -0.23 [1.08] (Figure 3.2.A). A quarter of patients (26.5%; 27/102) had a height SDS <-1 at diagnosis, with 8.8% (9/102) of patients having a SDS <-2.

The mean [SD] weight SDS at diagnosis was -0.61 [1.38] (Figure 3.2.B). As expected, there was a correlation between height and weight SDS at diagnosis ($r=0.71$; $p<0.001$, Pearson's) (figure 3.3)

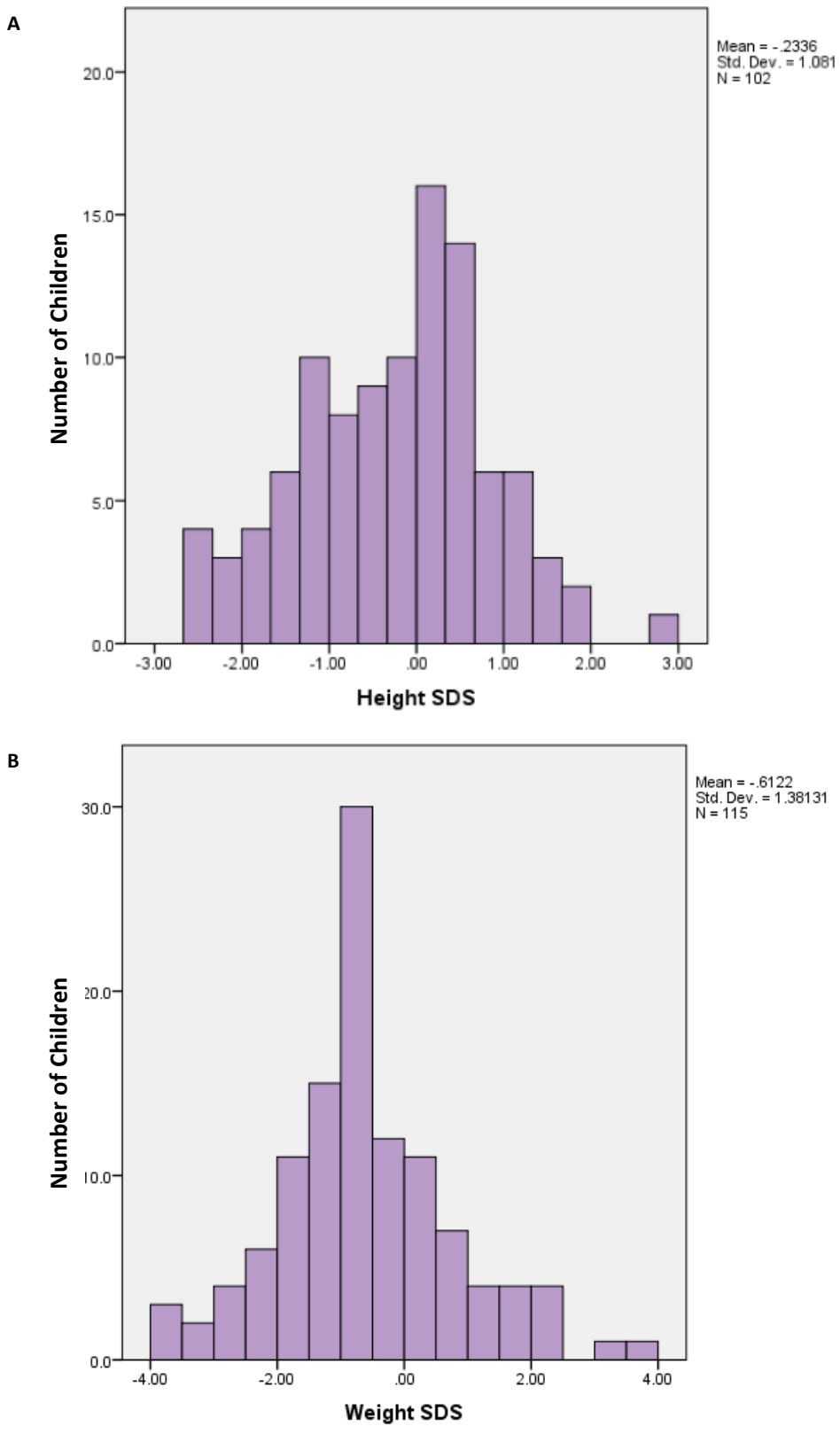

Figure 3.2 Height and weight SDS at diagnosis

A) height SDS at diagnosis was available in 102 patients diagnosed between 2006-2011. Mean SDS -0.23, SD 1.08. B) Weight SDS were available in 115 patients. Mean SDS -0.61 [1.38].

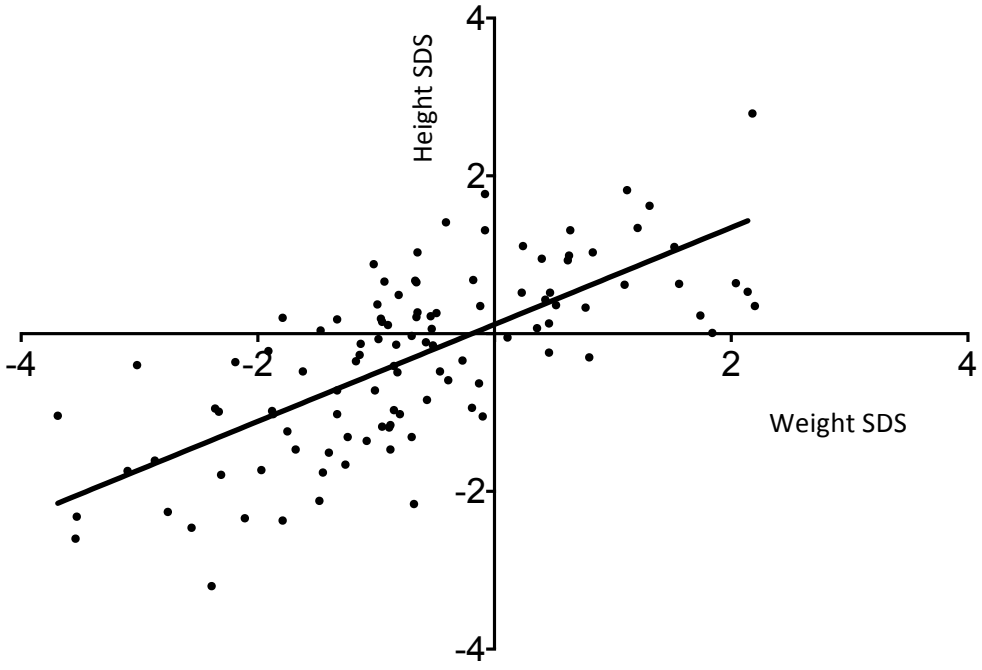

Figure 3.3 Height and weight correlation at diagnosis
Pearson's correlation found a significant relationship between height and weight SDS at diagnosis ($r=0.71$, $p<0.001$)

3.4.2 Aim 2: To determine the symptom duration at diagnosis in patients, and whether this is affected by presenting symptoms, disease phenotype or activity, age or gender

3.4.2.1 Symptom duration at the time of diagnosis

The median (range) duration of symptoms at diagnosis was 6 months (1-48 months) (Figure 3.4), with 33/122 (27.0%) of patients having symptoms for ≥ 12 months until diagnosis.

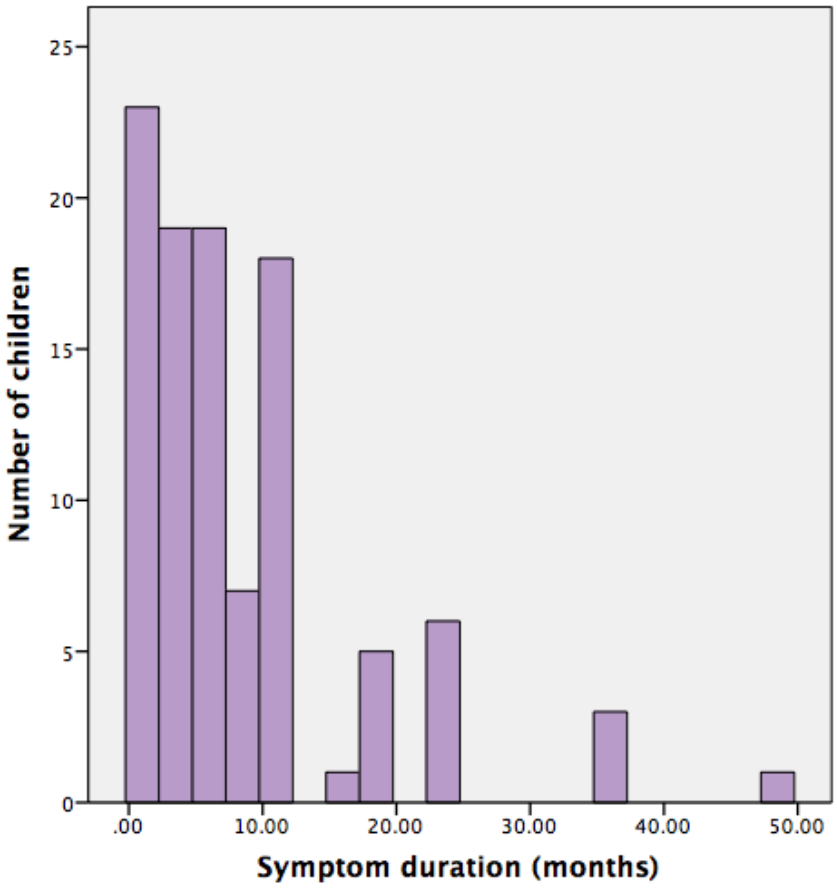

Figure 3.4 Symptom duration at the time of diagnosis for 122 children diagnosed with CD between 2006-2011 at Barts and The London Hospital for Children.
The median symptom duration was 6 months (range 1-48 months).

3.4.2.1 Presenting symptoms

The commonest presenting symptom was abdominal pain, with 74.6% of patients reporting this (Table 3.1). This was followed by diarrhoea (64.8%; 79/122) and then weight loss (63.9%; 78/122). Only 10 patients (8.2%) did not have any of these three symptoms, with 38.5% (47/122) presenting with all three. 35.2% (43/122) of patients had extra-intestinal manifestations at diagnosis, with 9.8% (12/122) having faltering growth as a presenting symptom.

3.4.2.2 Disease phenotype and activity

The majority of patients had ileo-colonic disease at diagnosis (63.1%; 77/122), of non-stricturing non-penetrating behaviour (74.6%; 91/122) (Table 3.1). 36.9% (45/122) of patients had upper GI disease present at diagnosis, and 30.3% (37/122) had perianal disease.

The mean [SD] ESR at diagnosis was 34.0 [28.0] mm/hr, CRP 41.2 [39.7] mg/l and PCDAI 30.1 [12.1]. The majority of patients (70/122; 57.3%) presented with moderate-severe disease activity as defined by a PCDAI>30. 40.1% (49/122) had mild-moderate disease (PCDAI 10-30), and only 2.7% (3/122) had inactive disease as defined by PCDAI < 10 at diagnosis.

Table 3.1 Demographics, disease phenotype, behaviour and activity and presenting symptoms in 122 children diagnosed with CD between 1st January 2006 to 31st December 2011

Demographics & Clinical detail	**Patients**
Median (range) age at diagnosis	12.46 (4.79 - 16.68) yrs
M:F	1.5:1
Ethnicity n (%)	
Caucasian	57 (46.7)
Asian	38 (31.1)
Afro-Caribbean	15 (12.3)
Other	12 (9.8)
Location n (%)	
L1 Ileal	30 (24.6)
L2 Colonic	15 (12.3)
L3 Ileo-colonic	77 (63.1)
Behaviour n (%)	
B1 Non-stricturing non-penetrating	91 (74.6)
B2 Stricturing	9 (7.4)
B3 Penetrating	22 (18)
Upper (L4)	45 (36.9%)
Perianal (p)	37 (30.3%)
Presenting symptoms n (%)	
Diarrhoea	79 (64.8)
Abdominal pain	91 (74.6)
Weight loss	78 (63.9)
Extra-intestinal manifestations *	43 (35.2)
Fatigue	43 (35.2)
Pyrexia**	20 (16.4)
Faltering growth***	12 (9.8)
Symptom duration in months median (range)	6.0 (1.0 - 48.0)
Height SDS mean [SD]	-0.23 [1.08]
Weight SDS mean [SD]	--0.61 [1.38]
ESR mean [SD] mm/hr	34.0 [28.0]
CRP mean [SD] mg/l	41.2 [39.7]
PCDAI mean [SD]	30.1 [12.1]

* oral ulcers, arthritis, uveitis, erythema nodosum, pyoderma gangrenosum

** Fever ≥ 38.5°C for 3 days over the course of a week

*** See section 1.3.2 for definitions of faltering growth

3.4.2.3 Factors associated with the duration of symptoms before diagnosis

There was no statistically significant correlation seen between the age at diagnosis and symptom duration (r=0.12; p=0.45, Spearman rank correlation) (Figure 3.5). As it was felt that the outcome could be associated with more than one predictor variable, multivariate analysis was performed. This analysis did not show any statistically significant association between symptom duration at diagnosis and gender (p=0.22), ethnicity (p=0.18), age (p=0.47),disease behaviour (p=0.63) or location (0.76) (Table 3.2). . Multivariate analysis found no association between symptom duration at diagnosis and ESR (p=0.26), CRP (p=0.39) or PCDAI (p=0.64).

Presence or absence of specific symptoms (diarrhoea, abdominal pain, weight loss, anorexia, EIMs) was not associated with symptom duration at the time of diagnosis (Table 3.2). The presence of the classic 'triad' of symptoms (diarrhoea, weight loss and abdominal pain), or the absence of any of them had no impact on time to diagnosis (p=0.95 and p=0.89, respectively; Mann Whitney U).

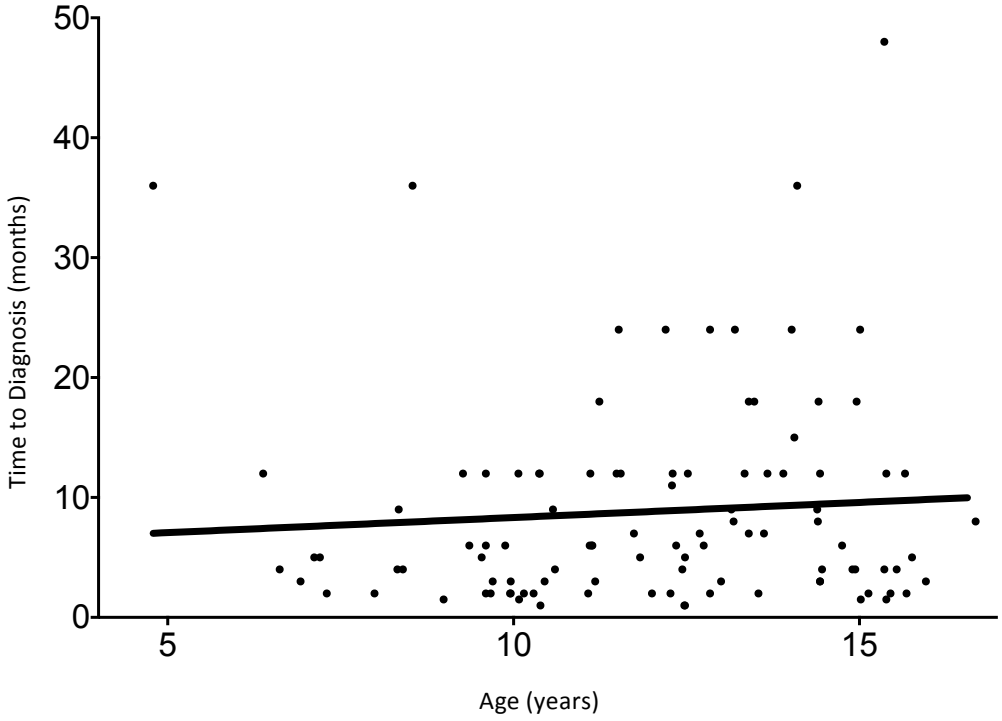

Figure 3.5 Correlating age and duration of symptoms at diagnosis.
Spearman's rank correlation found no statistically significant between the age at diagnosis and the duration of symptoms at the time of diagnosis (r=0.12, p=0.22)

3.4.3 Aim 3: To assess whether any of the above factors has an impact on the extent of growth failure

3.4.3.1 Relationship between presenting symptoms and height SDS at diagnosis

We hypothesised that patients who had longer symptom duration at diagnosis would have lower height SDS. There was a negative correlation between time to diagnosis and height SDS at diagnosis (r=-0.06, p=0.02) (Figure 3.6.A). The same was seen for weight SDS (r=-0.05, p=0.03) (Figure 3.6.B). However, as with time to diagnosis, multivariate analysis found no associations between any specific symptoms and height SDS at diagnosis (Table 3.2).

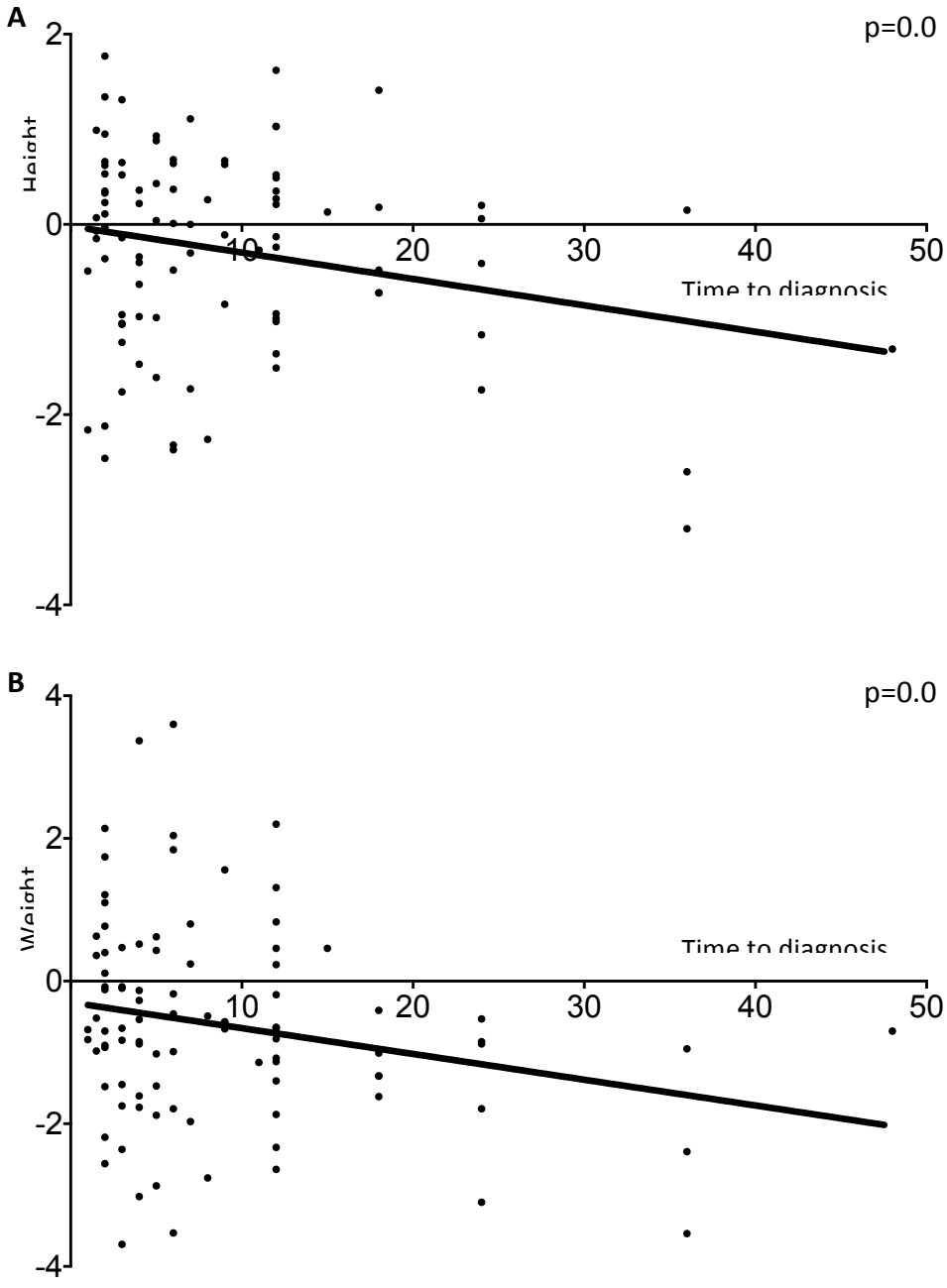

Figure 3.6 Correlating time to diagnosis with height and weight SDS.
There was a statistically significant negative correlation seen between the time to diagnosis (months) and both A) height SDS at diagnosis (r=-0.06, p=0.02; Spearman's rank correlation) and B) weight SDS (r=-0.05, p=0.03; Spearman's rank correlation)

3.4.3.2 Demographics with relation to growth failure

Multivariate analysis found no statistically significant associations between height SDS and age at diagnosis (p=0.27; Figure 3.7), or ethnicity (p=0.49) (Table 3.2).

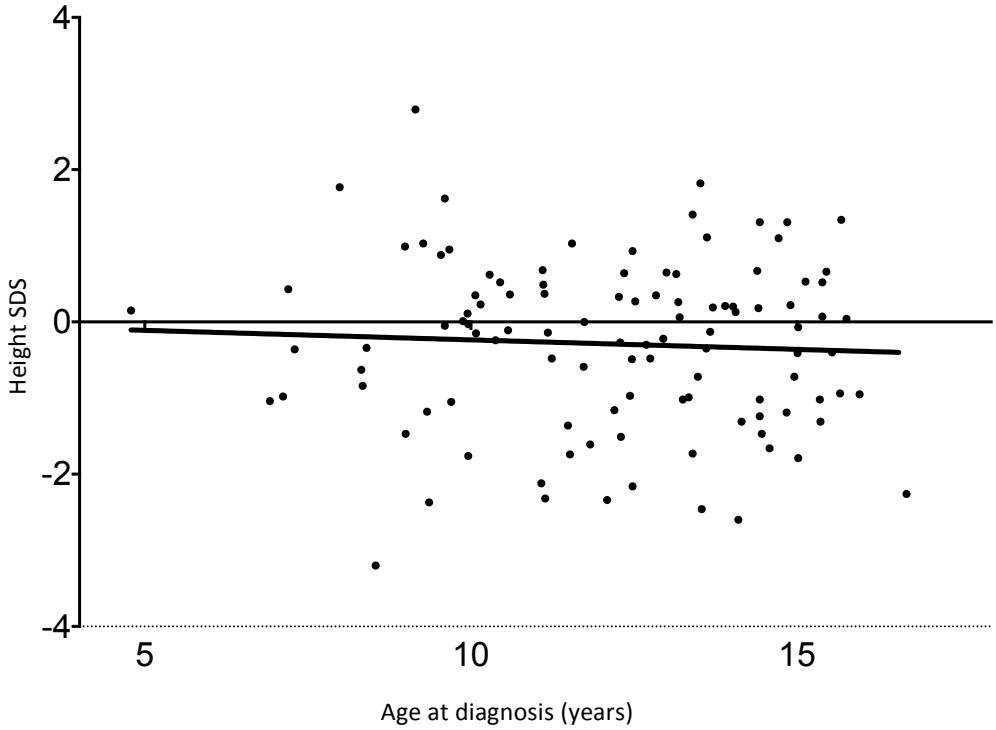

Figure 3.7 Correlating age at diagnosis and height SDS.
There was no statistically significant relationship seen between the age at diagnosis and height SDS at the time of diagnosis (p=0.27; GLM)

3.4.3.3 The impact of disease phenotype and activity

Neither disease phenotype or behaviour, or the presence of upper GI or perianal disease had a statistically significant association with height SDS at diagnosis, as determined by multivariate analysis (Table 3.2). There was no association seen between ESR or CRP at diagnosis and weight SDS. However, higher PCDAI scores were

associated with lower height SDS at diagnosis (p=0.01). However, it should be noted that growth assessments form part of the PCDAI (Appendix 2), and so this association is unsurprising.

Table 3.2 Multivariate analysis showing association between demographics, disease phenotype, behaviour and activity, and presenting symptoms with the time to diagnosis and height SDS at diagnosis (GLM)

	p-value	
	Time to diagnosis (yrs)	**Height SDS at diagnosis**
Age (years)	0.47	0.27
Gender	0.22	0.32
Ethnicity	0.18	0.49
Disease location	0.76	0.47
Disease behaviour	0.63	0.69
Upper	0.56	0.62
Perianal	0.91	0.76
PCDAI	0.13	0.01*
ESR	0.12	0.73
CRP	0.08	0.72
Diarrhoea	0.97	0.26
Abdominal pain	0.54	0.46
Weight loss	0.77	0.26
Anorexia	0.55	0.40
EIMs	0.32	0.40

* PCDAI was negatively associated with height SDS at diagnosis. However, part of the PCDAI scores includes an assessment of growth and so this finding is to be expected.

3.5 Discussion

In our cohort of paediatric CD patients diagnosed between 2006-2011, poor growth was very prevalent at diagnosis, with a quarter of patients having a height SDS <-1. In a normally distributed population, 13.6% is the usual percentage below -1 SD. The expected percentage below -2 SD is 2.1%. However, 9% of our patients fell into this category.

Despite the increase in prevalence [34, 35] and public awareness [281-283] of paediatric CD, children still experienced a considerable length of time from the onset of symptoms to diagnosis. In our patients, the median time to diagnosis was 6 months, with 27% of patients experiencing ≥12 months of symptoms before diagnosis. This is comparable to results of a study looking at presenting features of 739 children diagnosed with IBD in the UK between 1998-1999 [42]. This report found that the median time from onset of symptoms to diagnosis was 5 months, with 14% having had symptoms for 1-3 years beforehand. However, it should be noted that this study included both patients with CD and UC. A more comparable study to the one in this thesis is one performed previously at our centre with a cohort of 123 children with CD diagnosed before 1989 [101]. In this study, the mean interval from symptom onset to diagnosis was 12.5 (±12.9) months. However, the original individual patient data from this study is not available to us, and so any statistical analysis between the 2 cohorts is not possible.

In contrast to children with CD, children with UC often experience a shorter interval between onset of symptoms to diagnosis [42]. This is often speculated to be due to the differences in presenting symptoms between the two diseases, with CD patients

more likely to present with symptoms less conducive to speedy investigations [53]. However, our study did not find any association between any specific symptoms and time to diagnosis. This was even the case when children presented with the classic 'triad' associated with CD (diarrhoea, abdominal pain and weight loss) were present.

As has been previously described, we found that the interval between the onset of symptoms and diagnosis negatively correlates with the degree of growth retardation [42, 101]. Indeed, this was the only factor that we found predicted reduced height SDS at diagnosis. This provides an explanation as to why our group of patients overall had higher height SDS at diagnosis than the cohort from our centre diagnosed before 1989 (mean SDS in our patients -0.23, as compared to -0.50 in the previous study). However, as has been previously stated, 25% of our patients had height SDS <-1 at diagnosis. This is important because, even though some patients show catch-up growth, a significant number of patients remain growth retarded despite treatment of their CD [94, 101, 220]. This emphasises the importance not only of the early recognition and diagnosis of CD, but also of the implementation of therapies to promote growth as well as induce remission, from the very start of treatment.

3.6 Study strengths and limitations

This study follows up from previous research performed at the same centre. Barts and The London Children's Hospital provides tertiary paediatric care to a very diverse population, comprising many different ethnic groups, allowing us to assess the impact of this upon time to diagnosis and height SDS.

All patients had anthropometric data measured on the same scales, allowing for standardisation. This is of importance when assessing growth as different scales and assessors can yield significantly different results [284].

However, in comparison to Sawczenko *et al* (2003), who had a total of 739 new cases of IBD across many centres in Great Britain and Ireland [42], this was a single centre study. Therefore, although comparisons may be drawn against previous results from our centre [101], this cannot be generalised. Also, as with all studies of this nature, the problems of collecting data retrospectively were encountered. This research was dependent upon hospital records and clinical notes for data collection, which were in some cases incomplete.

3.7 Future directions

It would be interesting to follow this cohort of patients through to final adult height. Paediatric CD has now been recognised as presenting very different challenges to those experienced by our colleagues managing adults. Disease is often more extensive at diagnosis, and follows an aggressive course [26]. Therefore, treatments such as immunomodulators are being initiated earlier [217]. In addition, anti-TNF therapy is increasingly used. Sawczenko *et al* (2006) found that from 91 children diagnosed with CD before 1989, although height SDS did overall improve significantly from diagnosis to final height, mean final height was 2.4cm below target, with 19% of patients achieving a height less than -8.0cm below target height [101]. However, in view of the

changes in management of paediatric CD since that study was performed, it would be of interest to see if there were any improvements seen in this cohort.

3.8 Summary

Although improving, children diagnosed with CD at our centre still experienced a considerable length of time between the onset of symptoms and diagnosis. 25% of patients had at least 12 months of symptoms before diagnosis. Impaired growth was prevalent at diagnosis, with a quarter of patients having a height SDS <-1. As has been demonstrated before, there was a negative relationship between the length of the interval between the onset of symptoms and diagnosis with height SDS at diagnosis. This highlights the importance of early recognition of children with CD, and also the importance of considering growth in the management of these patients from the moment of diagnosis.

4 Results: long term clinical and growth outcomes following initial induction of remission therapy with exclusive enteral nutrition

4.1 Introduction

Exclusive enteral nutrition (EEN) using an elemental or polymeric formula has been shown to be effective treatment for children with active CD [164]. Meta-analyses have shown EEN to be as effective as CS in inducing remission in children [182, 183]. In contrast to CS which are associated with adverse effects upon growth [285] and bone density [286, 287], EEN has an excellent safety profile [105]. Recent guidelines published by the European Crohn's and Colitis Organisation (ECCO) and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have recommended EEN as the induction therapy of choice for children with CD [55]. However, despite this, EEN is not used as the therapy of choice worldwide. Although commonly used in Europe, it is rarely utilised in North America [170]. Reason stated for this include questions about efficacy and compliance [172].

Although there have been many studies demonstrating EEN's efficacy, and short term benefits to growth [136, 156, 164], there have been very few studies examining long term outcomes for children following initial induction therapy with EEN. One such study has been reported from a centre in England [193], and one from Scotland [194]. Both of these studies did not find any sustained benefit to height over 1 year and 2 years' follow-up, respectively. However, neither study assessed the impact on growth over a longer period of time. Additionally, both studies only followed up patients who

showed a response to EEN, with no comparison made to non-responders. There have been no studies investigating whether the children who do not respond have a different clinical course, and differing growth outcomes, to children who respond to EEN. This knowledge is both useful in supporting the use of EEN as an induction agent, and also for identifying patients early who may be at an increased risk of a poor outcome regarding disease activity and growth.

4.4 Results

104 children were diagnosed with CD at Barts and The London Hospital for Children between 1 January 2003 to 31 December 2008. 12 patients received neither EEN nor CS upon diagnosis (9 received 5-aminosalycilates (5-ASA) and 3 antibiotics) and so were excluded from the study. A further 3 patients transferred their care to other centres before the completion of the 5 years. Therefore 15 patients were excluded in total, leaving a cohort of 89 patients (Figure 4.1).

The median age (range) at diagnosis was 12.75 (4.84 - 15.86) years. 70.8% (63/89) patients were male, and 62.9% (56/89) Caucasian. At diagnosis, the majority of patients (68.5%) had ileo-colonic disease of non-stricturing non-penetrating behaviour (84.3%), as determined by Montreal classification [48]. Over half of the patients (55%) had Upper GI disease, and 28.1% perianal disease (Table 4.1).

Table 4.1 Demographics, disease phenotype, behaviour and activity, and growth parameters at diagnosis in 89 children with newly diagnosed CD, diagnosed between 1st January 2003 to 31st December 2008.

Demographics & Clinical Data	Patients (n=89)
Median (range) age at diagnosis / yrs	12.75 (4.84 - 15.86)
M:F	2.4:1
Ethnicity	
Caucasian	56 (62.9%)
Asian	20 (22.5%)
Afro-Caribbean	8 (9.0%)
Other	5 (5.6%)
Location	
L1 leal	20 (22.5%)
L2 Colonic	8 (9.0%)
L3 Ileo-colonic	61 (68.5%)
Behaviour	
B1 Non-stricturing non-penetrating	75 (84.3%)
B2 Stricturing	3 (3.4%)
B3 Penetrating	11(12.4%)
Upper (L4)	40 (44.9%)
Perianal (p)	25(28.1%)
Height SDS medium (range)	-0.29 (-3.2 to +2.82)
Weight SDS median (range)	-0.84 (-3.53 to +2.86)
ESR mean [SD] mm/hr	35.6 [31.2]
CRP mean [SD] mg/l	46.5 [44.1]
PCDAI mean [SD]	30.1 [12.1]

4.4.1 Aim 1: To assess tolerance and compliance to EEN in children with CD

All 89 patients were initially started on EEN using Modulen® (Nestle UK) as per unit protocol (Appendix 3). No child was started on CS as initial therapy. 72 patients (80.9%) took the Modulen orally, with 17 (19.1%) patients requiring an NG tube due to dislike of the taste or inability to consume the required amounts per day. EEN was generally well tolerated with only 5 patients (5.6%) unable to comply with the full 6 weeks of treatment. The median [IQR] age of patients who were intolerant of EEN was higher than those who tolerated the treatment (13.58 [2.23] vs 12.75 [4.17] respectively; p=0.02, Mann Whitney U). No side effects were reported during the period of use. At the end of 6 weeks of EEN, food was reintroduced progressively [164] with relatively good acceptance. According to unit policy, all patients were also started on 5-ASA at diagnosis.

4.4.2 Aim 2: To assess the efficacy of EEN as used for the induction of remission

Of the 89 patients included in the study, as defined by PGA; 62.9% (56) of patients had a complete response (CR), 21.3% (19) a partial response (PR), and only 15.7% (14) with no response (NR). The patients were separated into two groups for further analysis: 'responders' and 'non-responders'. Only the patients achieving CR were classified as 'responders'. Patients who showed PR or NR were collectively grouped as 'non-responders' (Figure 4.1).

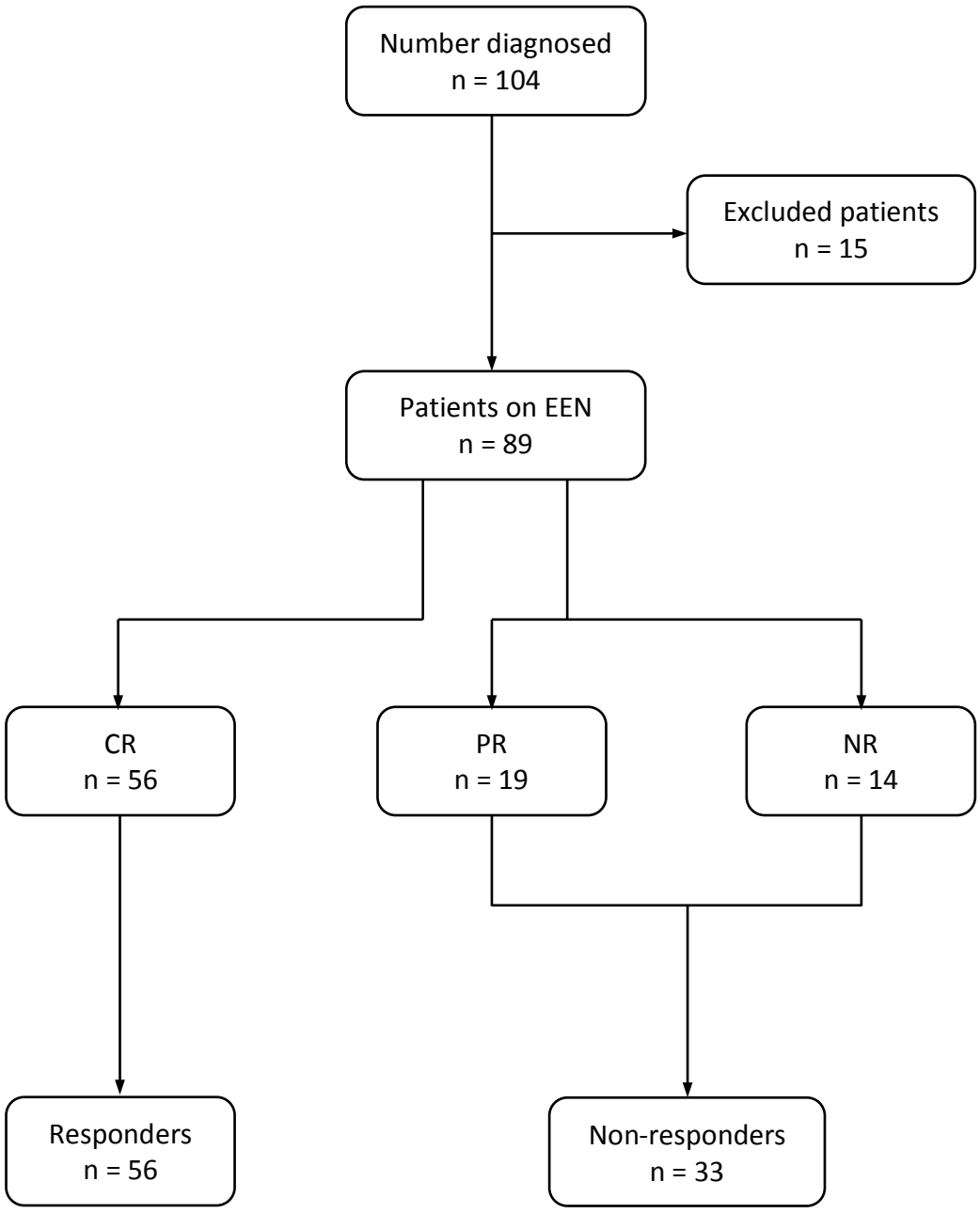

Figure 4.1 How children treated with EEN were defined as responders and non-responders. 104 children were diagnosed with CD 2003-2008. 15 children were excluded from the study due not starting either EEN or CS at diagnosis (12) or transfer of care to another centre within the 5 years (3). The remaining 89 patients were started on EEN. Following this, 56 patients were classed by PGA as being in complete remission. They were defined as responders. 19 patients had partial remission (PR), and 14 patients no response (NR). These 33 patients were collectively classed as non-responders.

It is important to be able to determine whether any particular variable about a patient will determine whether they are more or less likely to respond to a treatment. We felt that there were multiple factors that could possibly influence whether a patient responded to EEN or not. These included both demographic factors, such as age or gender, as well as disease-specific indices such as location, behaviour and activity. Therefore, a multivariate analysis was performed, allowing us to account for multiple predictor values simultaneously. The following factors were tested: age, gender, ethnicity, disease location and behaviour, CRP, ESR and PCDAI (Table 4.2).

Patients who did not respond to EEN had higher baseline PCDAI scores (mean [SD] 37.8 [SD]) than patients who responded (mean [SD] 30.8 [9.7]); p=0.005, Student's t-test (Figure 4.3.A). However, there was no significant difference between CRP (p=0.63, Mann Whitney U) or ESR (p=0.20, Mann Whitney U) between groups at diagnosis (Table 4.2).

There was no significant association seen between disease location and response to EEN (p=0.74). However, it must be noted that as there were only 8 patients in the colonic group, a type II error (failure to reject a false null hypothesis) may have occurred. Also, although patients with non-stricturing non-penetrating disease were found to be significantly more likely to respond to EEN than those with either stricturing or penetrating disease (p=0.001), the number of patients with stricturing (n=3) and penetrating (n=11) disease were low, and so this finding may be due to a type I error (the incorrect rejection of a true null hypothesis). Another statistically significant association from the multivariate analysis was that children with perianal disease were less likely to respond to EEN (p=0.001). The presence of perianal disease

was also associated with a higher PCDAI at diagnosis (mean [SD] 37.7 [11.4] in children with perianal disease as compared to 31.4 [9.5] in children without, p=0.02). There are limited reports describing the benefits of EEN in children with perianal disease [288]. Therefore the ECCO/ESPGHAN guidelines for the management of paediatric IBD state that there is no data to support its use in isolated perianal disease [55].

Responders to EEN were younger than non-responders (mean [SD] 11.85 [2.39] year vs 12.96 [2.50], p=0.03 Student's t-test) (Figure 4.3.B). Given the above results, we checked whether older children were more likely to have perianal disease or stricturing or penetrating behaviour. However, there was no association found between age and the presence of perianal disease (p=0.15) or disease behaviour (p=0.74). There was also no statistically significant correlation seen between age at diagnosis and PCDAI at diagnosis (r=-0.005, p= 0.97).

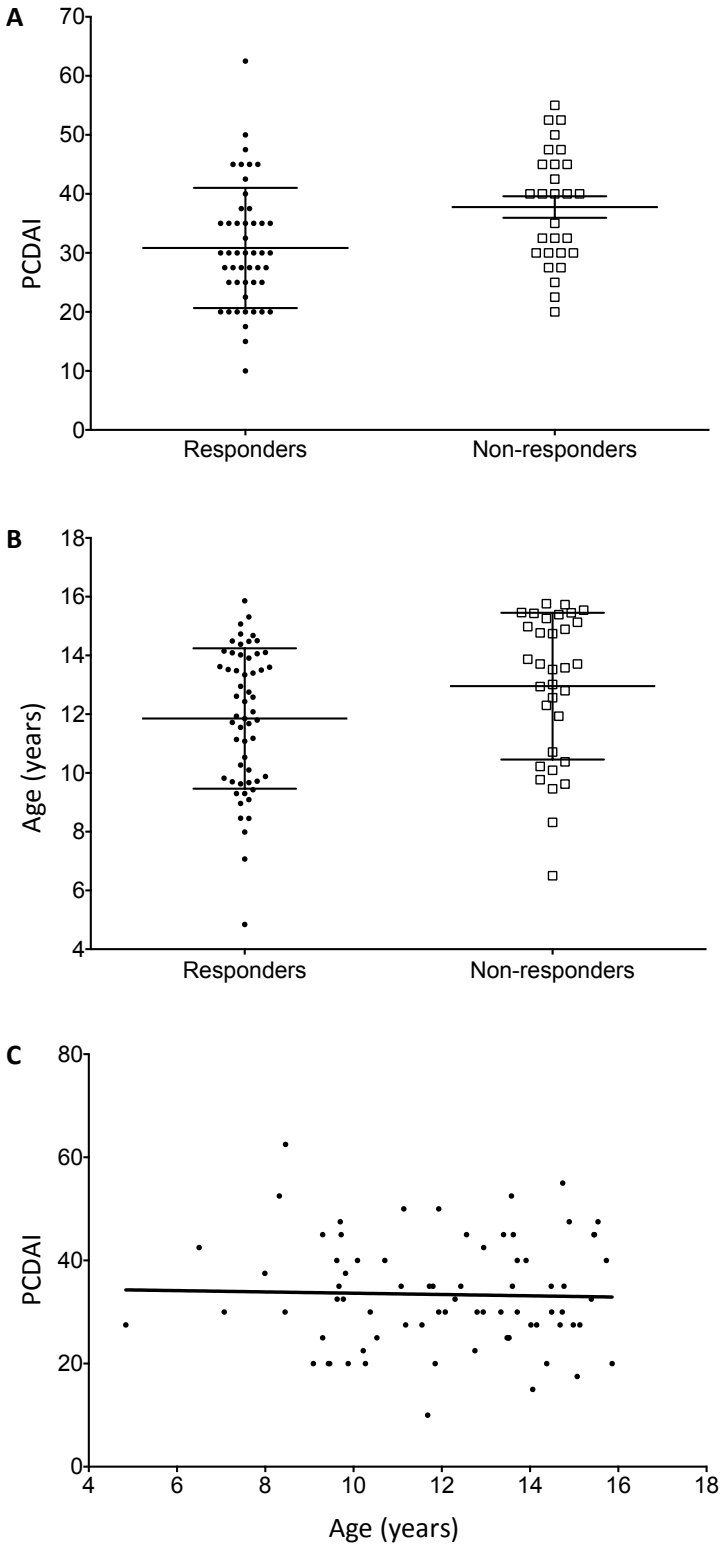

Figure 4.2 PCDAI and age in responders and non-responders to EEN.
A) PCDAI at diagnosis was significantly lower in responders (mean [SD] 30.8 [10.2]) when compared with non-responders (37.8 [9.7]); p=0.005. Mean and SD indicated by horizontal lines. B) Responders to EEN had a lower mean [SD] age of 11.85 [2.39] than non-responders (mean [SD] 12.96 [2.50]); p = 0.03. Mean and SD indicated by horizontal lines. C) There was no statistically significant correlation between age and PCDAI at diagnosis (r=-0.005, p=0.97).

Table 4.2 Demographics, disease location and behaviour, and inflammatory markers at diagnosis for 89 children started on EEN (2003-2008).

	Responders (n=56) (62.9%)	**Non-responders (n=33) (37.1%)**	**p value**
Median age (range) in yrs	12.01 (4.84-15.86)	13.58 (6.5 - 15.76)	0.03
M:F	38:18	25:8	0.54
Ethnicity			
Caucasian	30 (53.6%)	26 (46.4%)	0.08
Asian	15 (75%)	5 (25%)	
Afro-Caribbean	7 (87.5%)	1 (12.5%)	
Other	4 (80%)	1 (20%)	
Montreal disease classification: Location			
L1: ileal	15 (75%)	5 (25%)	0.74
L2: colonic	2 (25%)	6 (75%)	
L3: ileo-colonic	39 (63.9%)	22 (36.1%)	
L4: Upper	28 (70%)	12 (30%)	0.48
P: perianal	9 (36%)	16 (64%)	0.001
Disease Behaviour			
B1: non-stricturing, non penetrating	52 (69.3%)	23 (30.7%)	0.001
B2: Stricturing	2 (66.7%)	1 (33.3%)	
B3: Penetrating	2 (18.2%)	9 (81.8%)	
Inflammatory Markers at diagnosis			
Mean ESR (mm/hr) [SD]	47.1 [47.1]	40.4 [28.1]	0.20
Mean CRP (mg/l) [SD]	32.8 [32.8]	45.3 [38.8]	0.63
PCDAI [SD]	30.8 [10.2]	37.8 [1.83]	0.008

p-values were calculated using multivariate analysis testing all variables simultaneously**.**

Inflammatory markers were measured at baseline and at 6 weeks. Overall, they improved significantly. Paired data for ESR was available in 75 patients. The median ESR [IQR] at diagnosis for these patients was 27.0 [9.25 – 57.75] mm/hr, as compared to 10.0 [5.0 – 27.0] at 6 weeks ($p<0.001$, Wilcoxon signed rank test). Paired CRP results were also available in 75 patients. The median [IQR] CRP at diagnosis was 35.5 [14.75 – 58.25] mg/l, and 2.5 [0 – 14.5] at 6 weeks. However, the CRP and ESR at 6 weeks in the responders to EEN were significantly lower than those in the non-responders ($p<0.001$, Wilcoxon signed rank test) (Figure 4.3A and 4.3B).

The PCDAI (Appendix 2) also decreased in the group of patients from diagnosis to 6 weeks. Paired results were available in 67 patients. The median [IQR] PCDAI at diagnosis was 31.25 [25.63 – 40.0], and 5.0 [2.5 – 17.5] at 6 weeks ($p<0.001$, Wilcoxon signed rank).

PCDAI at 6 weeks was significantly lower in responders as compared to non-responders (median [IQR] 5.0 [0 - 7.5] vs 18.75 [9.5 – 27.5], $p<0.0001$, Mann Whitney U) (Figure 4.3C). As has been previously described in other studies [56], a Pearson correlation of our data showed that there was a statistically significant correlation between PGA and PCDAI, with PGA of inactive disease correlating with lower PCDAI scores, and active disease having the highest scores ($p<0.001$). As PCDAI data was not available for all patients, and it correlated so clearly with PGA, PGA alone was used for further analysis.

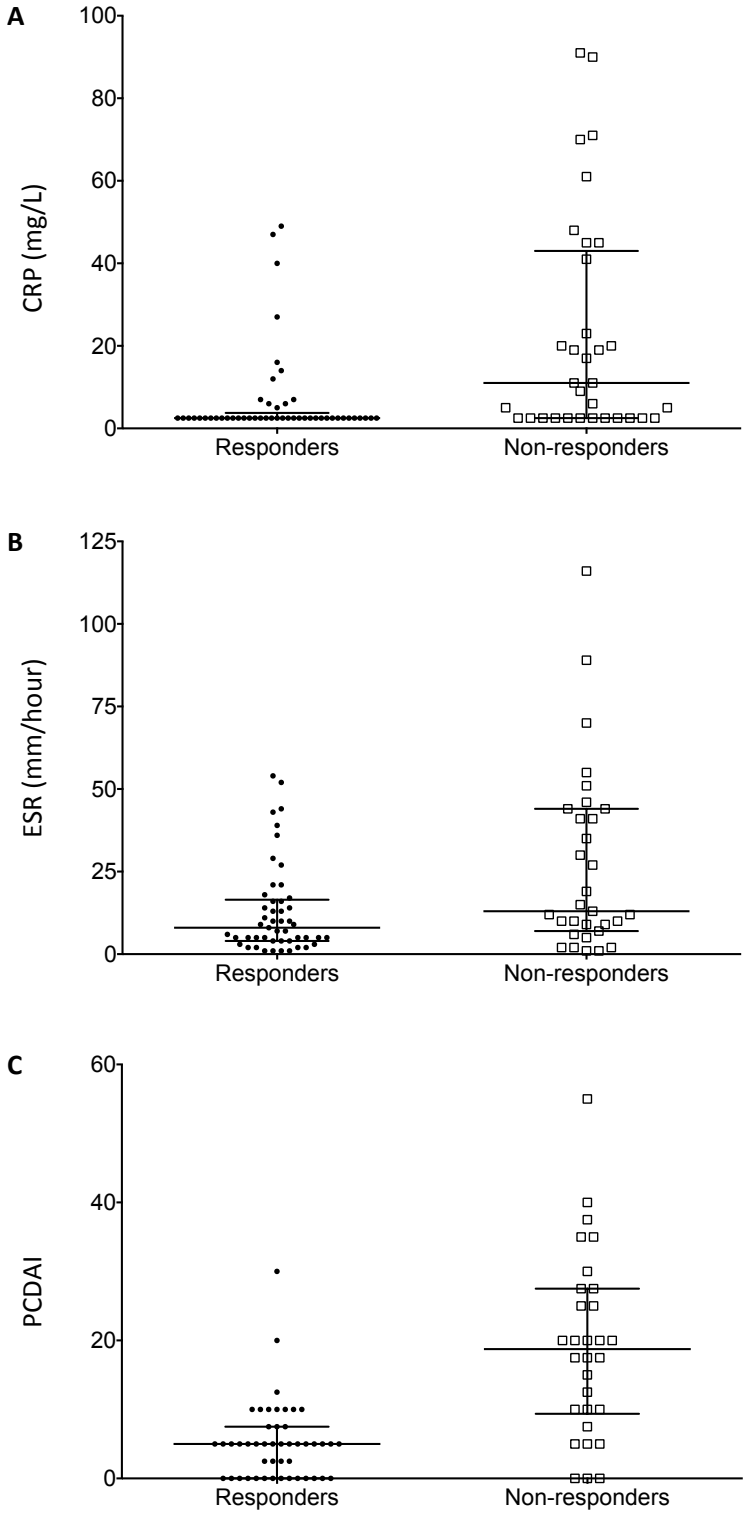

Figure 4.3 Markers of disease activity at 6 weeks were significantly lower in responders to EEN than in non-responders.

A) CRP at 6 weeks was lower in responders to EEN than in non-responders (median [IQR] 2.5 [2.5 – 3.75] vs 11.0 [2.5 – 43.0], p<0.0001, Mann Whitney U). B) ESR values at 6 weeks were also significantly lower in responders than non-responders (median [IQR] 8.0 [4.0 – 16.5] vs 13.0 [7.0 – 44.0], p<0.0001). C) PCDAI median [IQR] at 6 weeks was 5.0 [0.0 – 7.5] in responders as compared to 18.75 [9.375 – 27.5] in non-responders, p<0.0001. Medians and IQR are indicated on all graphs by the horizontal bars.

4.4.3 Aim 3: To compare the clinical course and outcomes between patients who entered remission following EEN to those who did not

Over the 5 year study period, 69.6% (39/56) of responders and 81.8% (27/33) of non-responders to EEN relapsed (p=0.32). Relapse in both responders and non-responders was defined as an increase in disease activity, as determined by PGA (from inactive to mild-moderate or moderate-severe disease), with changes in symptoms and/or necessitating an escalation in the on-going therapy. There was no significant difference between the time spent in remission between responders to EEN (mean [SD] 48.7 [11.7] months) and non-responders (mean [SD] 50.3 [10.4] months); p=0.5 (Figure 4.4).

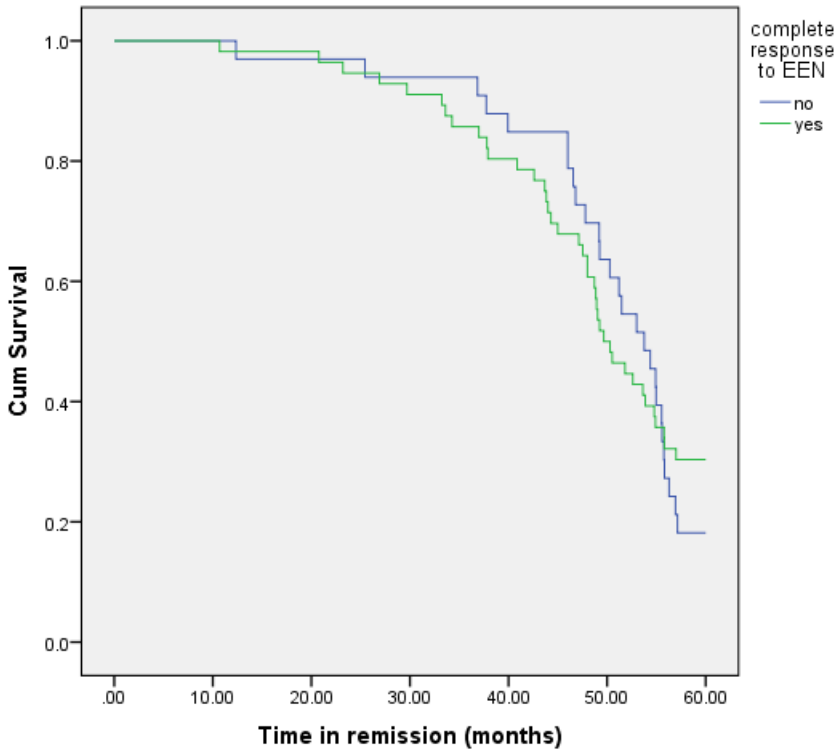

Figure 4.4 Time in remission following EEN induction therapy.
The responders and non-responders had comparable times spent in remission before first relapse (mean [SD] 48.7 [11.7] months vs 50.3 [10.4] in non-responders); p=0.5 [log rank test - Kaplan-Meier survival curve]. Baseline time-point 0 reflects time at starting EEN.

Treatment escalation to immunosuppressives, anti-TNF or surgery was comparable between responders and non-responders to EEN (Table 4.3).

4.4.3.1 Azathioprine:

Azathioprine (AZA) was prescribed in 70 (78.7%) of patients over the 5 years; 43 of 56 (76.8%) and 27 of 33 (81.8%) patients in the responder and non-responder groups, respectively. There was no significant difference in the number of patients starting AZA between the two groups (p=0.79, Chi squared test), nor in the time to initiating AZA treatment (mean [SD] time (months) in responders vs non-responders 20.8 [13.5] vs 22.2 [12.7]; p=0.69).

4.4.3.2 Biologics:

29 patients (32.6%) were started on anti-TNF therapy. Again, there was no difference between the percentages starting biologics in the responders as compared to the non-responders (18/56 (32.1%) vs 11/33 (33.3%), respectively; p=1.00, Chi squared test), nor the mean time (months) to starting anti-TNFs (mean [SD] in responders 30.5 [16.6] vs 33.4 [18.3] in non-responders; p=0.68).

4.4.3.3 Surgical resection:

Overall, 26/89 (29.2%) of patients had a surgical resection; 15/56 (26.8%) of responders and 11/33 (33.3%) of non-responders (p=0.63, Chi squared analysis). The time to surgery in both groups was comparable (mean [SD] in responders 24.7 [19.7] months in responders vs 33.3 [16.8] months in non-responders; p=0.27).

Table 4.3 Treatment escalation in responders and non-responders to EEN.

	Responders (n=56)	**Non-responders (n=33)**	**p-value**
Azathioprine	43 (76.8%)	27 (81.8%)	0.79
Anti-TNF	18 (32.1%)	11 (33.3%)	0.68
Surgery	15 (26.8%)	11 (33.3%)	0.63

p-values are a result of chi-squared analysis

4.4.4 Aim 4: To compare growth over 5 years between patients in whom remission was induced with EEN to those who did not enter remission.

The mean height SDS at diagnosis was -0.29 (SD 1.22) (Figure 4.5A). 82 patients had data available to calculate height SDS at 0, 1 and 5 years. Repeated measures one-way ANOVA analysis was performed on these patients. We found that in this overall cohort, there was no improvement of growth over the 5 years. The mean height [SD] SDS at diagnosis was -0.28 [1.23], -0.33 [1.23] at 1 year, and -0.29 [1.21] at 5 years (p=0.78) (Figure 4.5.B).

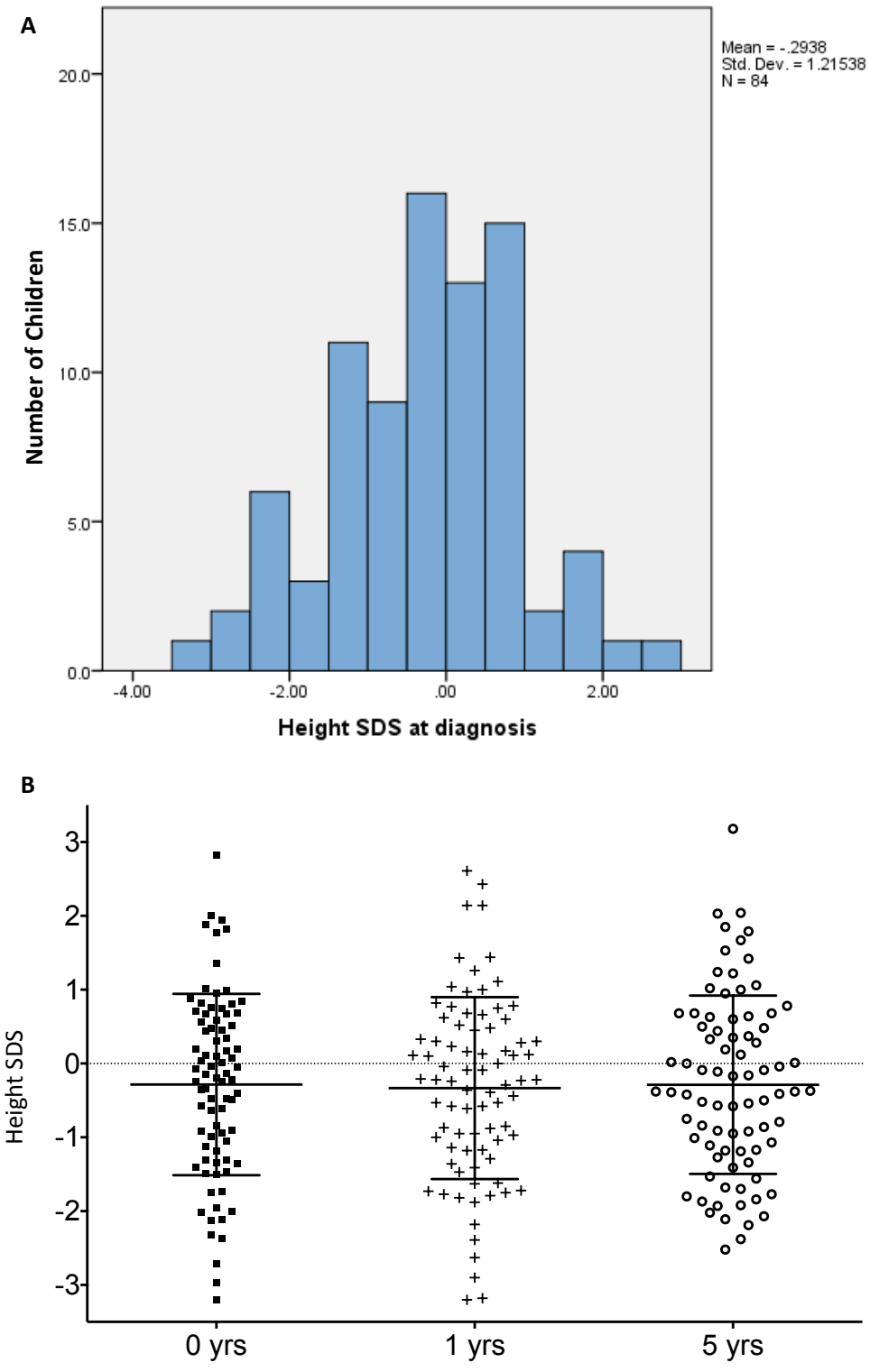

Figure 4.5 Linear growth in all children receiving EEN, regardless of their response
A) The mean [SD] height SDS at diagnosis was -0.29 [1.22]. B) There was no improvement in height SDS over the 5 years. The mean height [SD] SDS at diagnosis was -0.28 [1.23], -0.33 [1.23] at 1 year, and -0.29 [1.21] at 5 years (p=0.78). Mean and SD indicated by error bars.

However, when comparing growth between responders to EEN with non-responders, we found that patients who had responded to EEN grew significantly better than those who had not. This difference was evident after 1 year (Figure 4.6.A), and maintained over the 5 years (Figure 4.6.B). Patients in the responder group showed a slight increase in height SDS, from a mean [SD] of -0.34 [1.33] to -0.29 [1.37] at 1 year and -0.15 [1.31] at 5 years, $p<0.001$, repeated measures ANOVA (Figure 4.7.A). However, in contrast, the children in the non-responder group had a more negative height SDS at each time point (mean [SDS] at baseline -0.18 [1.01] vs -0.41 [0.95] at 6 months vs -0.55 [0.96] at 5 years, $p <0.001$), repeated measures ANOVA (Figure 4.7.B).

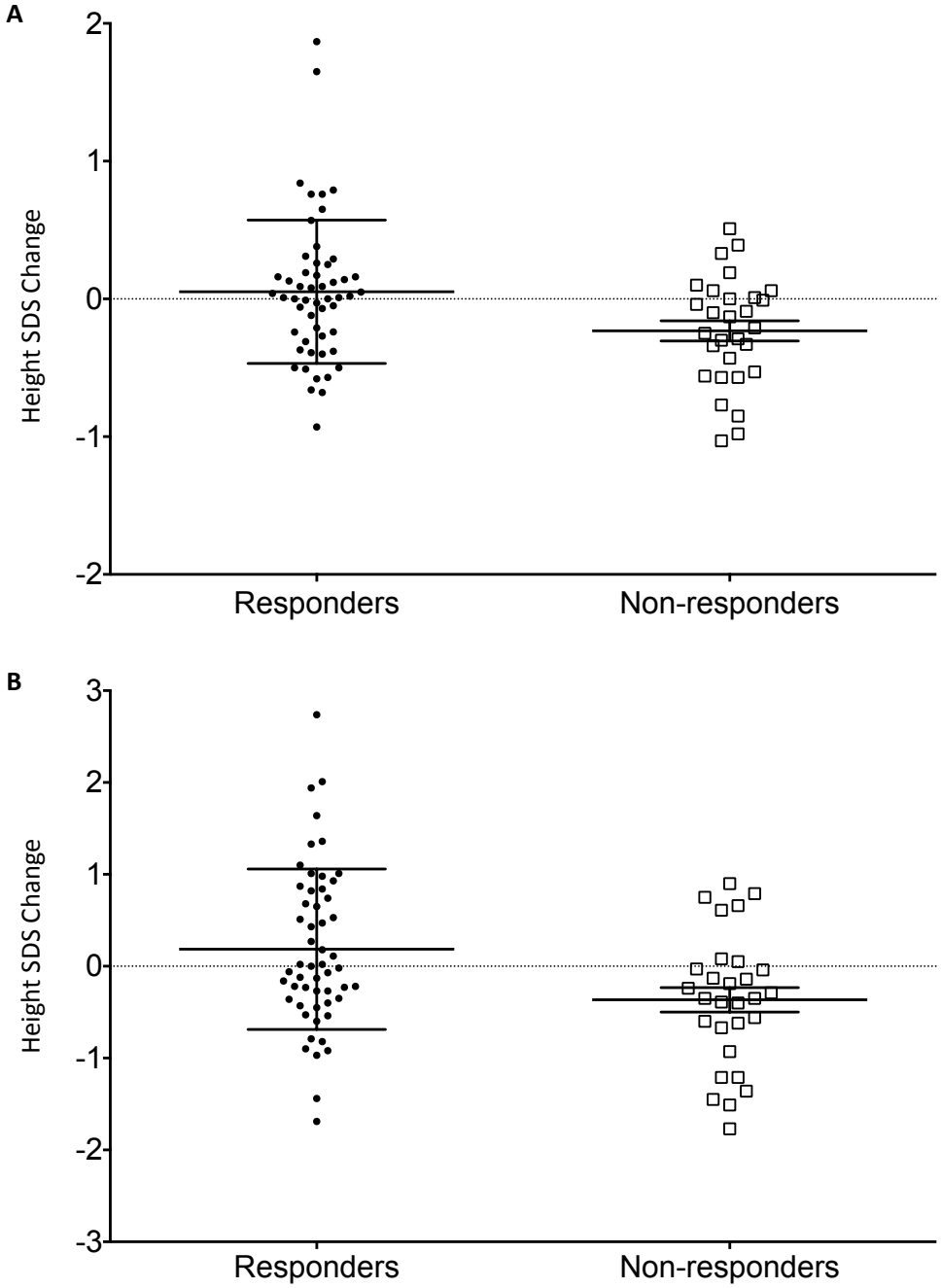

Figure 4.6 Changes in height SDS between responders and non-responders to EEN.
Responders to EEN had significantly greater increases in height SDS than non-responders: A) From diagnosis to 1 year after follow-up. The mean [SD] change in height SDS was +0.05 [0.39] as compared to -0.23 [0.39] in non-responders (p=0.01, Student T-test). B) From diagnosis to the end of the 5 years. The mean [SD] change in height in responders was +0.18 [0.12] as opposed to -0.37 [0.13] in non-responders; p=0.005, Student T-test). Means and SD indicated by horizontal bars.

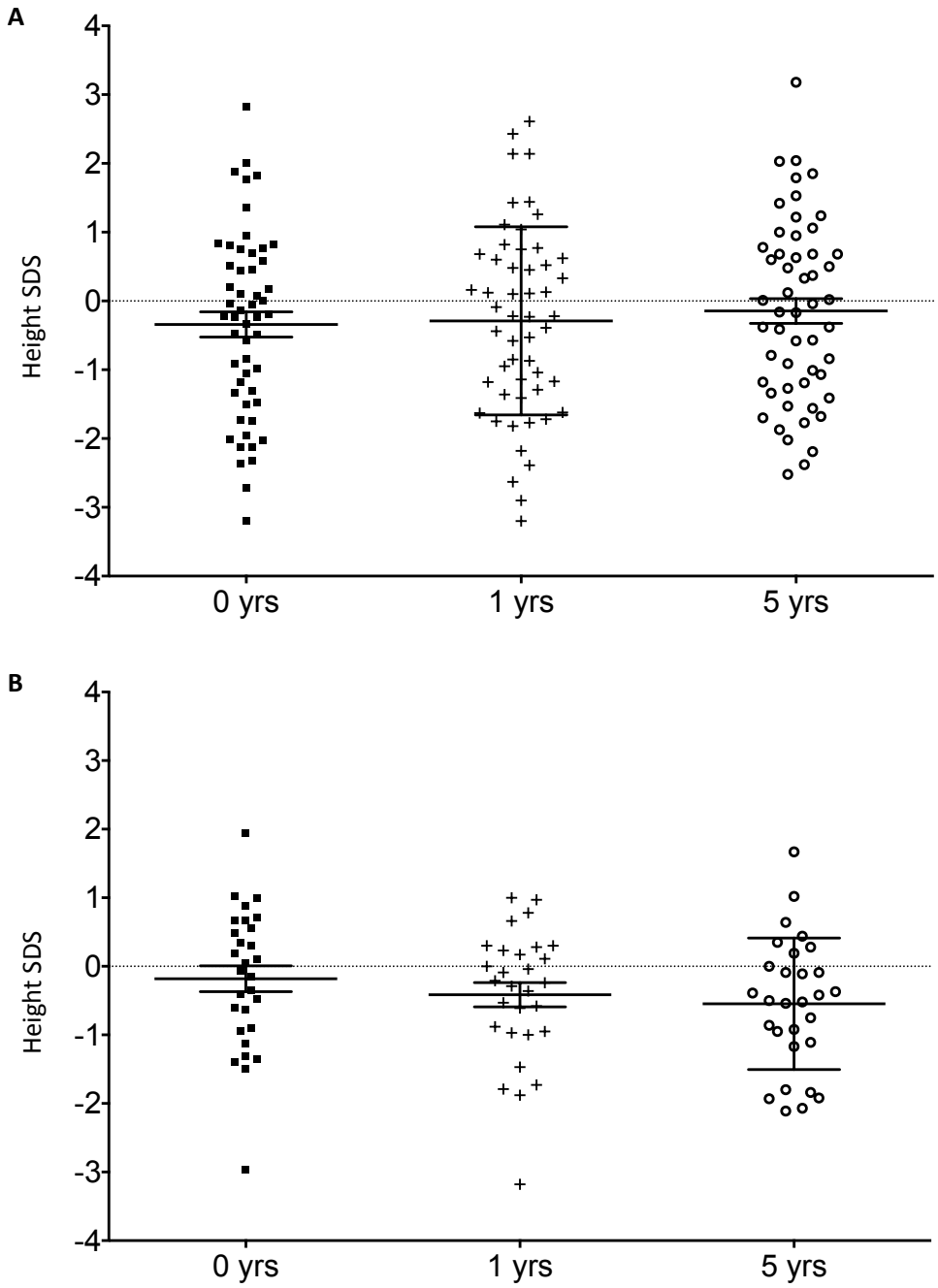

Figure 4.7 Changes in height SDS over 5 years.

A) Responders to EEN had a significant increase in height SDS over the 5 years, from a mean [SD] of -0.34 [1.33] to -0.29 [1.37] at 1 year and -0.15 [1.31] at 5 years, p<0.001; repeated measures ANOVA. B) By contrast, non-responders to EEN had more negative height SDS across the 5 years. Mean [SDS] at baseline -0.18 [1.01] vs -0.41 [0.95] at 1 year vs -0.55 [0.96] at 5 years, p <0.001; repeated measures ANOVA.. Means and SD indicated by horizontal bars.

Within the cohort of 89 patients, multivariate analysis showed that there were no factors present at diagnosis that were significantly associated with change in height SDS at 1 year (Table 4.4). However, there was an association seen between CRP at diagnosis (p=0.035) and ESR at diagnosis (p=0.045) and change in height SDS over 5 years (Figure 4.8). There was no such relationship with PCDAI at diagnosis (p=0.29) (Figure 4.5.C).

Table 4.4 Multivariate analysis using GLM showing association between baseline demographics, disease location or behaviour, and inflammatory markers with change in height (ht) SDS.

	p-value	
	Change in ht SDS 0-1 year	**Change in ht SDS 0-5 years**
Age (years)	0.28	0.61
Gender	0.85	0.18
Ethnicity	0.35	0.59
Disease location	0.87	0.11
Disease behaviour	0.91	0.39
Upper	0.59	0.66
Perianal	0.33	0.19
PCDAI	0.53	0.29
ESR	0.34	0.045
CRP	0.28	0.035

p-values determined by multivariate analysis.

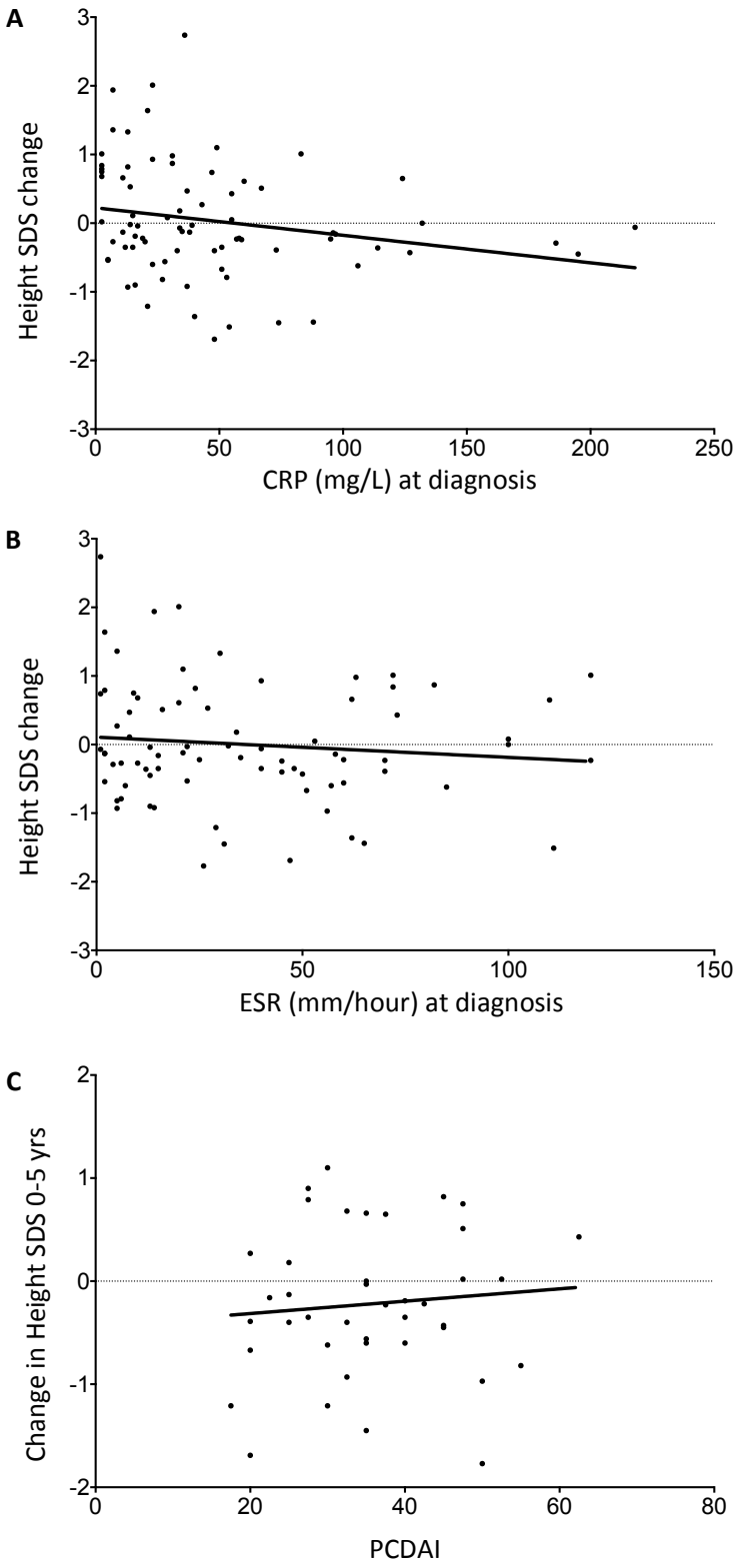

Figure 4.8 Correlation between inflammatory markers at diagnosis and change in height SDS. A) There was a statistically significant negative correlation seen between CRP at diagnosis and change in height SDS over 5 years. Higher CRP values were associated with poorer growth (r=-0.28, p=0.035). B) The same was seen with ESR at diagnosis. Low ESR levels at diagnosis were associated with better growth across the 5 years (r=-0.1, p=0.045). C) There was no statistically significant correlation seen between PCDAI at diagnosis and change in height SDS over the 5 years (r=0.07, p=0.29).

Taking the responders alone, a statistically significant correlation was again seen between the CRP at diagnosis and poor growth, as defined by changed height SDS (r=-0.29, p=0.05). However, there was no statistically significant correlation between raised ESR at diagnosis and change in height SDS (r=0.03, p=0.45). Using multivariate analysis, there were no significant associations found with any of the other variants, including PCDAI. However, within the non-responders, as well as the statistically significant association with baseline CRP (p=0.04), lower changes of height over 5 years were also seen in patients with perianal (p<0.0001) and upper GI disease (p<0.0001).

4.4.4.1 The impact of corticosteroid usage upon growth

29 patients (32.6%) out of the 56 responders did not require any CS over the 5 year study period. However, there was no significant difference seen in change in height SDS between patients who avoided CS use (mean [SD] +0.03 [0.65]) and those who did (mean [SD] -0.03 [-.95]); p=0.78, Student t-test.

In responders who required CS, the median (range) time to CS was 47.6 (1.5 - 59.52) months. However, a longer duration of time to the start of CS did not result in improved growth (Figure 4.9) (r=0.19, p=0.35, Spearman), nor was there any association between the number of courses of CS used and change in height SDS (p=0.85).

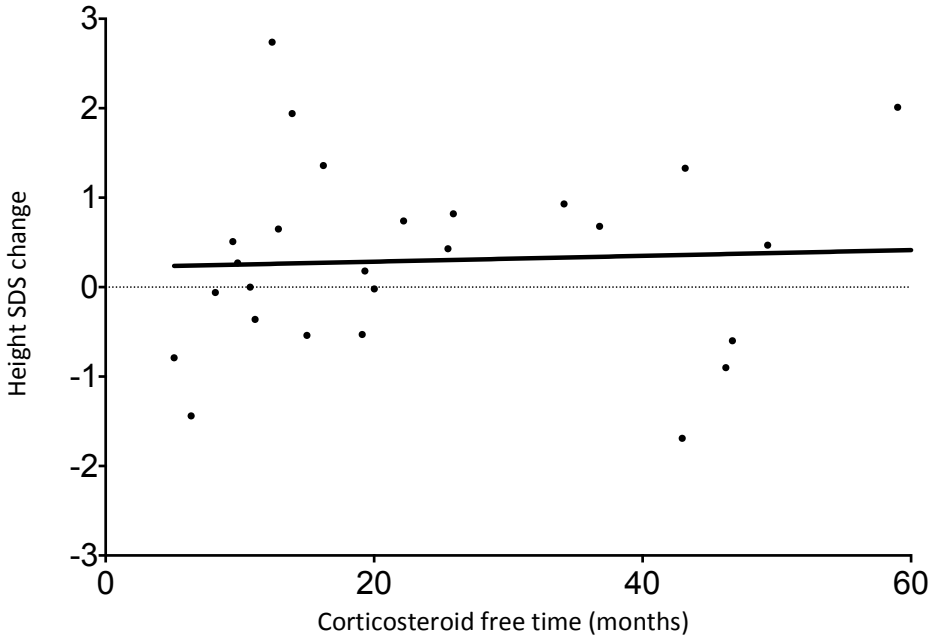

Figure 4.9 Correlation between change in height SDS and time to first CS use
22/56 (42.9%) patients who responded to EEN at diagnosis without requiring any CS usage did not require any CS during the 5 year study period. However, there was no statistically significant correlation between CS-free time and change in height SDS 0-5 years, p=0.53; Spearman's rank correlation.

4.4.4.2 Weight:

The mean [SD] weight SDS at diagnosis was -0.68 [1.28] (Figure 4.10.A). Overall, there was a significant improvement in weight SDS at 1 year (mean [SD] at 1 year -0.24 [1.36]; p<0.001, paired t-test, which was sustained over the 5 years (mean [SD] at 5 years -0.24 [1.39]; p<0.001, one-way repeated measure ANOVA) (Figure 4.10.B). However, there was no difference between change in weight SDS in responders and non-responders to EEN, either at 1 year (mean [SD] +0.41 [0.09] vs +0.47 [0.17], respectively; p=0.77) or after 5 years (mean change in weight SDS [SD] in responders +0.58 [0.13] vs +0.24 [0.24] in non-responders; p=0.17) (Figure 4.11). As expected,

there was a statistically significant positive correlation between increases in weight SDS over the 5 years and change in height SDS; $r=0.45$ $p<0.0001$ (Figure 4.12).

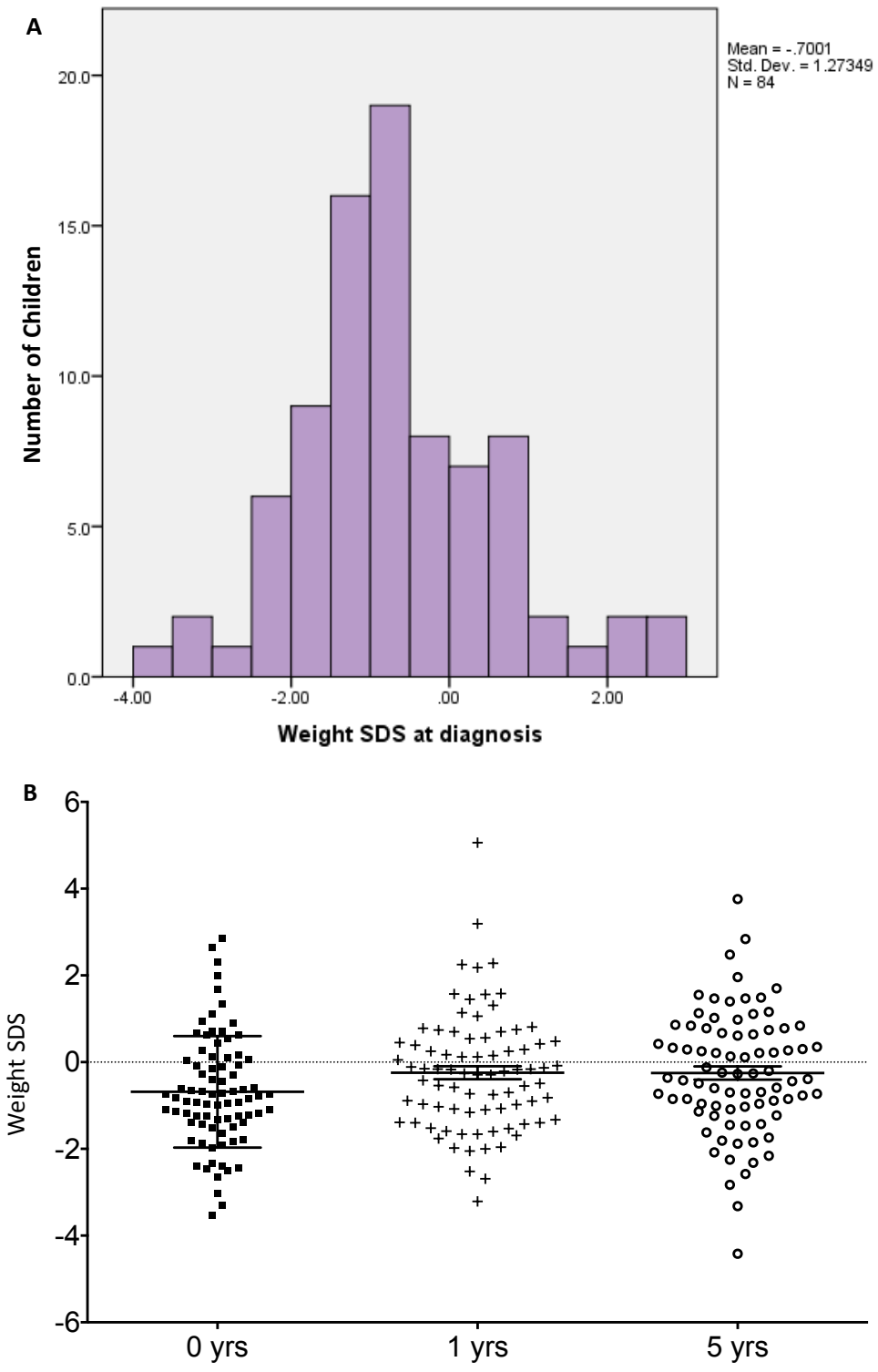

Figure 4.10 Weight SDS in patients treated with EEN.

A) The median weight SDS at diagnosis was -0.84 (range -3.53 to +2.86), mean [SD] -0.68 [1.28]. B) Weight SDS improved over the 1 year (mean [SD] at 1 year -0.24 [1.36]; p<0.001), which was sustained over the 5 years (mean [SD] at 5 years -0.24 [1.39], p<0.001. Means and SD shown

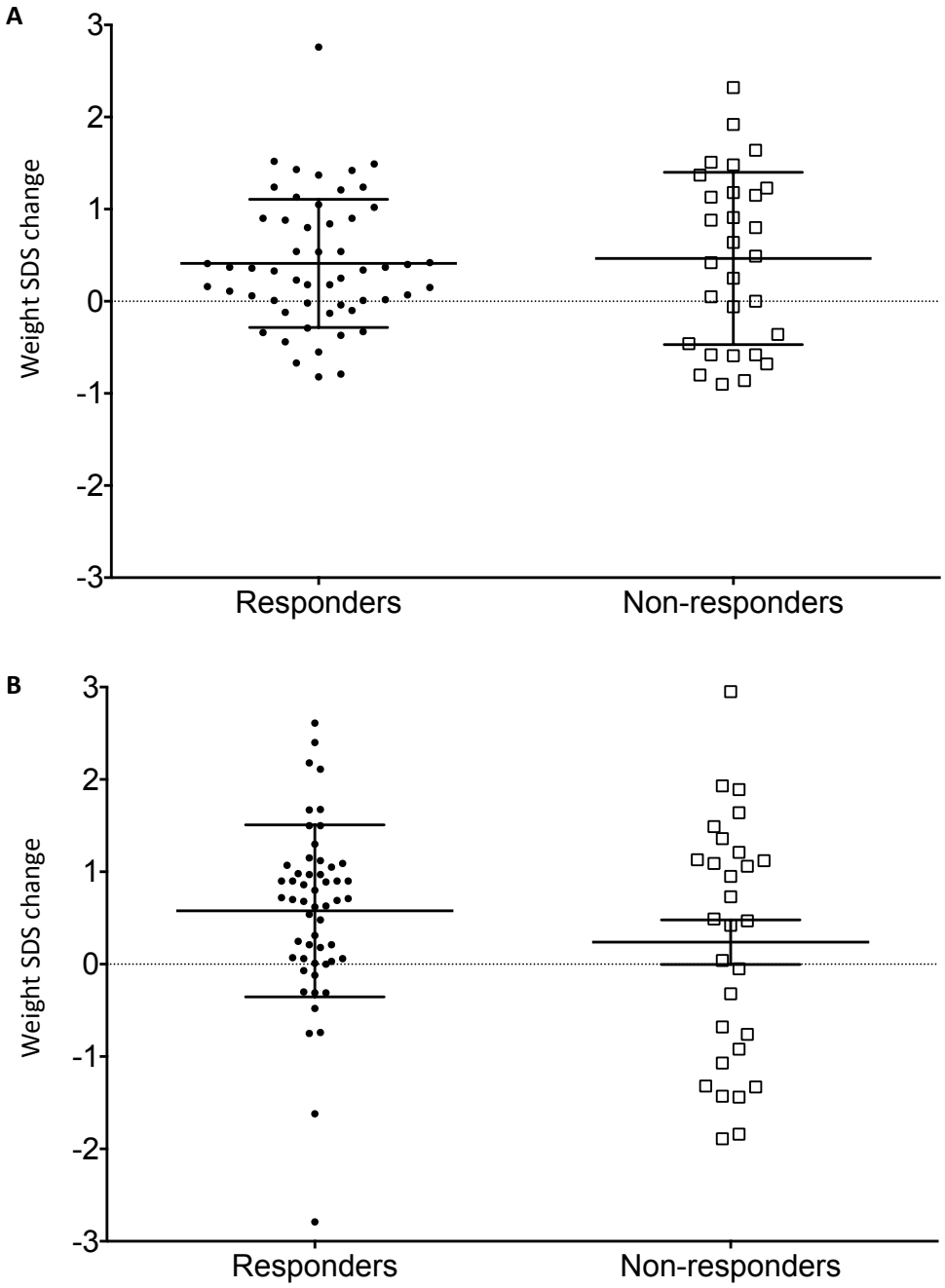

Figure 4.11 Change in weight SDS in responders and non-responders to EEN.
There was no difference between change in weight SDS in responders and non-responders to EEN, either at(A) 1 year (mean [SD] +0.41 [0.09] vs +0.47 [0.17], respectively; p=0.77) or after (B) 5 years (mean change in weight SDS [SD] in responders +0.58 [0.13] vs +0.24 [0.24] in non-responders; p=0.17) (Figure 4.11).

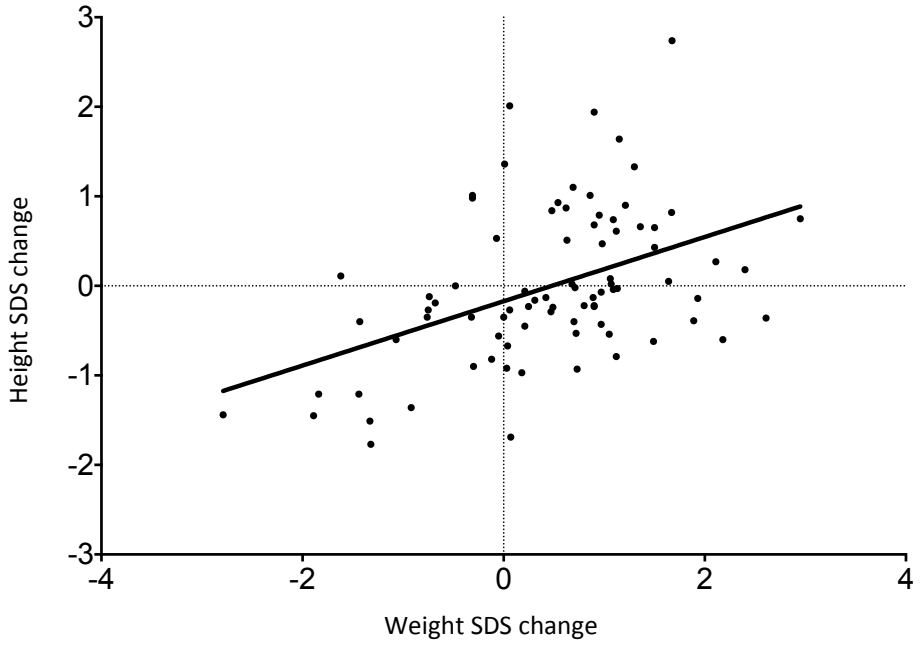

Figure 4.12 Correlation between changes in height and weight SDS 0-5 yrs.
There was a statistically significant positive correlation between changes in weight SDS and height SDS 0-5 years, r=0.45, p<0.001.

4.5 Discussion

One of the commonest reasons given for not using EEN as induction therapy are concerns about compliance [170]. However, around 95% of the children in our study completed the 6 week course of EEN. This is a similar percentage to that seen in previously published data. Indeed, compliance greater than 90% is often described when using EEN in children [156, 164, 174]. None of our patients was given CS as primary therapy, rather than EEN.

63% of our patients achieved complete remission following treatment with EEN. Only 15.7% (14/89) of patients had no response to EEN therapy. Although the percentage achieving complete remission is lower than those reported in some other studies [174,

193], it is similar to the findings of a recent study from Scotland, which reported a remission rate of 60% in 109 newly diagnosed children with CD treated with EEN [194]. It has to be noted that all of the children in our study were prescribed EEN as induction therapy. Therefore, we have no way of knowing whether similar remission rates would have been seen following CS therapy. However, two meta-analyses comparing the use of EEN and CS in inducing remission in children with CD have concluded that there is no difference in effectiveness between the two therapies [156, 183].

In our group of patients, children with perianal disease and with penetrating disease were less likely to respond to EEN. There has been very little information published on the efficacy of EEN in either of these categories, and so there is no consensus as to whether EEN is recommended first line therapy in these situations [55, 288]. We had a small number of patients in each group (25 patients with perianal disease, and 11 with penetrating behaviour), and so no conclusions can be reached for this particular group. However, it does highlight the need for further studies to be conducted in this aspect of CD in order to enable us to start appropriate treatment for these patients at diagnosis.

Interestingly, we found that age was correlated with response to EEN in our cohort of patients. We examined if these findings were due to older children being more likely to have perianal disease or disease with a penetrating phenotype. However, there was no significant correlation between age and either of these factors. Adult studies generally tend to show lower response rates to EEN than those seen in paediatric studies [289]. This is very often due to issues with tolerance, with adult studies having far higher rates of treatment withdrawal due to this [289]. Indeed, in our study, the 5

patients intolerant to EEN did have a mean higher age than those who tolerated the treatment. However, overall tolerance was till 95%, and as there were such a small number in the intolerant group, we cannot draw conclusions from this.

Unfortunately, there are few reports available from other studies with which to compare our results to, to help ascertain whether age was a common factor associated with response. Knight *et al* (2005) found that non-responders to EEN had a lower median age at diagnosis (median [range] 9.2 [6.6-14] years as compared to 12.6 [7.7-15.8] years in responders). However, the numbers from this study were low, with only 4 patients in the non-responder group, and 44 patients in total [193]. It would be interesting to be able to review the ages of patients from other studies investigating response of children with CD to EEN, but these are not available to us. Therefore, we recommend that age is considered when performing future studies regarding the effectiveness of EEN.

In our cohort of patients, multivariate analysis found that PCDAI was significantly associated with response. Children with higher PCDAI scores were less likely to go into complete remission with EEN. However, our results also found that children with perianal disease on average had a higher PCDAI on diagnosis; a fact most likely due to the inclusion of perianal disease assessment in the calculation of the disease activity index [56]. Therefore, the presence of perianal disease may explain our findings. In addition, similar to the question of response, it is important to remember that all of our patients had EEN as induction therapy at diagnosis, and so there is no other treatment group to compare them to. It is reasonable to suppose that children with worse disease are less likely to respond to any induction agent.

Many studies have demonstrated the beneficial effects of EEN to growth over the short term [133, 164]. EEN is known to cause mucosal healing, decrease inflammatory markers and increase levels of anti-inflammatory cytokines and growth-stimulating factors, with effects seen as early as 3 days into treatment [136]. However, there are very few studies investigating the long-term effect of EEN upon growth, and those that there are report no sustained benefit to height SDS over a 1 [193] or 2 year follow up period [194]. In our study, we found that children who responded to EEN had better growth than non-responders, and that this was maintained over the 5 years (Figures 4.6) . This occurred despite no differences in time to relapse (Figure 4.4) or treatment escalation between the groups. In addition, although increased weight was, as expected, significantly correlated with better growth, there was no significant difference in weight gain between responders and non-responders to EEN. Of note, our study followed up both responders and non-responders over the 5 years. If investigated in isolation, our responders showed a significant, but relatively small mean change in height of +0.18 SDS over 5 years (Figure 4.7.A). However, this figure is more striking when compared to non-responders, whose height SDS decreased by a mean of -0.37 (Figure 4.7.B); the resulting comparison finds that responders on average grew +0.5 SD better than non-responders, which corresponds to a significant amount of growth.

As we have discussed, PCDAI scores were negatively associated with response to EEN, with higher scores correlating to no response. Therefore, a possible explanation for the poor growth in the non-responders could be that they had worse inflammation than responders, and this was the factor affecting their growth rather than anything

related to EEN. However, change in height SDS over 5 years did not correlate with the PCDAI at diagnosis. There was, however, a correlation seen between both CRP and ESR at diagnosis and height (Figure 4.8). However, these factors did not correlate with response to EEN.

In our cohort of patients, we found no difference in growth between patients who had been treated with CS during our study period, and those who had not. This is in contrast to the findings of other studies. However, these studies often compare growth in children with CD treated with CS as compared to those treated with EEN [156, 164, 174] or contain patients with a longer duration CS use [220]. In our study, all children were given EEN as primary induction therapy, with CS only being introduced as necessary. Active inflammation has a direct effect upon growth, both through anorexigenic effects, and through direct effects upon the GH / IGF-1 axis [53]. Therefore, it is important that active inflammation is treated. Our study suggests that judicious use of CS at appropriate times may not hinder growth in children with CD.

4.6 Study strengths and limitations.

This is the first study investigating 5 year outcomes in children with newly diagnosed CD treated with EEN as induction therapy. All of the children in the study had 60 months of data available. Data was analysed at specified time points in each patient. At Barts and The London Hospital for Children, children with newly diagnosed CD are all started on EEN instead of CS, regardless of age, disease activity or disease phenotype. This allowed us to investigate the short- and long-term effects in a heterogeneous cohort of patients. In addition, in contrast to other studies, we also collected 5 year data on the non-responders to EEN in order to compare how their disease progressed over time. Whereas the short term differences between responders and non-responders to EEN have been described before, this has not been conducted over a period of years previously. We were able to show that response to EEN was associated with better growth, even if it had no impact on other long-term outcomes, such as surgery rates or time in remission.

However, this was a retrospective study. Therefore, data collected for this study was completely dependent upon clinical notes and electronic records. Unfortunately, information was missing for some patients at some time points. Also, although there was a standardised protocol for EEN therapy and subsequent food reintroduction, other treatments were instigated at the treating physician's discretion. In addition, although we did have a very varied cohort of patients, this study was conducted at a single centre. It would be very interesting to compare our results with findings from elsewhere to see if the results are the same, or in the event of differences, to try and ascertain as to why.

4.7 Future directions

In our cohort of patients, those who responded to EEN showed better growth over a period of 5 years than patients who did not respond. It would be very interesting to see whether this difference continued through to affect final adult height. It would be ideal to conduct this study prospectively, following children from the time of diagnosis through to final height, allowing for standardised assessments at standardised time points. This would minimise the amount of missing data.

The study raises two important questions: first, why do some patients respond to EEN and others not? In our study, we found that younger age was associated with response, something that has not been reported before. We also found that our patients who were intolerant of EEN had a higher mean age than those who tolerated the treatment. However, as we had only 5 children who were unable to tolerate the EEN, this may have been an incidental finding. A prospective multicentre study containing a wide age range of patients would help determine whether age is, indeed, a factor related to response to EEN, and whether this is in part due to problems of tolerance.

Secondly, although we have an idea as to why treatment with EEN improves growth in the short term, it would be interesting to see why response to EEN has such a sustained impact upon growth, independent of CS use or time in remission.

4.8 Conclusion

EEN was well tolerated, and induced complete remission in over 60% of our patients. In our cohort of patients, we found that younger children, and children with lower PCDAI scores were more likely to respond to EEN. Response to EEN was associated with improved growth, which was sustained over the 5 year study period. This occurred despite no significant difference between time spent in remission, CS use or treatment escalation in each group. This study provides further evidence of the benefits of inducing remission in children with CD with EEN, as well as highlighting directions for future research in this area.

5 Results: The impact of thiopurines upon disease activity and growth in children with CD

5.1 Introduction

Azathioprine (AZA) and its active metabolite 6-mercaptopurine (6-MP) are frequently used for the maintenance of CS-free remission in both adults and children with CD [55, 211]. Although the side effects profile include bone marrow suppression, hepatotoxicity and pancreatitis, children are reported to have lower rates than adults of intolerance and subsequent discontinuation due to adverse effects [50, 290].

Several studies have described the efficacy of thiopurines in children [210, 212, 216]. This includes an American randomised controlled trial performed by Markowitz *et al* [209] which found that following induction of remission with CS, relapse rates in patients in the 6-MP arm were only 9%, as compared to 47% in the placebo arm. However, in recent years, the efficacy of thiopurines has been questioned. Indeed, a recent paediatric study found no statistically significant difference in remission rates between those treated with early immunomodulators as compared to no immunotherapy use at all [219], while recent studies in both adults [215] and children [219] have found that anti-TNF use is superior to thiopurine use in newly diagnosed patients with CD. In our centre, every patient who is started on anti-TNF therapy is also on concurrent thiopurine treatment, either already established or initiated at the same time as the anti-TNF. Therefore, it was not possible in this thesis to be able to analyse the effects of thiopurines and anti-TNFs independently.

In contrast to studies looking at the impact of surgery [291, 292] and anti-TNF therapy [222, 293], no study has demonstrated a beneficial effect of either AZA or 6-MP on growth in children with CD [220], even when the thiopurine was efficacious at maintaining remission [209]. This is a surprising finding. Given that pro-inflammatory cytokines have been shown to have a direct impact upon growth in children with CD, both through anorexigenic effects [119, 120], and direct action on the GH/IGF-1 axis [89], any therapy that results in suppression of inflammation without the use of CS could be expected to cause improvement in growth. One possible explanation for these findings is that the studies evaluating thiopurines have always occurred at centres, which use CS as primary induction to remission therapy. In addition to having known adverse effects upon growth [287], CS do not result in the mucosal healing that is known to occur with EEN [174]. It is not known what effect thiopurines would have upon growth in children with CD treated in a centre such as ours that utilises EEN as its primary form of induction therapy.

5.4 Results

There were 59 patients aged <17 years who were started on thiopurine therapy between 1st January 2007 to 31st December 2010 at the paediatric IBD clinic at Barts and The London Hospital for Children. All patients were started on azathioprine. One patient transferred her care to another centre within a year, and so was excluded from the study.

60.3% (35) patients were male and 53.4% (31) Caucasian. The majority of patients had ileo-colonic disease (79.7%), of non-stricturing, non-penetrating behaviour (72.4%) as defined by Montreal classification [48] (Table 5.1). The median (range) age at starting AZA was 12.70 (6.65 - 16.71) years, with a median (range) disease duration of 0.97 (0.07 - 6.38) years at initiation of therapy (Figure 5.1 The mean starting dose was 1.8mg/kg (Figure 5.2). Children were initiated on the dose that they were to be maintained on.

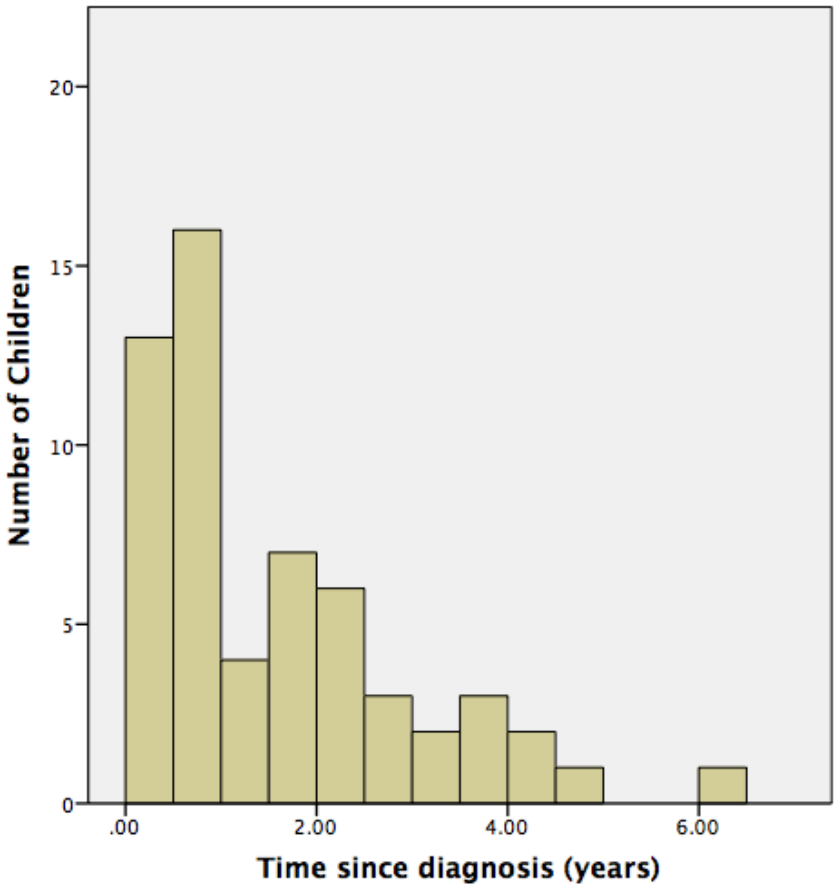

Figure 5.1 Disease duration at initiation of thiopurine therapy.
Children had been diagnosed with CD for a median (range) of 0.97 (0.07 - 6.38) years at the time thiopurine therapy was started.

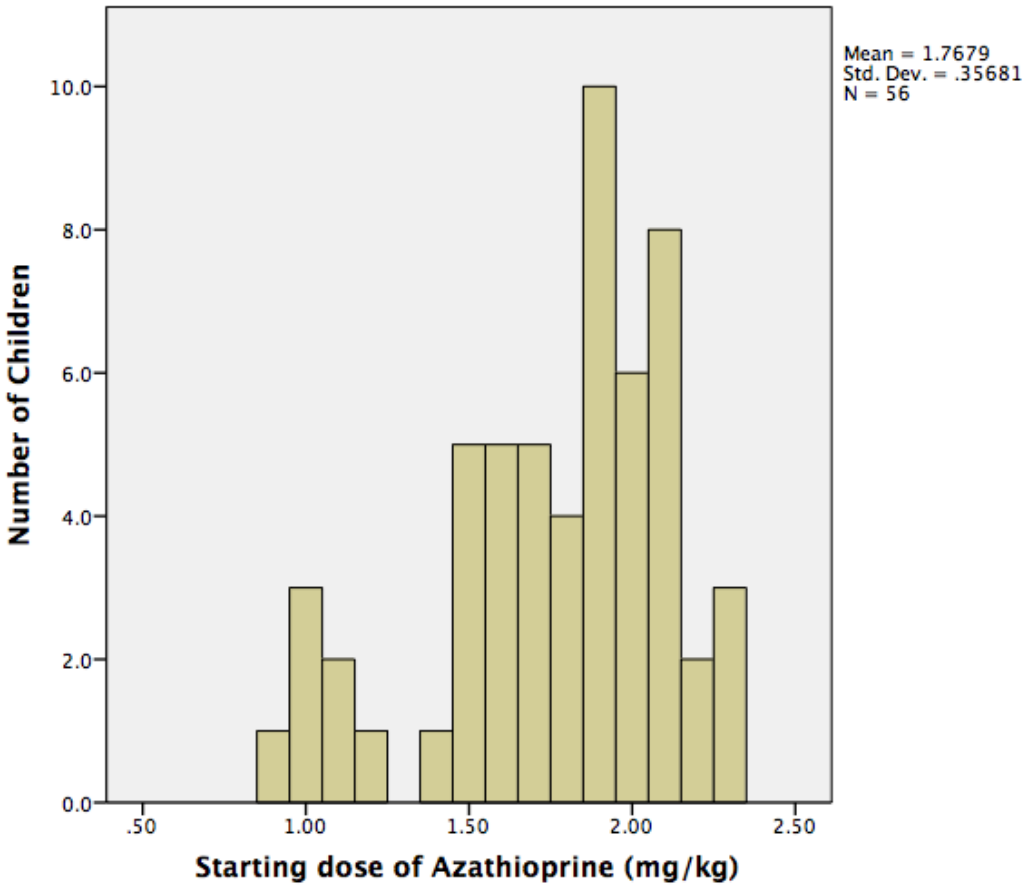

Figure 5.2 Starting dose of azathioprine.
The mean [SD] starting dose was 1.8 [0.36] mg/kg. However, there was a smaller peak around 1.0mg/kg. This reflects the patients with TPMT heterozygosity. These patients are started on a lower dose of thiopurine due to the higher risk of toxicity [271]. The starting dose was unavailable in 2 patients.

Table 5.1 Demographics, disease phenotype, behaviour and activity, medications and TPMT activity at the time of starting thiopurine therapy.

	Patients (n=58)
M:F	1.5 : 1
Age	
Median age (range)	12.70 (6.65 – 16.71) yrs
Median (range) age at diagnosis	11.19 (4.84 – 15.38) yrs
Median (range) disease duration at start AZA (yrs)	0.97 (0.07 – 6.38)
Ethnicity	
Caucasian	31 (53.4%)
Asian	21 (36.2%)
Afro-Caribbean	2 (3.4%)
Other	4 (6.9%)
Location	
L1 Ileal	11 (19.0%)
L2 Colonic	6 (10.3%)
L3 Ileo-colonic	41 (70.7%)
Behaviour	
B1 Non-stricturing non-penetrating	42 (72.4%)
B2 Stricturing	3 (5.2%)
B3 Penetrating	13 (22.4%)
Upper (L4)	24 (41.4%)
Perianal (p)	19 (32.8%)
Medications	
EEN	10 (17.2%)
Prednisolone	26 (44.8%)
5-ASA	49 (84.5%)
Infliximab	4 (6.9%)
Surgery	5 (8.6%)
Height SDS mean [SD]	-0.42 [1.21]
Weight SDS mean [SD]	-0.41 [1.31]
ESR median [IQR] mm/hr	23.0 [8.0 – 34.0]
CRP median [IQR] mg/l	15.0 [5.0 – 28.0]
PCDAI median [IQR]	21.25 [15.0 – 31.5]
Mean [SD] starting dose AZA mg/kg	1.8 [0.4]
TPMT mean [SD] units/ml RBC	32.5 [6.9]

5.4.1 Aim 1: To assess the tolerance to thiopurines of children with CD started on therapy between 2007-2010

15 of the 58 patients (26%) followed up for 1 year had adverse reactions to azathioprine: 4 patients developed transaminitis, 6 lymphopenia, 2 raised amylase and 3 headaches. The 3 patients with headaches were switched to 6-MP, which resolved these symptoms in 2 of the patients. From the 58 patients, 7 patients (12%) discontinued therapy within the year's follow-up period: 4 due to lymphopenia, 2 due to raised amylase and 1 because of headaches.. The ability to measure levels of the thiopurine metabolite 6-thioguanine nucleotide (6-TGN) would have enabled us to determine whether this was related to high 6-TGN levels [208]. However, this test was not routinely undertaken during the study period of 2007-2010.

5.4.2 Aim 2: To determine the response of CD activity to thiopurine therapy

The 7 patients who discontinued thiopurines were excluded from further analysis, leaving 51 patients remaining in the study. 49 of these patients were on AZA and 2 on 6-MP.

The median CRP [IQR] of patients at initiation of therapy was 15.0 [5.0 - 28.0] and ESR 23.0 [8.0 - 34.0]. 41.2% (21) of patients had moderate-severe disease as determined by PGA, and 47.1% (24) of patients mild-moderate disease. Only 11.7% (6) patients had inactive disease. AZA was started in 3 of these patients as prophylaxis following bowel resection, and 3 because of frequent relapses. PCDAI scores were available in 48 out of the 51 patients. The median [IQR] was 21.25 [15 - 31.5].

Overall, the parameters of disease activity improved significantly over the course of a year. At 3 months, 11 (22.6%) patients were in remission, as determined by CS-free PGA of no disease activity, as compared to 15 (29.4%) at 6 months, and 26 (51.0%) patients at 12 months ($p<0.001$, Chi-squared analysis). PGA, CRP and PCDAI improved at each time point (Figure 5.3). However, even though ESR did show a significant decrease from baseline to 12 months, this was small (median [IQR] at 0 months 23.0 [8.0 - 34.0] vs 14.0 [8.0 - 31.0] at 12 months; $p<0.0001$). PGA was available for each patient at every time point. CRP, ESR and PCDAI were analysed using the Friedman test. 43 patients for CRP analysis, 44 patients for ESR and 42 for PCDAI had data available at each time point.

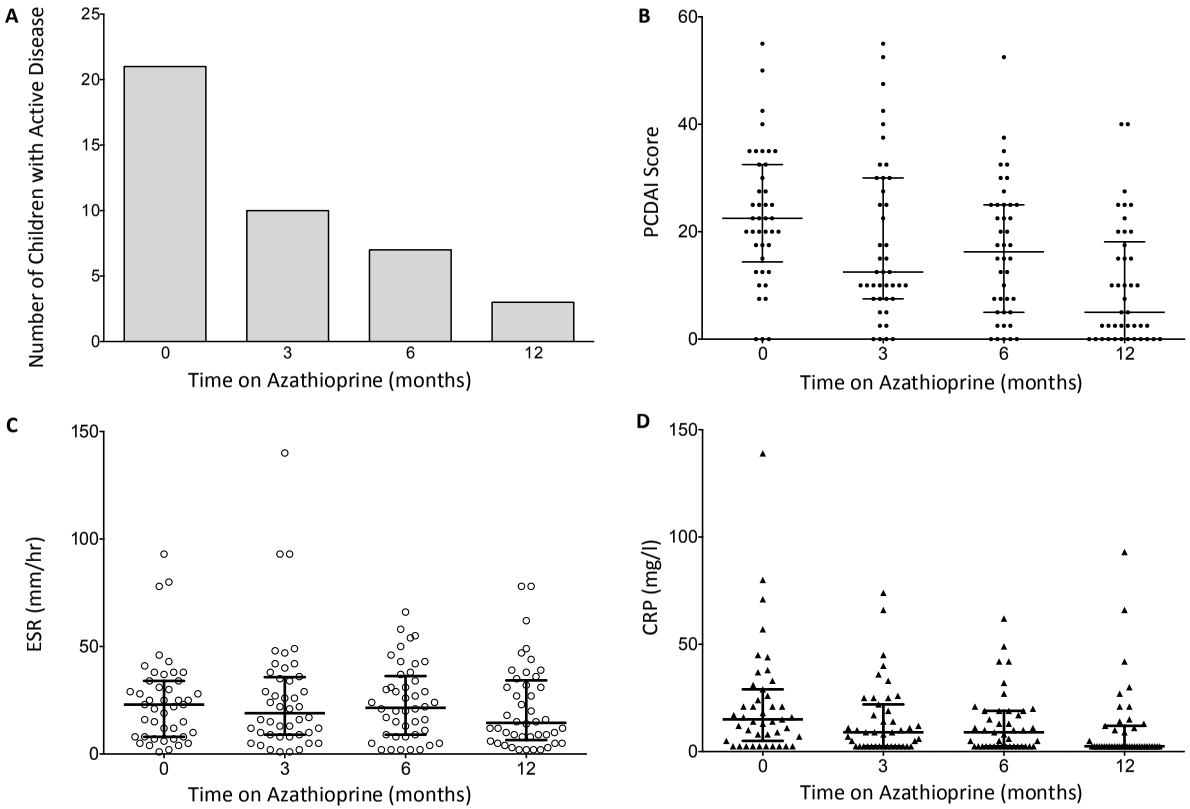

Figure 5.3 Disease activity improved in the year following initiation of AZA therapy.

A) Children with moderate-severe disease, as determined by PGA decreased from 41.2% (21) at the start of AZA therapy to 27.5% (14) at 3 months, 15.7% (8) at 6 months and 11.8% (6) at 12 months (Chi squared analysis, $p<0.0001$). B) The median [IQR] PCDAI decreased at each time point, from 21.15 [15.0 - 32.5] at the start of treatment to 12.5 [7.5 - 25.0] at 3 months, 15.0 [5 - 25] at 6 months, and 8.75 [0 - 15] at 12 months ($p<0.0001$). C) Although median ESR decreased from baseline (median [IQR] 23.0 [8.0 - 34.0]) to 12 months (14.5 [6.5 - 34.25]) $p<0.001$, the median ESR remained elevated throughout the year (median [IQR] at 3 months 19.0 [9.0 - 35.75]] and 21.5 [9.0 - 36.25] at 6 months). D) Median [IQR] CRP decreased from 15 [5.0 - 29.0] at 0 months to 9.0 [2.5 - 19.0] at 3 months, 9.0 [2.5 - 19.0] at 6 months, and 2.5 [2.5 - 12.0] at 12 months ($p<0.0001$). Medians and IQR are indicated by the error bars.

Within this cohort, the MCV also increased significantly over the 12 months, from a median [IQR] of 73.7 [69.7 - 80.2] fl at baseline to 80.7 [76.1 - 84.8] fl at 1 year ($p<0.0001$).

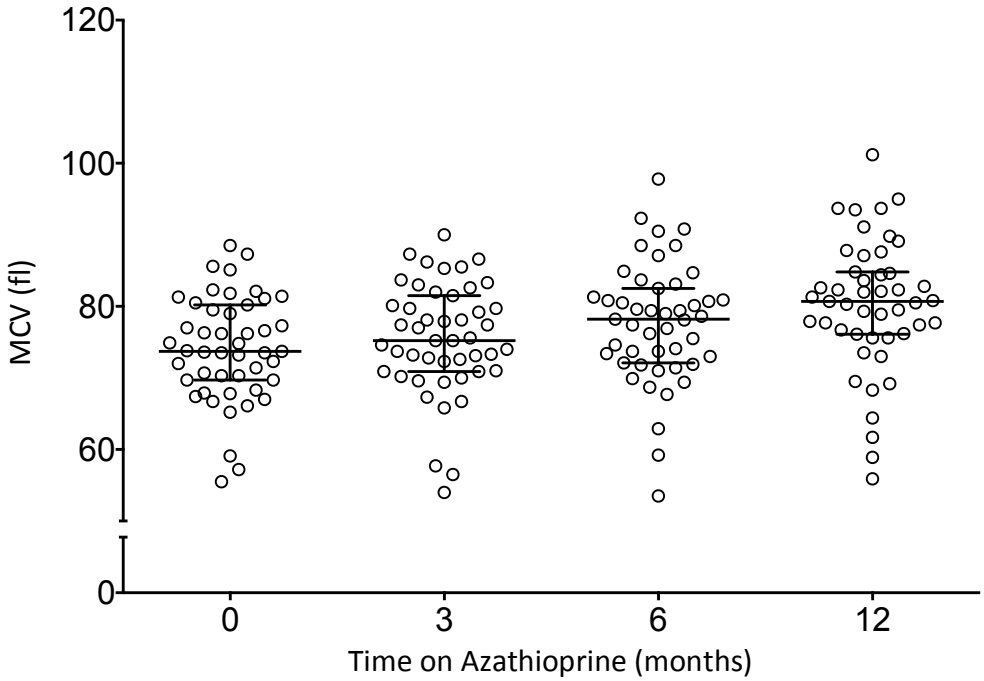

Figure 5.4 Change in MCV following initiation of thiopurine therapy.
The median [IQR] MCV at the start of thiopurine treatment was 73.7 [69.7 - 80.2] fl. It significantly increased over the year to 75.2 [70.9 - 81.5] fl at 3 months, 78.2 [72.1 - 82.5] fl at 6 months, and 80.7 fl [76.1 - 84.8] fl at 12 months ($p<0.0001$, Friedman test).

CS use decreased over the year. At baseline, 52.9% of patients (27/51) were on CS, as compared to only 13.7% (7/51) at the end of the 12 month follow-up period ($p<0.0001$) (Figure 5.4)

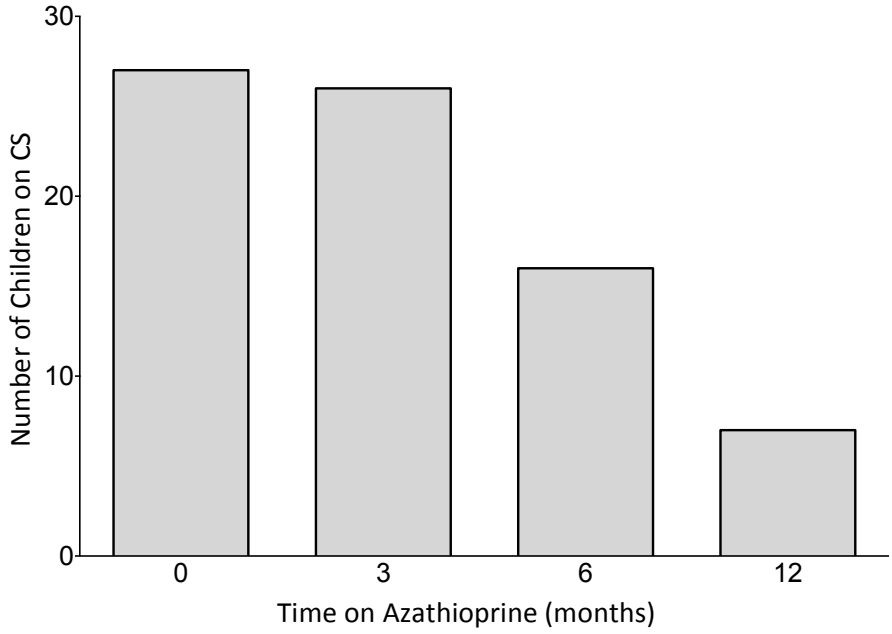

Figure 5.5 CS use over 12 months in children with CD treated with AZA
CS use decreased over the 12 months, from 52.9% [27] of patients at the start of thiopurine therapy, to 51.0% at 3 months, 31.4% at 6 months and 13.7% at 12 months (Chi squared analysis $p<0.0001$)

Approximately half of the patients (25/51 patients; 49%) started on AZA had either bowel resection or anti-TNF therapy in addition to AZA over the 1 year study period. This, of course, makes it very difficult to properly assess the response to thiopurine treatment alone. However, when these 25 patients who had additional treatment were excluded from analysis, the same pattern of results was seen (Figure 5.5).

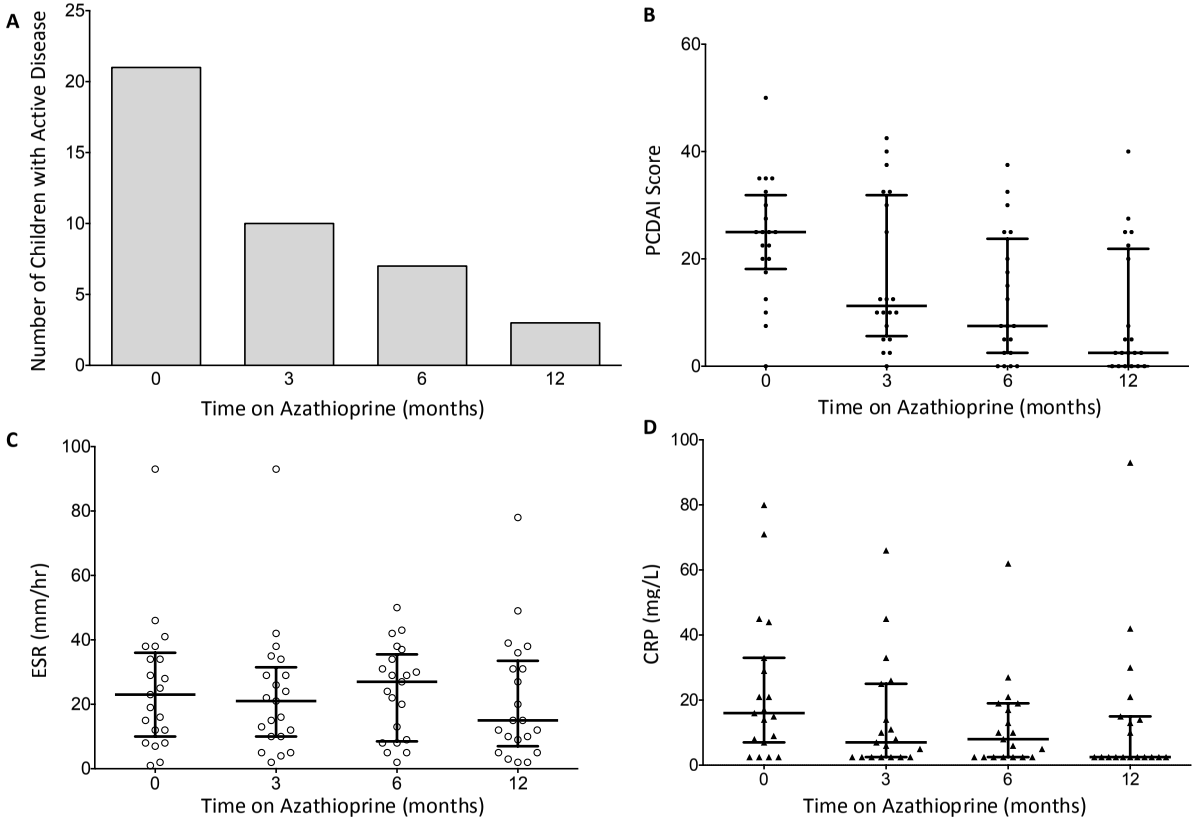

Figure 5.6 Disease activity in patients treated with AZA alone

A) Children with moderate-severe disease decreased from 80.8% (21) at the start of therapy to 38.5% (10) at 3 months, 26.9% (7) at 6 months and 11.5% (3) at 12 months ($p<0.001$). B) Changes in PCDAI scores in 20 children at each time point. Median [IQR] PCDAI decreased from 25 [18.75 - 32.5] at the initiation of AZA to 11.25 [5.625 - 32.25] at 3 months, 7.5 [2.5 - 23.75] at 6 months and 2.5 [0 - 22.5] [12.2] at 12 months (Friedman test $p=0.003$). C) Median ESR remained elevated throughout the 12 months, but decreased from median [IQR] 23.0 [10 - 36.0] at baseline, to 21.0 [10.0 - 31.5] at 3 months. It increased at 6 months (median [IQR] 27.0 [8.5 - 35.5], decreased at 12 months (median [IQR] 15.0 [7.0 - 33.5]) $p<0.001$, Friedman test. D) Median [IQR] CRP decreased over 12 months, from 16.0 [7.0 - 33.0] at 0 months to 7.0 [2.5 - 25.0] at 3 months, 8.0 [2.5 - 19] at 6 months, and 2.5 [2.5 - 15.0] at 12 months ($p<0.001$). Median and IQR are indicated by the error bars

5.4.3 Aim 3: To assess the impact of thiopurine therapy on growth in these patients

The mean [SD] height SDS at the initiation of thiopurine therapy was -0.35 [1.20] (Figure 5.7) There was no association seen between ESR and height SDS (p=0.17, multivariate analysis). Surprisingly, multivariate analysis found no association between PCDAI score and height SDS (p=0.34). However, there was a trend towards CRP being negatively associated with height SDS at diagnosis, but this failed to reach statistical significance (p=0.06).

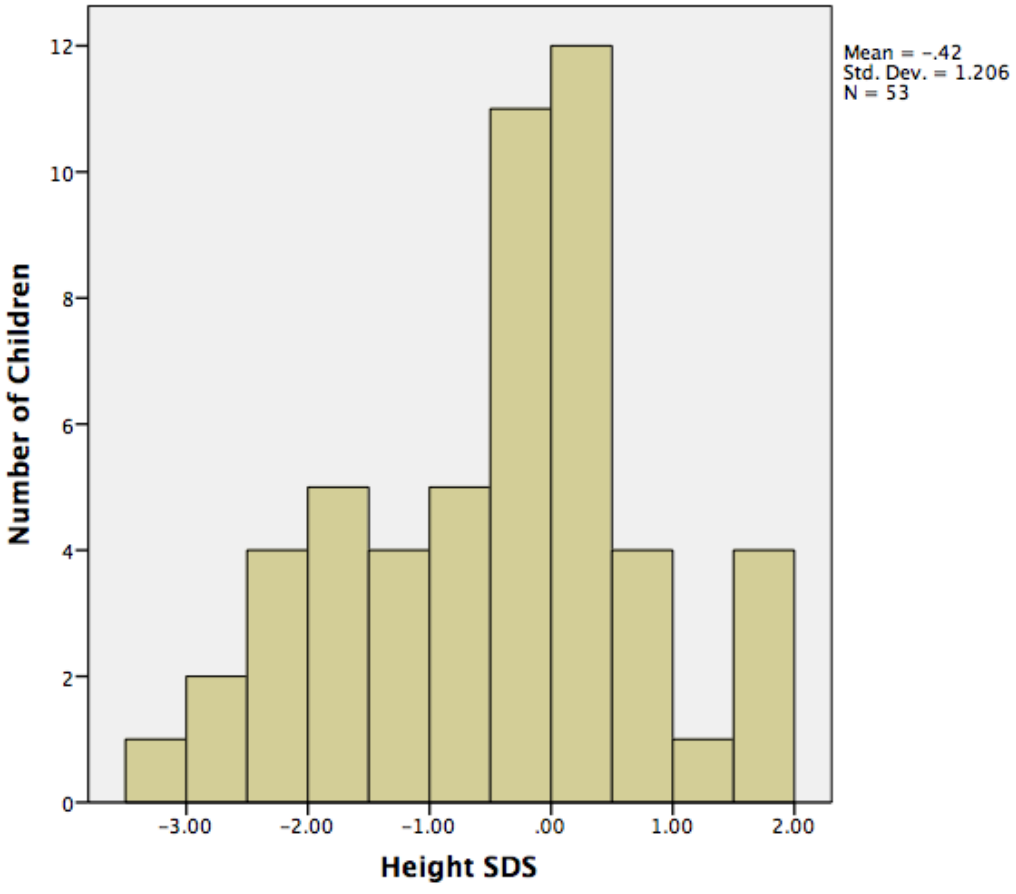

Figure 5.7 Baseline height SDS at start of azathioprine treatment

Despite the improvements seen in markers of disease activity over the year, overall, there was no improvement seen in height SDS scores. In fact, height SDS significantly decreased during this time from a median [IQR] of -0.26 [-1.19 to +0.38] at 0 months, -0.49 [-1.63 to +0.31] at 6 months and -0.50 [-1.30 to +0.31] at a year ($p<0.0001$, Friedman test) (Figure 5.8.A). This occurred despite a reduction in CS usage over the year (Figure 5.5) as well as approximately 50% of patients also having anti-TNF therapy or surgical resection.

However, when growth was compared between the 26 patients who had responded to thiopurine therapy at 12 months, as compared to the non-responders, a significant difference was seen in change of height SDS over the 12 months between the groups. The responders had a median [IQR] change in height SDS of 0.08 [-0.06 – 0.19], as opposed to -0.24 [-0.61 - -0.07] in non-responders ($p=0.001$, Mann Whitney) (Figure 5.8.B).

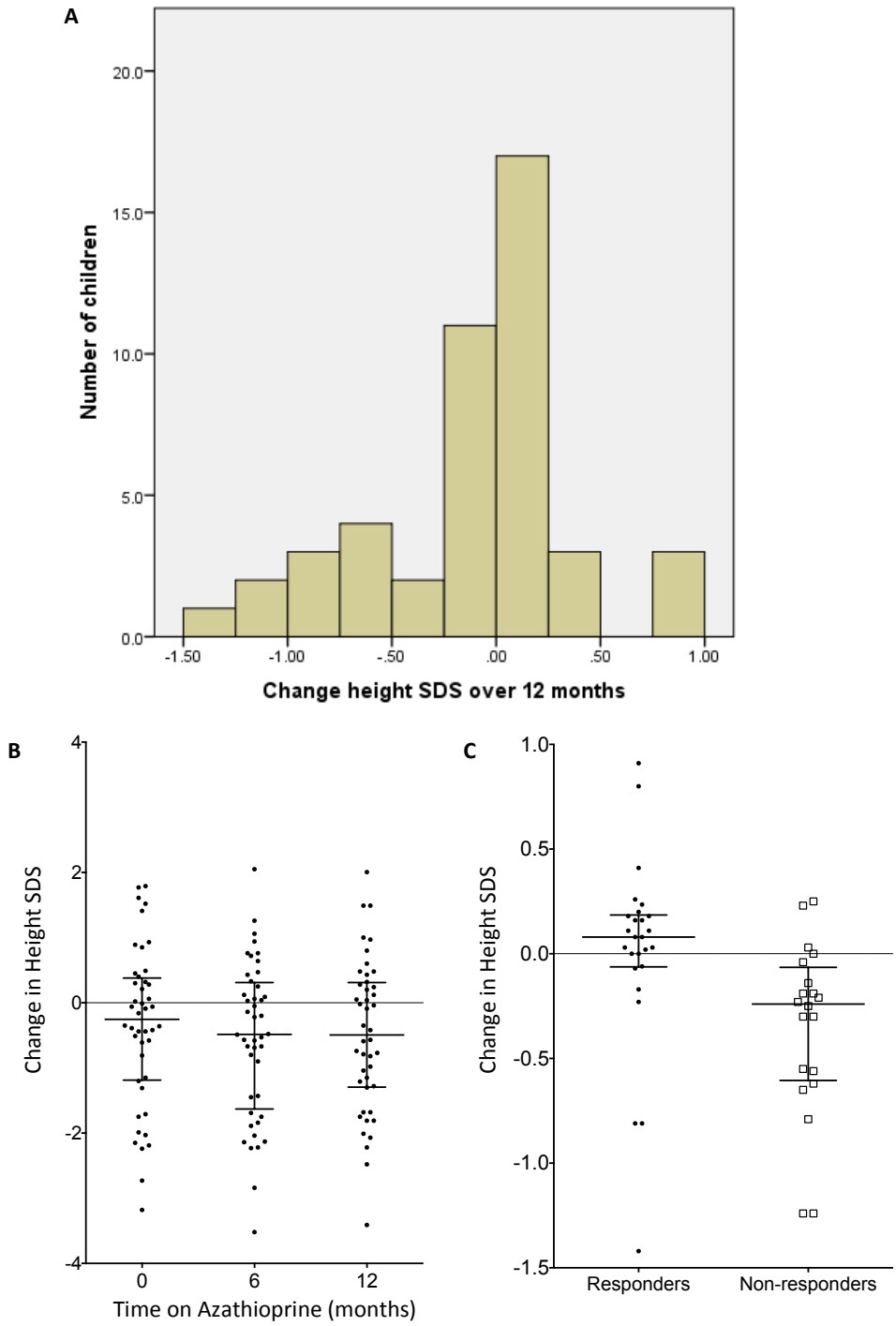

Figure 5.8 Change in height SDS in the year following azathioprine treatment

There was no improvement in height SDS seen over the course of 12 months following initiation of thiopurine therapy. A) The change in height SDS over 12 month. Data was available from these two time points in 46 patients. The median change in SDS was -0.02, with a range of -1.42 to +0.91. B) Height SDS at 0, 6 and 12 months were available in 44/51 (86.3%) of patients. The height SDS decreased significantly over the year, from median [IQR] -0.26 [-1.19 to +0.38] at 0 months to -0.49 [-1.63 to +0.31] at 6 months and -0.50 [-1.30 to +0.30] at 12 months ($p<0.001$). C) Responders to thiopurines had better growth over the 12 months than non-responders (median [IQR] 0.08 [-0.06 – 0.19] vs -0.24 [-0.61 - -0.07] in non-responders; $p=0.001$, Mann Whitney). Error bars show medians and IQR.

Our definition of response was CS-free PGA of inactive disease. Therefore, it is possible that the differences in height SDS between responders and non-responders to thiopurines might be a reflection of CS use. Overall, 58.8% (30/51) of patients received a course of CS at one time during the study period. However, although the non-CS group had a higher median [IQR] change in height SDS than the children who had received CS (+0.03 [-0.19 to +0.16] vs -0.17 [-0.55 to +0.16], respectively) (Figure 5.9), this difference was not statistically significant when groups were compared using the Mann-Whitney U test (p=0.27).

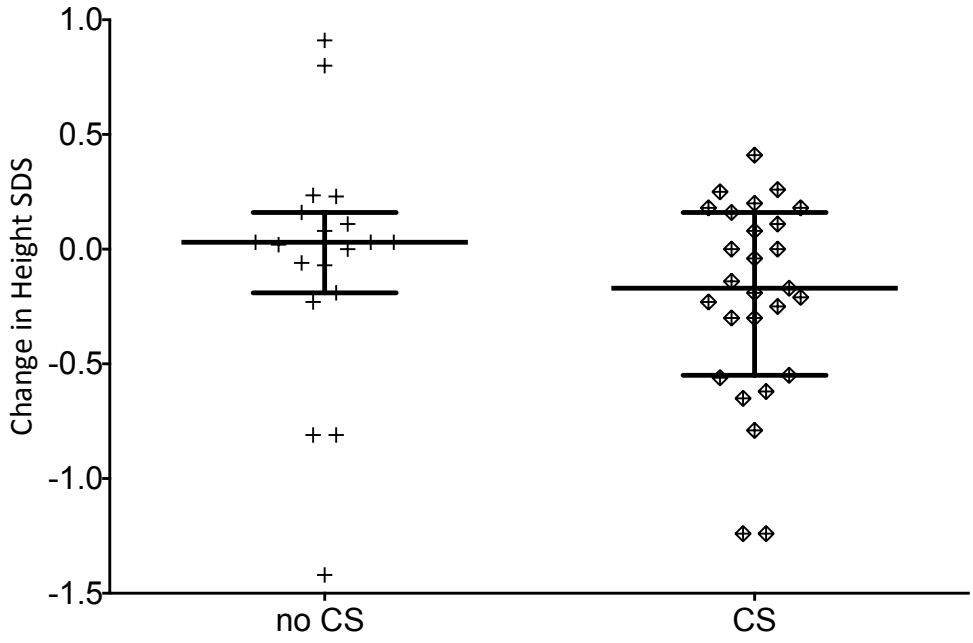

Figure 5.9 Change in height SDS in children treated with azathioprine who received corticosteroids compared with those who did not
Although the non-CS group had a higher median [IQR] change in height SDS (+0.03 [-0.19 to +0.16] vs -0.17 [-0.55 to +0.16], respectively), this difference was not statistically significant (p=0.27). Medians and IQR indicated by error bars.

Changes in MCV can be used as an indicator of 6-TGN levels. It has been reported that an increase in MCV of at least 6 fl is expected to reflect a 6-TGN level of around 175 pmol/8 x 10^8 RBCs [270]. Therefore within the 26 responders to azathioprine, we investigated whether there was a difference in growth between the 15 (57.7%) patients who increased their MCV >6fl over the 12 months, and the 11 (42.3%) who did not. However, there was no statistically significant difference between these groups (median [IQR] change in height SDS in responders +0.16 [0 to +0.24] vs +0.03 [-0.23 to +0.11] in non-responders; p=0.13, Mann Whitney) (Figure 5.10).

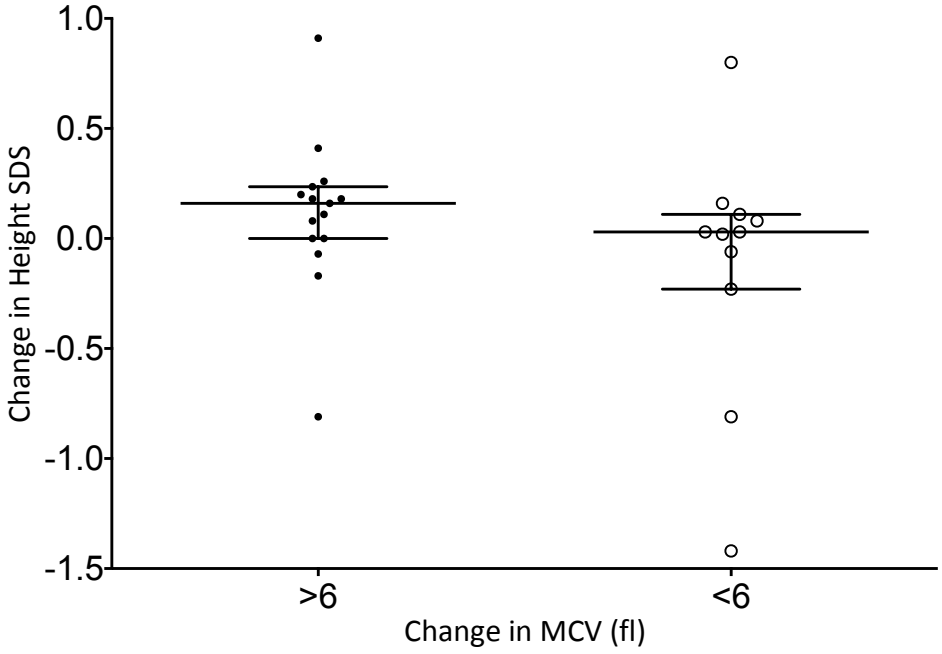

Figure 5.10 Within responders, 6 fl change in MCV and change in height SDS
Within the responder group, there was no significant difference between change in height SDS over 12 months in patients who increased MCV >6 fl, as compared to those who did not (median [IQR] change in height SDS in responders +0.16 [0 to +0.24] vs +0.03 [-0.23 to +0.11] in non-responders; p=0.13, Mann Whitney). Median and IQR indicated by error bars.

Mutlivariate analysis did not show any statistically significant association between change in height SDS and gender (0.58), ethnicity (0.24), disease location (p=0.43) or disease behaviour phenotype (according to Montreal classification) (p=0.82), age at starting AZA (p=0.60) or disease duration at the time of starting AZA (p=0.41). However, there was a statistically significant association with TPMT (p=0.04). Higher TPMT levels were associated with smaller changes in height SDS. It is possible that, because of the increased enzyme activity, the circulating concentrations of active thiopurine metabolites were lower. However, as 6-TGN levels were not routinely measured in this cohort of patients, we cannot test this hypothesis in this study.

50% (25/51) of patients had either surgery or anti-TNF therapy in addition to AZA during the year. However, there was no significant difference in change in height SDS over the year between these patients and the cohort who remained on AZA alone (median [IQR] +0.001 [-0.26 to +0.17] vs -0.07 [-0.36 to +0.13], respectively; p= 0.86) (Figure 5.11).

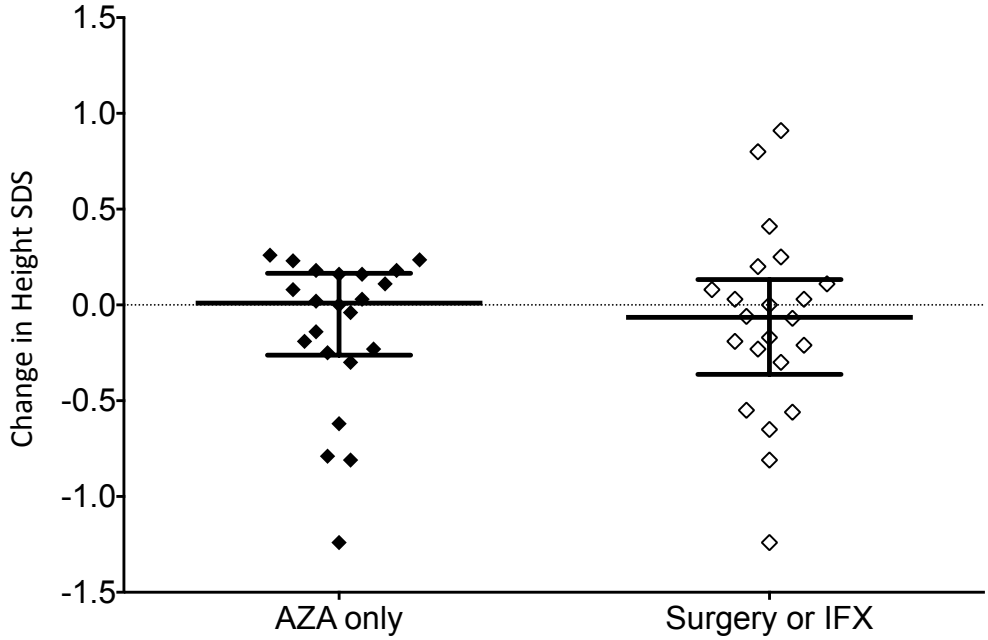

Figure 5.11 Change in height SDS in patients who remained on azathioprine alone as compared to those who underwent surgical resection or infliximab treatment
There was no significant difference in change in height SDS over the year between patients that remained on AZA alone, and those who also received surgery or anti-TNF therapy with the 12 month study period (median [IQR] +0.001 [-0.26 to +0.17] vs -0.07 [-0.36 to +0.13], respectively; p= 0.86). Median and IQR indicated by error bars.

5.4.4 Aim 4: To assess the impact of anti-TNF therapy on growth.

As previously discussed, all children in our centre who are started on anti-TNF therapy also receive thiopurines. Therefore, it is not possible to separate the effects of the thiopurine from the anti-TNF effects in the group receiving an anti-TNF. However, when comparing changes in height SDS between patients who were started on anti-TNF therapy and those who were not, no statistically significant difference was seen (p=0.71) (Figure 5.12.A).

We further examined the 20 patients who were commenced on anti-TNF therapy. These patients were also followed up for a 12-month period following initiation of therapy. Due to the small number of patients, in order to try and avoid type I or type II errors, data was only collected at 0 and 12 months. 50% (10/20) patients had moderate-severe disease at diagnosis, as classed by PGA. This significantly decreased to 15% (3/20) after 12 months of treatment (p=0.04, Fisher's exact test). 8/20 (40%) of patients had a complete response to anti-TNF therapy at 12 months, as defined bby CS-free PGA of inactive disease. However, there was no improvement seen in ESR (median [IQR] 40.0 [12.75 - 39.25] at 0 months vs 27.5 [8.75 - 55.5] at 12 months, p=0.43, Wilcoxon signed rank), or CRP median [IQR] at 0 months 8.5 [3.625 - 23.0] vs 2.5 [2.5 - 30.5] at 12 months; p=1, Wilcoxon signed rank), or PCDAI (median [IQR] at 0 months 23.75 [12.5 - 25.5] and 15.0 [5.0 - 25.0] at 12 months; p=0.34, Wilcoxon signed rank) over the 12 months. However, it must be noted that the numbers included in each analysis were small, with 15 patients having paired data available for ESR analysis, 16 for CRP, and 14 for PCDAI.

Only 13 patients had height SDS available at 0 and 12 months. In addition, there was no significant change in height SDS from the start of anti-TNF therapy to the end of the 12-month follow-up period (median [IQR] at 0 months -0.38 [-1.73 to -0.10] as compared to -0.61 [-1.72 to -0.09] at 12 months; p=0.43, Wilcoxon signed rank) (Figure 5.12.B). There was no significant difference seen between growth in patients who had responded to anti-TNF therapy, and those who did not (median [IQR] change in height SDS in responders -0.23 [-0.29 to +0.34] vs -0.19 [-0.39 - +0.14] in the non-responders;

p=0.75, Mann Whitney) (Figure 5.12.C). However, as only 13 patients were involved in this analysis, this might be due to a type II error.

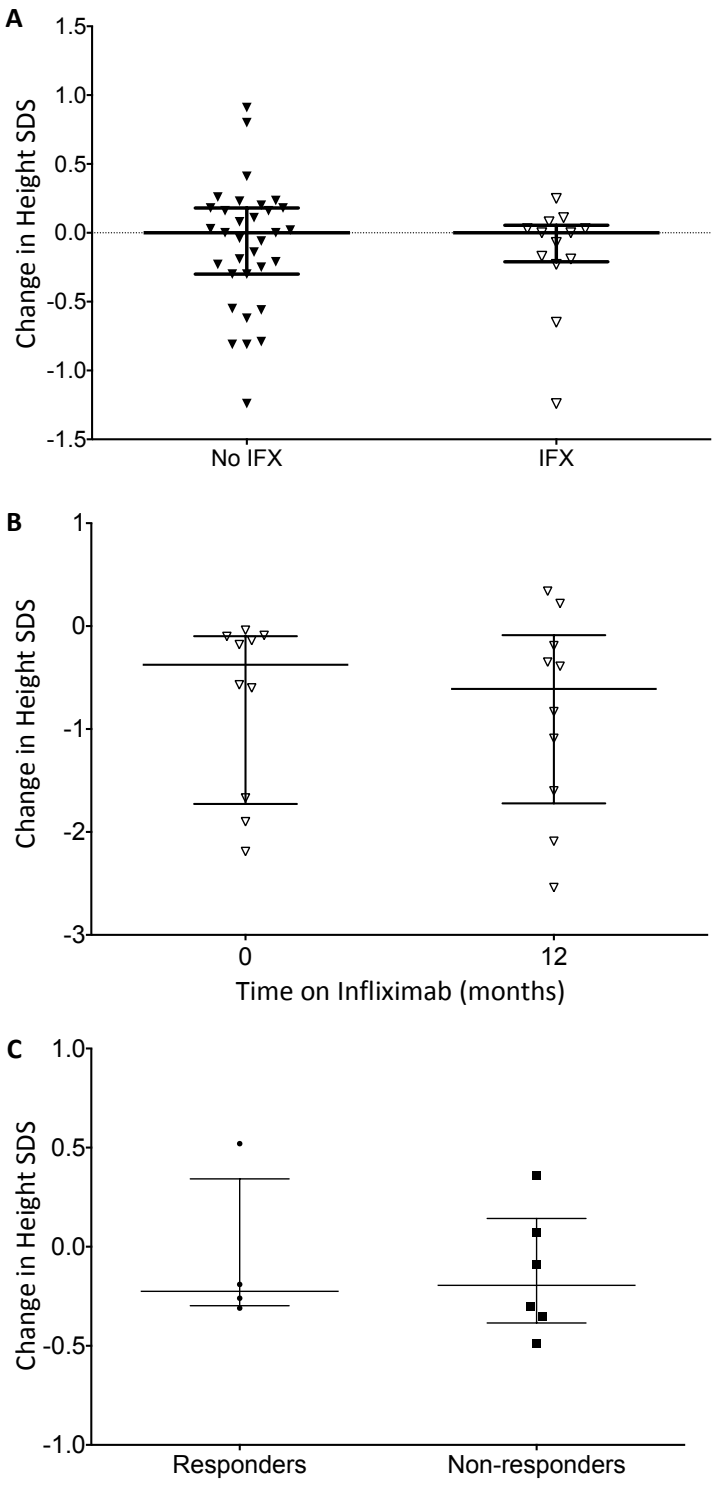

Figure 5.12 Anti-TNF therapy and change in height over 12 months

A) Height SDS at 0 and 12 months was available in 46 patients, 13 of which were started on anti-TNF treatment. There waso statistically significant difference in change of height SDS between patients who had no anti-TNF therapy (median [IQR] 0.00 [-0.30 to +0.18]) and those who did (media [IQR] 0 [-0.21 to +0.06]), p=0.71. B) There was no improvement in height SDS from 0 – 12 months of anti-TNF therapy (median [IQR] at 0 months -0.38 [-1.73 to -0.10] vs -0.61 [-1.72 to -0.09] at 12 months; p=0.43, Wilcoxon signed rank). C) Responders to anti-TNF therapy showed no improvements in growth as compared to the non-responders, p=0.75. Medians and IQR indicated by horizontal bars

5.5 Discussion

Thiopurines were well tolerated in our cohort of patients, with 12% of patients discontinuing treatment over the 12 months due to adverse reactions. These figures are in accord with previously published data [290, 294]. Adverse drug reactions have been reported in up to 46% of treated patients, resulting in a discontinuation rate of 18-43% [55].

Thiopurines were efficacious in our cohort of patients. Overall, markers of disease activity reduced across the 12 month follow up period. The number of patients with moderate to severe disease, as determined by PGA, decreased significantly from 41.2% at the start of thiopurine treatment to 11.8% at the end of the 12 month study period. In addition, PCDAI, CRP, and to a lesser extent, ESR all decreased significantly. As was seen in the Markowitz randomised controlled trial (2000) [209], CS usage decreased over the year..

A major difficulty in interpreting these results comes from the fact that 50% of this cohort had either surgery or anti-TNF therapy within a year of starting AZA. The reasons for this were varied. Some patients started anti-TNF due to the presence of perianal disease and some as treatment escalation. In 3 of the 11 patients who had a surgical resection, AZA was started at the time of surgery as prophylaxis against post-surgical relapse [295]. Therefore, it is difficult to determine the effect of the thiopurine therapy alone. However, when the cohort of patients who remained on AZA alone were analysed separately, comparable results were seen (Figure 5.5). These patients still showed a significant decrease in markers of disease activity over the 12

months. Of course, it is likely that several patients would have been started on anti-TNF therapy or have had surgical resection due to the presence of active disease despite AZA, and therefore removing them from analysis does not leave a representative group. Nevertheless, overall, our results suggest that thiopurines do reduce disease activity in children with active CD.

No previous studies have demonstrated any improvement in growth in children with CD as a result of thiopurine therapy [191, 209, 219]. However, these studies have all been performed in centres that use CS as primary induction therapy for remission in children with CD. At our centre, we use EEN, which has well documented anti-inflammatory [136, 188] as well as growth promoting [164] effects. Therefore, we hypothesised that in our cohort of patients, improved growth would occur alongside improvements in disease activity. Although overall our patients showed a slight decrease in height SDS over the 12 months, which occurred regardless of whether CS were used or not, patients who were classed as responders to thiopurines at 12 months showed better growth than the non-responders (Figure 5.8.C). This is the first study that has demonstrated an improvement in growth related to thiopurine therapy at recommended doses. However, these results need to be interpreted with caution due to the high numbers of patients who escalated to anti-TNF therapy in our cohort, and the lack of standardisation of the treatment protocol. A prospective study is needed to further investigate this.

Several studies have demonstrated how linear growth is improved following the initiation of anti-TNF therapy [293, 296]. All patients at our centre who are started on anti-TNF treatment also receive a thiopurine. Therefore, we are not able to investigate the effect that anti-TNF therapy or thiopurines alone have upon linear growth in our children with CD. In our patients, we did not find any improvement in height SDS following 12 months of treatment with anti-TNF therapy. This data agrees with results described by Pfeffercon *et al* (2009),who found no sustained benefit from this either [220]. However, our numbers were very small, with only 13 patients treated with infliximab having growth data available at 1 and 12 months. Indeed, Pfeffercorn's study only contained 18 children who had been treated with infliximab for > 12 months with growth data available [220]. Studies that have contained larger numbers of patients have found beneficial effects of anti-TNF therapy upon growth [219, 296].

5.6 Strengths and limitations

This study has re-evaluated the effectiveness of thiopurines in children with CD in everyday clinical use. There was a diverse population of patients included in this study, with varying disease location, activity and duration, amongst other factors. Therefore, this study was able to demonstrate thiopurine efficacy on disease activity markers across a spectrum of disease, which provides useful information for clinical practice. In addition, whereas most studies evaluating the effectiveness of thiopurines investigated the effects of early initiation of therapy (treatment started <3 months of diagnosis), with mixed results [209, 216, 219], the mean time to starting therapy in our

cohort of patients was 1.56 years. This suggests that thiopurines are still of clinical benefit in a 'step up' approach to treatment of CD rather than only in a 'top down' setting. This is also the first study to evaluate growth in children with CD treated with thiopurines in a centre that commonly utilises EEN as opposed to CS.

However, this study had several limitations. This was a retrospective study, and so was completely dependent upon hospital records and clinical notes for data collection, which were in some cases incomplete. There was no standardised management regimen, and all therapies were initiated at the treating clinician's discretion. The patient variability makes these results interesting at a clinic level, but difficult to apply to a specific patient.

A major limitation of this study was that it was performed before the measurement of thiopurine metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) was routinely available. This allows clinicians to determine whether lack of or response to treatment is due to suboptimal dosing or non-responsiveness to medication. The normal therapeutic range for 6-TGN levels is 200-400 pmol/RBC x 10^8, with lower levels indicating either non-compliance or under dosing [272, 297, 298]. It would be been very interesting to see how clinical and laboratory indices of disease and changes in height SDS correlated with 6-TGN levels. Although MCV changes have been reported as being correlated to TGN levels [270] and clinical response, this has been found to be a less useful guidance regarding clinical decisions than TGN levels [208]. However, not all of the patients started on thiopurines routinely had 6-TGN measured.

5.7 Future directions

Now that thiopurine metabolite testing is easily available, it would be useful to repeat this study with this tool available. Of particular interest would be to look for any correlation between change in height SDS and 6-TGN levels, especially given how in our cohort of patients, higher TPMT levels were significantly associated with poorer linear growth. If it was found that height SDS were higher in patients in whom therapeutic levels of 6-TGN were achieved, then this provides important information regarding the management of children with CD who have poor growth.

As mentioned above, the mean time from diagnosis to starting AZA therapy was approximately a year. It is possible that if AZA was started alongside EEN to induce remission in CD, then further beneficial effects on linear growth will be seen. Mucosal healing is associated with prolonged remission in patients with CD [215, 299], and in children, improved growth [296]. As does IFX, EEN is known to promote mucosal healing [174, 188], but without the adverse effects associated with anti-TNFs [300-304]. Therefore, a randomised controlled trial comparing the effects of EEN used in combination with AZA from diagnosis to that of EEN alone would provide some useful answers.

5.8 Summary

Thiopurine therapy improved clinical and laboratory markers of disease activity. PGA, PCDAI, CRP, and to a lesser extent ESR, all showed improvement after initiation of therapy. Although responders to thiopurines at 12 months showed better growth than non-responders, overall height SDS significantly decreased over the 12 month study period. Treatment with biologics conferred no improvement in height SDS over a 12 month period, although the numbers involved were small. This study highlights the difficulties in maintaining normal growth in children with CD, and suggests the needs for further strategies to assist with this.

6 Results: Mathematical modelling to restore circulating IGF-1 concentrations in children with CD-induced growth failure

6.1 Introduction

Inflammation *per se* has direct effects upon growth in children with CD and growth failure, which occurs separate from its effects on appetite and nutritional intake [123]. Therefore, treatments that resolve the inflammation, such as EEN [164] or anti-TNF therapy [293], result in improved growth. Therefore the optimum treatment for improving growth is the same as the treatment for CD in general: to eliminate the inflammation. However, there are some patients whose disease remain intractable to treatment despite our best efforts, and so therefore continue to grow poorly despite fully optimising treatment [220]. There is currently no agreed treatment to improve growth in these patients. Studies performed on children with constitutional delay in growth and puberty have found that these children are at increased risk of bullying, poor self-esteem and depression than those who grow normally [305-307]. Although no such studies have been performed in children with CD, it is likely that poor growth provides an additional burden to young people whose lives are already affected by having a chronic disease [308, 309].

Also, although CD is a lifelong disease, we only have a limited period of time in which we can impact upon growth. Children CD often do not achieve their growth potential [101].

The GH/IGF-1 axis is integral for normal linear growth in childhood, with any interruption to this pathway resulting in poor growth [65, 68, 86]. Children with active

CD and growth retardation seem to have a functional GH insensitivity. They have normal GH levels [137] [138] alongside low IGF-1 levels [133]. This occurs concurrently with elevated pro-inflammatory cytokines. The link between pro-inflammatory cytokines, IGF-1 and poor growth has been demonstrated in CD and also in other inflammatory conditions of childhood [131, 134, 310] and in animal studies. [124] [123]. In children with CD, treatment of inflammation causes a reduction in cytokines and concomitant increase in IGF-1 levels, with changes being seen within 3 days [136].

It is possible that restoring IGF-1 levels back to the normal range will improve growth in children with CD. When exogenous IGF-1 was given to the rats with TNBS-induced colitis, it significantly improved their growth [123]. This response was unrelated to any change in disease activity or nutritional status. Recombinant human IGF-1 (rhIGF-1) is a recognised therapy to improve growth in children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [236, 237]. However, restoring IGF-1 levels in children with CD is not so straightforward. There are multiple differences between children with CD and those with GHIS. Children with GHIS are significantly younger at the time of receiving treatment. The compartments of the IGF-1 system, including the IGF binding proteins, cannot be assumed to distribute as they do in younger children. Also, GHIS is not associated with the presence of inflammation. As has been discussed, pro-inflammatory cytokines have deleterious effects on the GH/IGF-1 axis. Therefore, this may also alter the pharmacokinetics of rhIGF-1. In addition, CD is often associated with the presence of protein-losing enteropathy (PLE) [311]. As IGF-1 is a protein, which predominantly exists in the

circulation bound to binding proteins, the presence of PLE may have a significant effect upon rhIGF-1 pharmacokinetics.

As opposed to children with GHIS, children with CD still have endogenous production of IGF-1. In addition, high levels of IGF-1 may be harmful. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over several decades, have double the incidence of colon cancer. Studies have shown that the increased cancer risk was seen in individuals whose circulating IGF-1 was ≥ +2.5 SDS [238]. This needs to be considered in children with CD as inflammation is also a risk factor for intestinal cancer.

Therefore, for rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, but not given in excess. A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics (PK) would help resolve these issues. This would enable us to determine a dosing regimen tailored to an individual patient in order to restore circulating IGF-1 levels into the normal range.

6.4 Results

Eight children with active CD and height velocity <-2 SDS for age and gender were recruited into the study. The median (range) age was 12.97 (10.67 - 14.82) years. At the time of recruitment into the study, all 8 patients had HV SDS <-2. All of the children commenced the trial within 4 weeks of recruitment. As the severity of disease in any one child varies over time, in some patients there was a difference between HV SDS at recruitment and that at the start of the trial. For the same reason, there were also differences seen in disease activity in the same patient between admissions (Table 6.1). The HV SDS and disease activity at the moment of participation in the trial were used in the mathematical model, and so these differences did not affect the model results. In addition, having differences in disease activity in the same patient during the different admissions (inter-occasion variability) further strengthened the model.

Table 6.1 Patient demographics.

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
	Baseline characteristics							
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	PI BI	PI BI	PIII GIII	PI GI	PII BII	PII BII	PII GII	PI GI
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (mg/g)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
	Montreal Classification							
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N
	1^{st} Part of trial (single dose rh-IGF-1)							
HV SDS	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
CRP mg/l	36	<5	26	<5	82	12	10	<5
ESR mm/hr	14	27	23	6	40	25	40	4
	2^{nd} Part of trial (multiple doses of rh-IGF-1)							
HV SDS	-1.42	-3.91	1.55	-4.04	5.97	2.33	-1.04	0.82
PCDAI	15	20	17.5	5	7.5	35	0	0
CRP mg/l	69	5	22	<5	<5	12	<5	<5
ESR mm/hr	17	20	26	5	20	37	11	4

Tanner stage: P:pubic hair, B: breast stage, G:genitals . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: inflammatory, B2: stricturing, B3: penetrating

6.4.1 Aim 1: To assess baseline IGF-1 and IGFBP-3 levels in children with active CD and faltering growth

Baseline IGF-1 levels were measured in each patient at each admission. They were then converted to SDS according to the patient's age and gender (Appendix 5).

7 of the 8 patients had baseline IGF-1 SDS <0 at their first admission (Figure 6.1.A), with a median [range] SDS of -2.02 [-3.19 - 1.05]. All 8 patients had baseline IGF-1 SDS <0 at the second admission, with a median [range] of -2.42 [-3.21 - -0.91] (Figure 6.1.B).

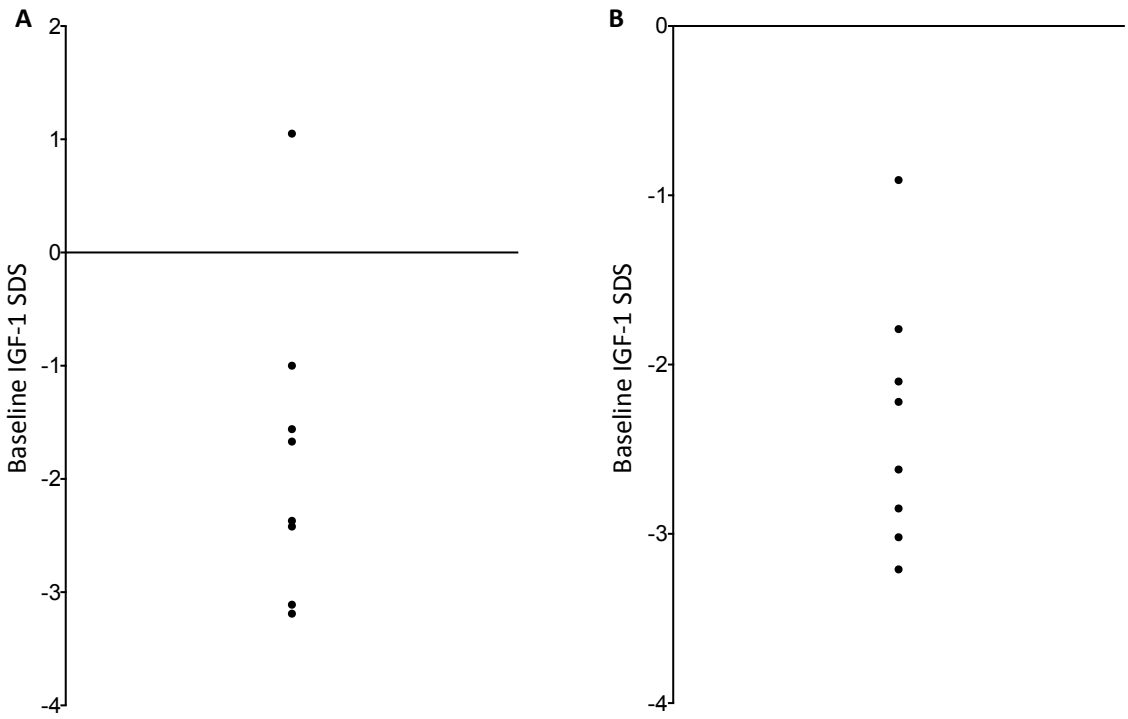

Figure 6.1 Baseline IGF-1 SDS.
A) 7 out of 8 children had baseline IGF-1 SDS <0 at the first admission (median [range] SDS -2.02 [-3.19 - 1.05]). B) All of the children had IGF-1 SDS <0 at the start of the second admission (median [range] of -2.42 [-3.21 - -0.91])

Spearman's rank correlation showed no statistically significant correlation with baseline IGF-1 SDS and either HV SDS (r=0.11, p=0.59) (Figure 6.2.A) or PCDAI (r=-0.01, p=0.99) (Figure 6.2.B). There was no statistically significant correlation between baseline IGF-1 SDS and either ESR (r=0.12, p=0.67) (Figure 6.2.C) or CRP (r=-0.30, p=0.26) (Figure 6.2.D).

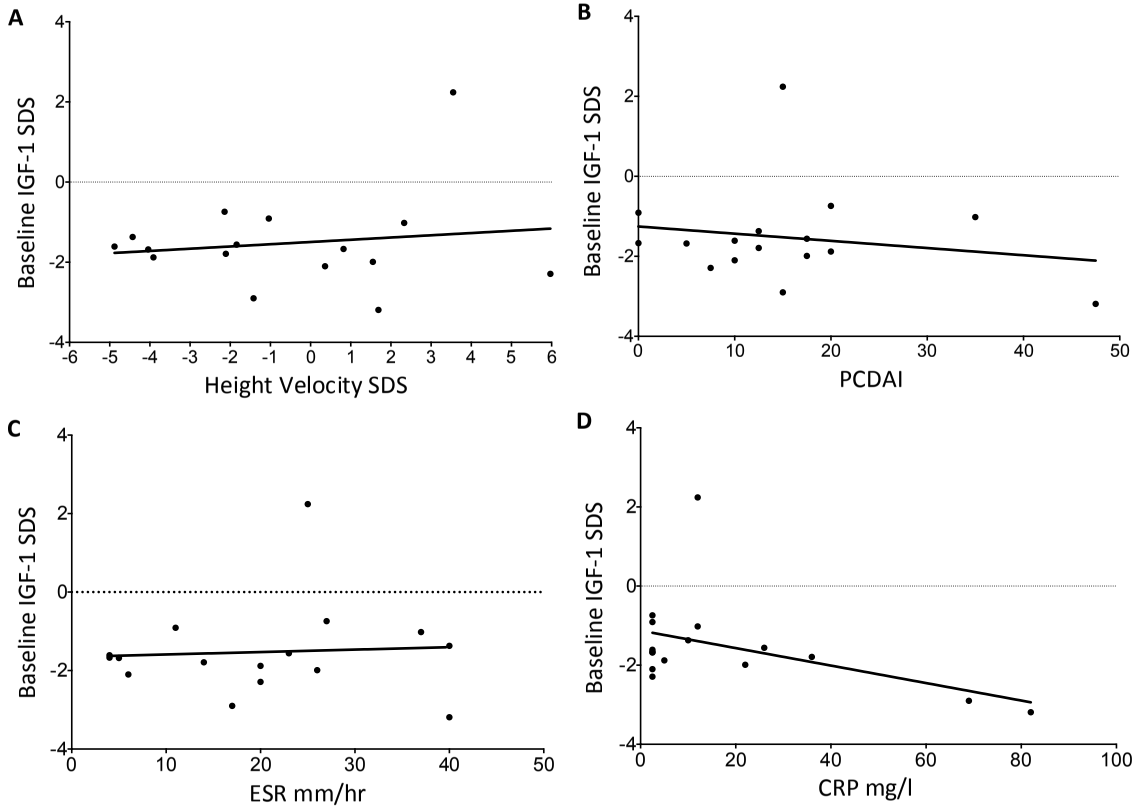

Figure 6.2 Correlation between baseline IGF-1 SDS and height velocity and PCDAI

Spearman's found no statistically significant correlation between IGF-1 SDS and A) HV SDS (r=0.11, p=0.59), B) PCDAI (r=-0.01, p=0.99), C) ESR (r=0.12, p=0.67) or D) CRP (r=-0.30, p=0.26)

IGFBP-3 is the main carrier of IGF-1 in the circulation. We measured levels in each patient. These values were also converted to SDS according to each patient's age (Appendix 5). Interestingly, we found that IGFBP-3 SDS were low in all patients, with a median [range] value of -1.71 [-2.70 to -1.08] (Figure 6.3.A). PLE is commonly present in patients with CD [312], and so we quantified the degree of this by measuring the concentration of α-1-antitrypsin (A1AT) in stool for each patient. Although we found that A1AT levels were elevated in 3 patients (median [range] 0.85 [0.08 - 3.34] mg/g) (Figure 6.3.B), we found no statistically significant correlation between these levels and IGFBP-3 SDS ($r=-0.14$, $p=0.63$, Spearman's) (Figure 6.3.C).

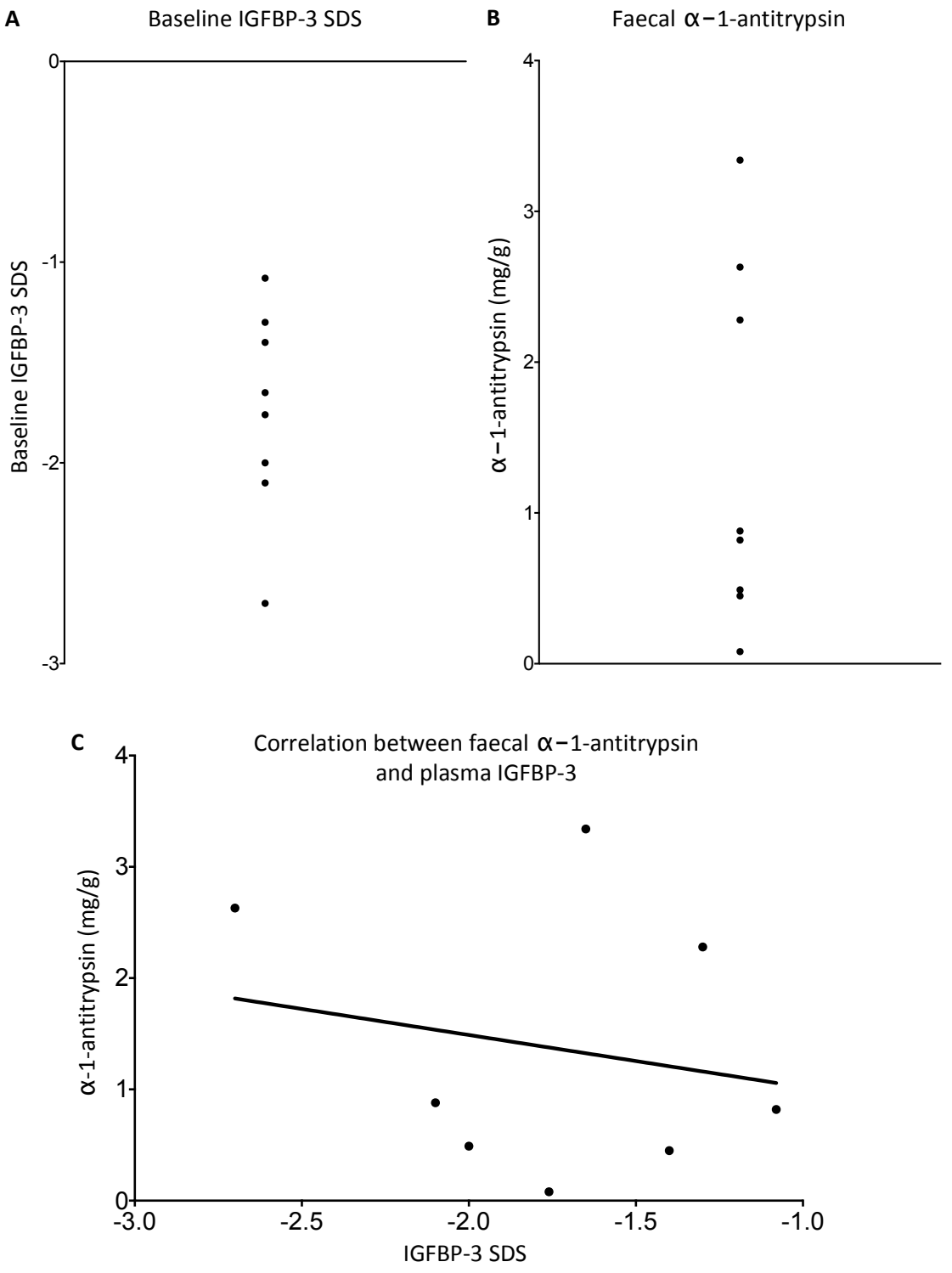

Figure 6.3 IGFBP-3 SDS and faecal A1AT levels (mg/g).
A)IGFBP-3 SDS were <0 in all 8 patients median [range]-1.71 [-2.70 to -1.08}). B) Median [range] faecal α-1-antitrypsin 0.85 [0.08 - 3.34] mg/g , C) there was no statistically significant correlation found between A1AT levels and IGFBP-3 SDS (r=-0.14, p=0.63)

As the majority of IGF-1 circulates in ternary complexes bound to IGFBP-3 and ALS, ALS levels were also measured in each patient, and converted to SDS according to the patient's age (Appendix 5). All except one patient had ALS SDS within the normal range (median range] +0.35 [-0.55 to +1.39]), suggesting that in our patients, not all of the proteins in the IGF-1 pathway were affected.

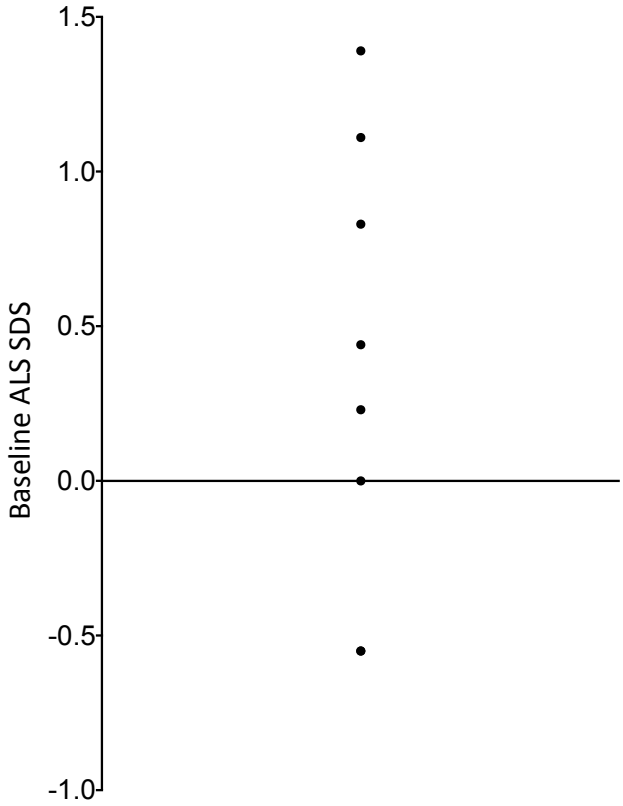

Figure 6.4 Baseline ALS SDS
ALS SDS median +0.34, range -0.55 to +1.39)

6.4.2 Aim 2: To assess the tolerance and safety of using rhIGF-1 in children with active CD.

All of the participants completed both parts of the study. The rhIGF-1 was generally well tolerated. The most common side effect experienced by patients with GHIS was hypoglycaemia [236]. There were no episodes of hypoglycaemia in any patient following a single dose of rhIGF-1 (Figure 6.4.A). However, during the second admission, one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood glucose <3.5 mmol/l) following the second dose of rhIGF-1 at 12 hrs (Figure 6.4.B), having not eaten before the dose was administered. This was corrected with oral glucose. No further episodes of hypoglycaemia occurred (Figure 6.4.C). There were no other adverse effects.

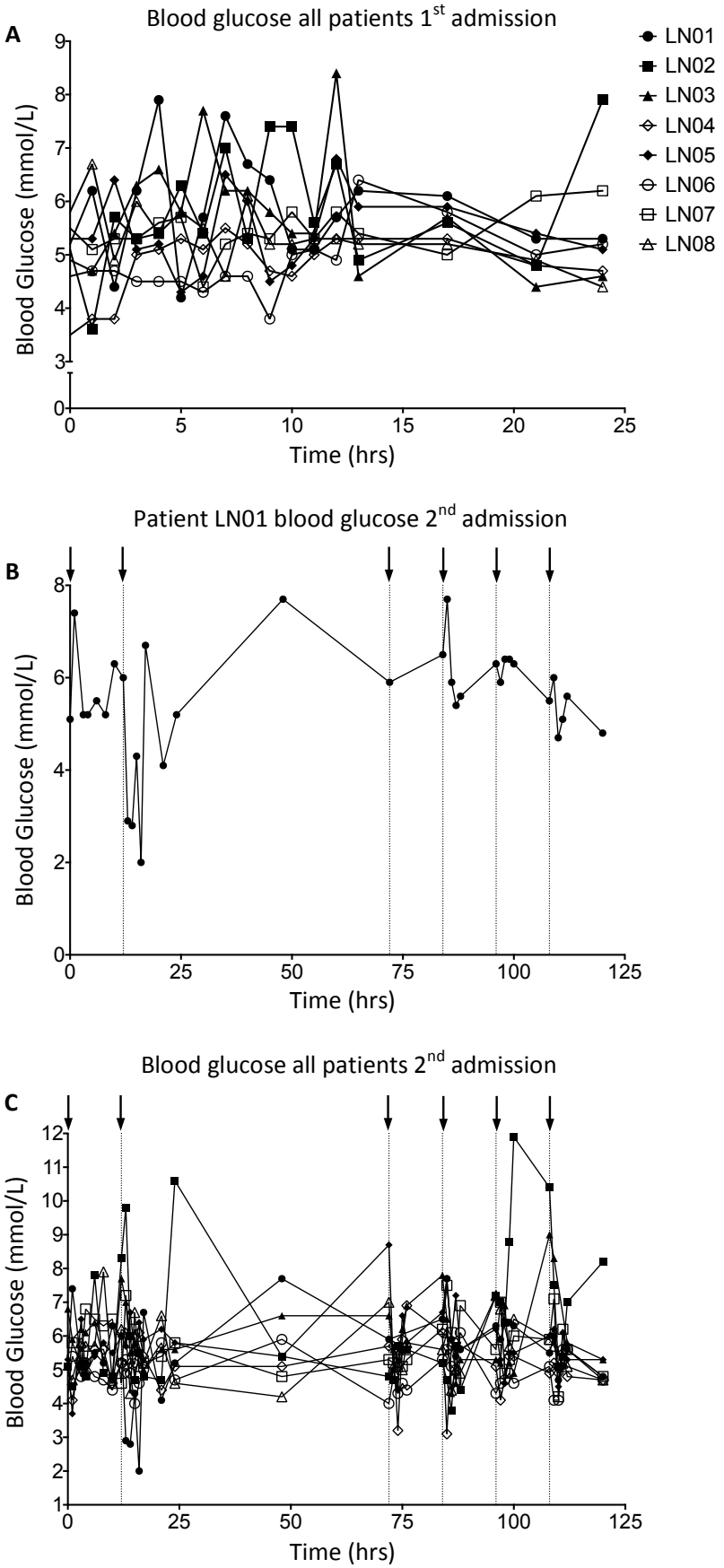

Figure 6.5 Blood glucose (mmol/l) following sc injections of rhIGF-1 (indicated by arrow). A) No episodes of hypoglycaemia in any of the patients following a single sc dose of rhIGF-1. B) LN01 had a single asymptomatic episode of hypoglycaemia (blood sugar < 3.5mmol/l) following the second dose or rhIGF-1 at 12 hours. This was corrected with oral glucose. C) There were no other episodes of hyopglycaemia during the study.

6.4.3 Aim 3: To determine whether exogenous administration of subcutaneous rhIGF-1 can restore IGF-1 levels to the normal range in children with active CD

Despite the variability in disease activity and location between patients, a single sc injection of rhIGF-1 at a dose of 120 μg/kg significantly increased circulating IGF-1 levels, with peak concentrations being reached between 3-4 h after injection (Figure 6.6). Plasma IGF-1 SDS were increased from a median (range) of -2.02 (-3.19 to +1.05) to 1.81 (-1.01 to +7.67) at the first admission (p=0.008, Wilcoxon signed rank) (Figure 6.6.A), and -2.42 (-3.20 to -0.91) to +1.17 (-0.04 to 2.89) (p=0.008, Wilcoxon signed rank) (Figure 6.6.B). The dose of 120 μg/kg was chosen because it was successfully used in a recent trial for the treatment of primary IGF-1 deficiency in children (industry data). However, in some children in whom the baseline IGF-1 was less depressed, this dose increased IGF-1 levels to above normal. There was a question as to whether the presence of PLE would blunt the ability of sc rhIGF-1 to achieve significant peak concentrations. However, no statistically significant correlation was found between stool A1AT levels and the change in IGF-1 induced by a sc injection (r=0.17; p=0.70, Spearman) (Figure 6.6.C) In all patients, the IGF-1 concentrations returned to baseline over the following 24 h (Figure 6.6.D).

IGF-1 levels vary during childhood, reflecting the different phases of growth. However, all of the children in our study were prepubertal, raising the question as to whether chronological age was the best marker to use when calculating IGF-1 SDS. However, even though the normal range of circulating IGF-1 concentrations for specific age and gender are known (Appendix 5), no such values are known according to pubertal stage. It has been demonstrated that bone age correlates well with pubertal status [150, 313-

315]. Therefore, we recalculated each child's IGF-1 SDS according to their bone age, and compared this to the IGF-1 SDS according to their chronological age. However, there was no statistically significant difference between these values, either at baseline. (median [range] IGF-1 SDS as baseline according to chronological age -2.02 [-3.19 - 1.05] vs -1.59 [-3.19 - 2.24] IGF-1 SDS calculated according to bone age, p=0.43, Mann Whitney U), or at peak concentration 3 h post-dose (median [range] IGF-1 SDS chronological age 1.81 [-1.01 - 7.67] vs 3.51 [-0.30 - 11.85], p=0.25, Mann Whitney U). (Figure 6.7)

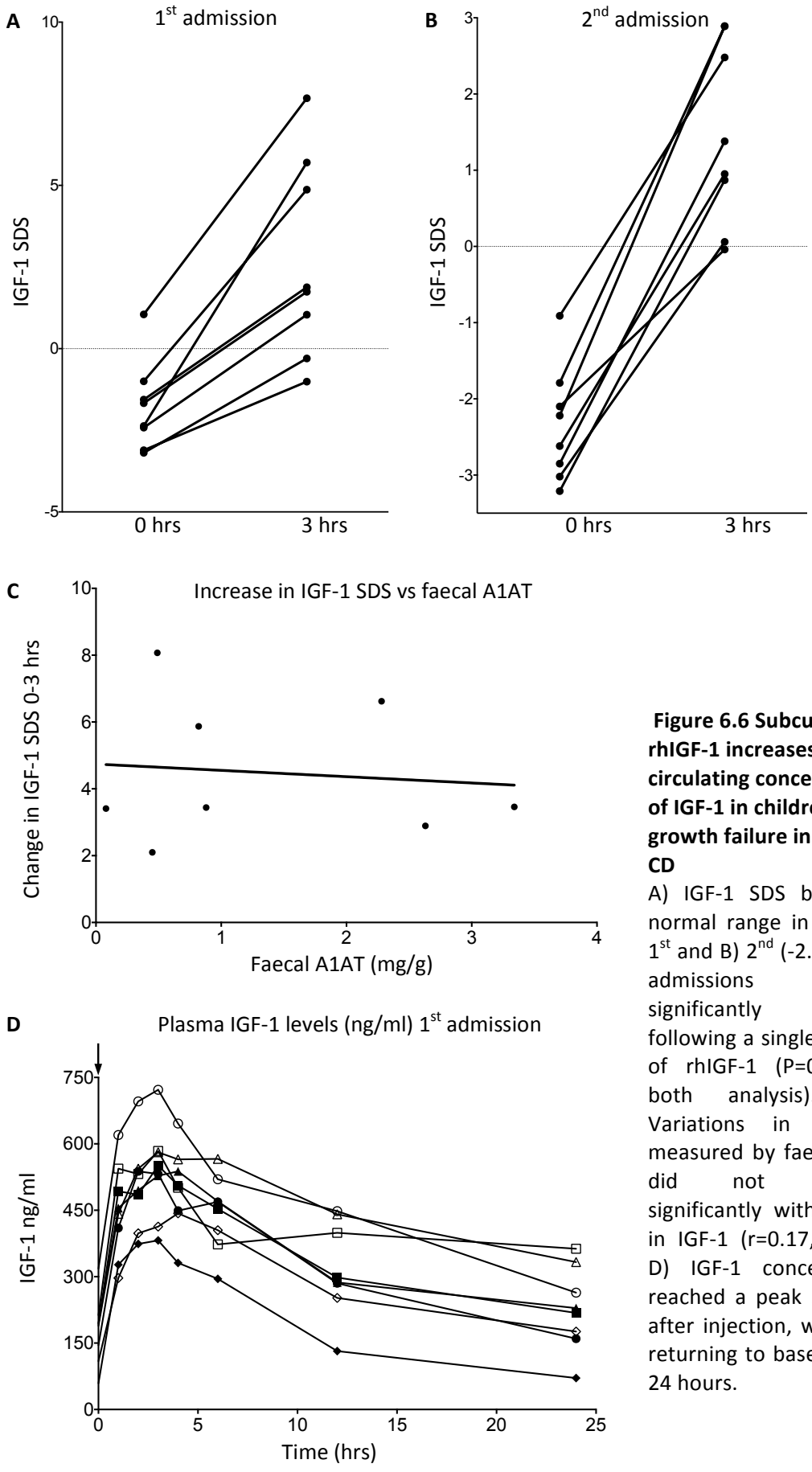

Figure 6.6 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by CD

A) IGF-1 SDS below the normal range in both the 1st and B) 2nd (-2.34 [0.75]) admissions were significantly increased following a single injection of rhIGF-1 (P=0.008 for both analysis). C) Variations in PLE, as measured by faecal A1AT, did not correlate significantly with changes in IGF-1 (r=0.17, p=0.70). D) IGF-1 concentrations reached a peak 3-4 hours after injection, with levels returning to baseline after 24 hours.

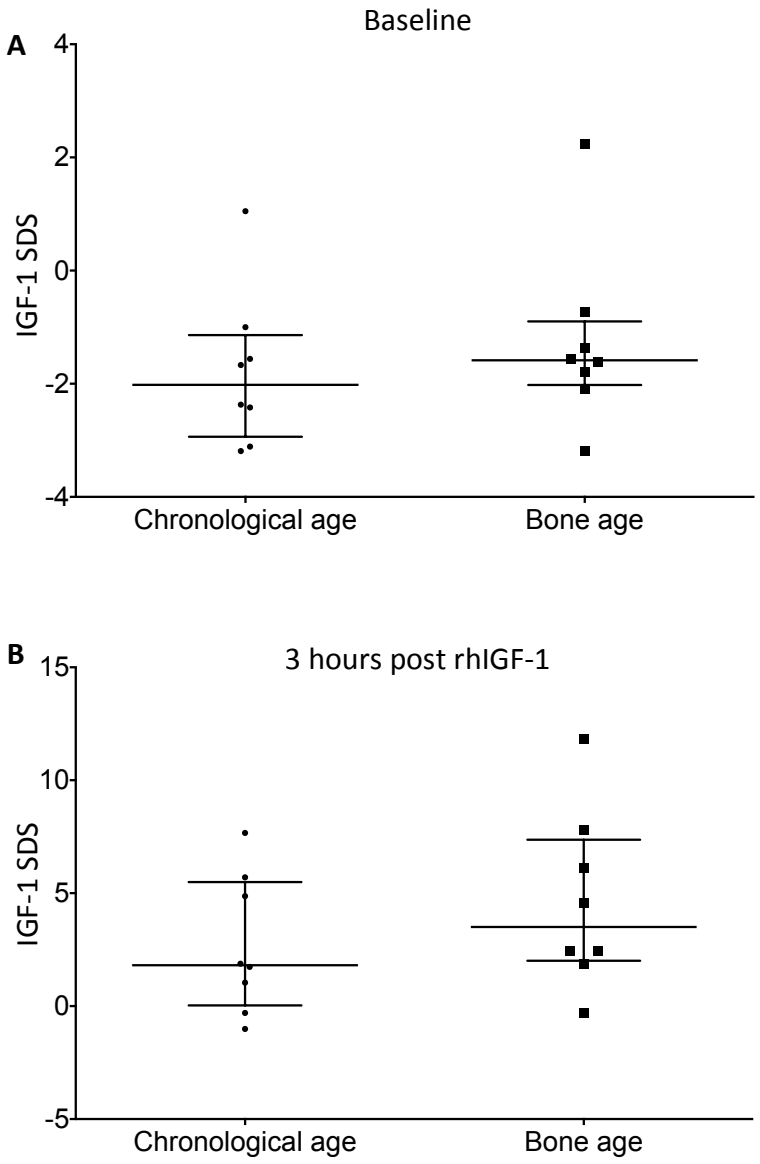

Figure 6.7 Change in IGF-1 SDS 0-3 hours in chronological age as compared to bone age
A) Median [range] IGF-1 SDS chronological age -2.02 [-3.19 - 1.05] vs -1.59 [-3.19 - 2.24] IGF-1 SDS calculated according to bone age, p=0.43, B) Median SDS at 3hrs IGF-1 SDS chronological age 1.81 [-1.01 - 7.67] vs 3.51 [-0.30 - 11.85], p=0.25).

During the second admission, the children received twice-daily doses of rhIGF-1 at a dose of 120 µg/kg, as used in children with GHIS (Figure 6.8). IGF-1 SDS were calculated from the results of each sample collected from every patient (Figure 6.8.B). From this section of the study we determined the average IGF-1 concentration by

using the area under the curve divided by time. This value was then converted to a SDS, according to each patient's age and gender, thus giving us an average IGF-1 SDS for each patient following BD dosing of rhIGF-1 at 120 μg/kg/dose (Table 6.2). We found that some patients had appropriate SDS, whereas others had an average IGF-1 SDS of >+2.5; the level known to be associated with increased colonic cancer risk in acromegalic patients. This thus demonstrated to us that the dosage used to treat children with GHIS is not suitable for all children with CD.

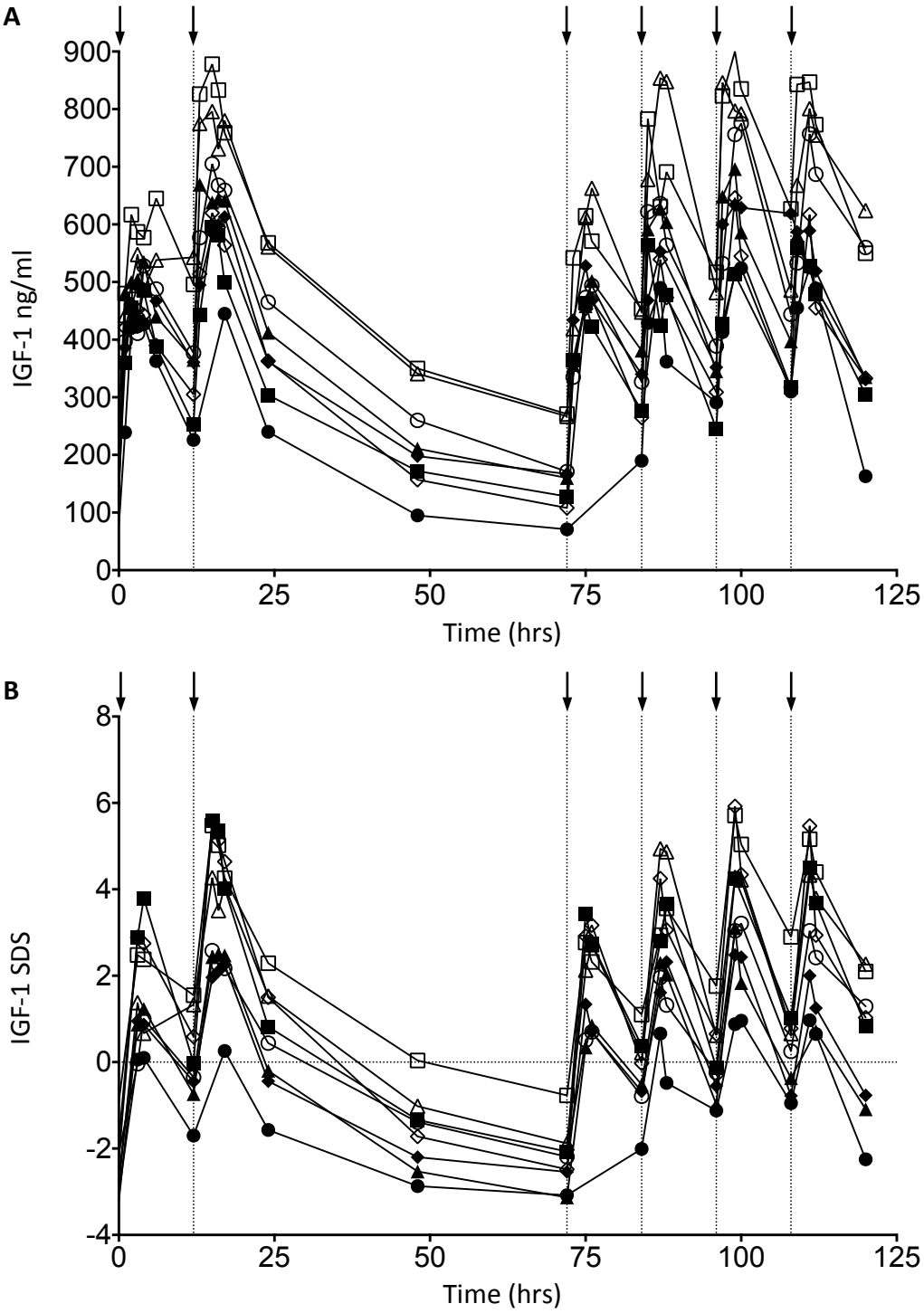

Figure 6.8 Twice daily injections of rhIGF-1 increased the circulating IGF-1 over a sustained period.

rhIGF-1 was given on day 1. Circulating concentrations were allowed to fall, before giving twice daily injections on days 4 and 5. A) Circulating IGF-1 concentrations over time B) IGF-1 SDS. rhIGF-1 doses are indicated by the arrows.

Table 6.2 Average IGF-1 plasma concentrations following BD rhIGF-1 at 120 µg/kg/dose.

Patient	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Average IGF-1 SDS	-1.27	1.93	0.98	3.75	-0.2	4.12	5.21	2.24

6.4.4 Aim 4: To develop a new mathematical model to determine an individualised dosing regimen to restore circulating IGF-1 concentrations to within the normal range for age.

From our study results, we developed a model from which the dose-concentration relationship between rhIGF-1 and circulating IGF-1 concentrations could be derived. A turnover model, which accounted for both endogenous production and exogenous dosing of IGF-1 (Figure 2.3), was fitted to the data using non-linear mixed effects analysis. Four fixed effects were estimated: K_{syn} (endogenous synthesis rate, K_a (exogenous absorption rate following subcutaneous injection), C_L (IGF-1 clearance), and V_D (volume of distribution). The model considered two levels of random effects, which resulted in improved fit: the random effects of inter-subject variability on K_{syn} and C_L, and inter-occasion variability on K_{syn}. By extrapolating the model to hypothetical patients with real Crohn's disease demographics the robustness of the model fit was demonstrated. The effects of potentially relevant covariates were also included to improve the model fit. These included ESR, CRP, the degree of PLE and PCDAI. Only PCDAI, when added to K_{syn}, significantly improved fit. Increased PCDAI scores correlated with lower K_{syn} (Figure 6.9.A; $p<0.001$). However, linear growth assessment is included in the PCDAI scoring system. To determine whether this was the reason for correlation between PCDAI and $K_{syn,}$ we repeated our covariate analysis

using the weighted PCDAI (wPCDAI) [316], which does not include growth in its assessment. However, testing the model with wPCDAI produced comparable results to the standard PCDAI. Using PCDAI gave a 76.9 point drop in OFV whereas wPCDAI gave a drop of 80.3, and so the p-values for the difference in the two values taken from the chi squared 1 distribution is 0.07. This means that whilst both wPCDAI and PCDAI score significantly improved the fit, there was no significant difference between these improvements. Hence using either PCDAI or wPCDAI will give the same result. This suggests that disease severity is the major driver of IGF-1 synthesis rate both between patients and in the same patient on different occasions. Including PCDAI score significantly eliminated inter-individual variability in the model. Model predictions were graphically compared to observed data with good agreement observed (Figure 6.9.B & C). The absence of a trend in the distribution of the standardised residuals meant the parametric model assumptions were correct (Figure 6.9.D & E). The two medians of the model predicted and observed data sets were comparable (Figure 6.9.F).

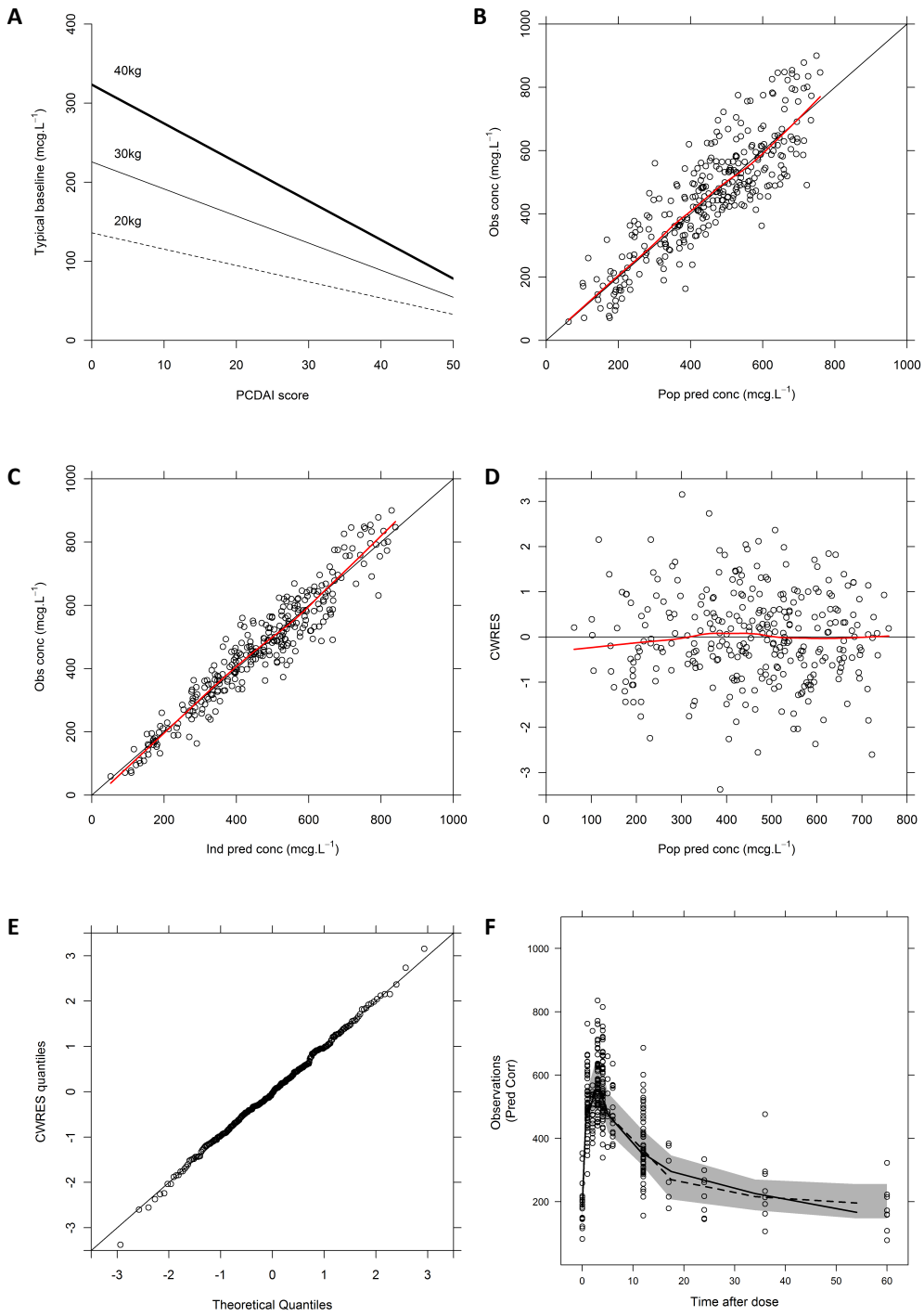

Figure 6.9 IGF-1 in a mathematical model.
(A) Increasing PCDAI significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (B) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (C) Individual model predicted concentrations are in agreement with observed concentrations. (D) Conditional weighted residuals (CWRES), (a form of standardised residuals) expected to follow the Normal Independent Distribution lay within −2 and +2 SDs and did not change with model predictions, indicating good structural model fit. (E) QQ plot of CWRES indicate that the assumption of normality of residuals is met; (F) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% CI of the model simulations (grey-shaded area).

The purpose of the modelling approach was to be able to quantify the relationship between endogenous IGF-1 production and exogenous dosing and to understand how IGF-1 average concentrations (area under curve/time) can be adjusted. Current understanding of IGF-1 concentrations and thresholds of safety derived from acromegaly patients. Although we do not understand the exact pathogenesis of the increased malignancy risks, it is thought to be likely to decades worth of sustained exposure to elevated IGF-1 levels >+2.5 SDS [238]. Even though our patients would receive a much shorter duration of treatment, due to the risks of malignancy associated with chronic intestinal inflammation, we defined the optimal IGF-1 concentration range as 0 to +2 SDS. We simulated different dose scaling regimes to predict and maximise the likelihood that an individual will maintain IGF-1 levels within the target range. 92.7% of children were predicted to have IGF-1 levels within the target range by individualising dose based on weight, age and PCDAI score (Figure 6.10). As the PCDAI score will vary in each patient during the course of treatment, this can be recalculated, and the rhIGF-1 dose adjusted accordingly, at 3 monthly intervals. The two different age ranges deflects the difference in target IGF-1 values at different ages (Appendix 5). At certain ages, children experience an increase in circulating IGF-1 levels, reflecting the growth spurt seen at this time [317]. This enables us to suggest a dosing regimen that restores IGF-1 plasma concentrations within safe levels (Table 6.3).

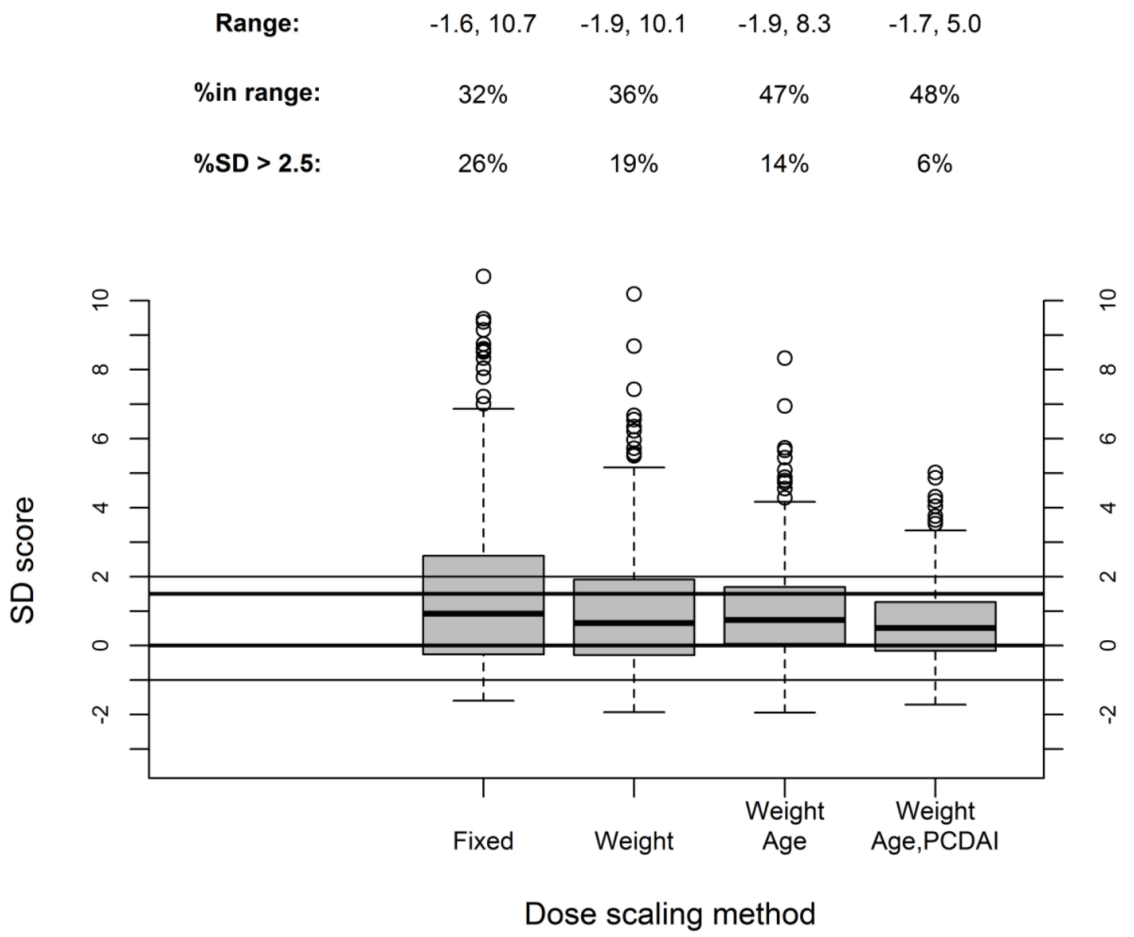

Figure 6.10 rhIGF-1 dose scaling.
Incorporation of a disease activity index into dose calculations allowed an accurate prediction of circulating IGF-1. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SD score (SDS). Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference −2 SDS and +2.5 SDS.

Table 6.3 The rhIGF-1 dosing regimen recommended by the mathematical model

Age (years)	Dose
10 to <12	21 µg/kg + 1 µg/kg/PCDAI point
12 to <14	41 µg/kg + 1.4 µg/kg / PCDAI point

6.5 Discussion

As has been previously described [133], the children in our cohort who had active inflammation and growth retardation also had low circulating concentrations of IGF-1 (Figure 6.1). The best strategy to increase IGF-1, and therefore to improve growth, is the resolution of the inflammation [136]. However, there are some children whose inflammation remains intractable to treatment. We need a distinct strategy to accelerate growth in the group.

RhIGF-1 has thus far only been used as a treatment in children with GHIS. Although it has been shown to be very efficacious at increasing IGF-1 levels in these children, children with CD present a different challenge due multiple reasons, including differing age range, the presence of inflammation and protein-losing enteropathy, amongst other things. However, all of the children in our study increased their circulating IGF-1 concentrations in response to exogenous rhIGF-1, with levels being maintained following repeated dosing (Figure 6.8). This was also well tolerated. Even though hypoglycaemia has been reported as occurring in approximately 50% of children with GHIS treated with rhIGF-1 [236], despite some children reaching high peak IGF-1 SDS (Figure 6.5), we only had a single incidence of hypoglycaemia in our study, and this too occurred in a child who had not eaten for several hours prior to administration of the sc dose (Figure 6.4).

As rhIGF-1 has only been used in children with GHIS, it is difficult to decide on an appropriate dosing regimen for children with CD. Indeed, even in GHIS patients, the optimal dosing guidelines are still being debated [277, 318, 319]. It is important to be

able to increase IGF-1 levels to within the normal range and no higher as sustained high IGF-1 concentrations are associated with increased risk of colonic cancer in middle aged and older patients with acromegaly [238, 320]. Normal IGF-1 concentrations vary at different ages of childhood (Appendix 5), and so rhIGF-1 dosage needs to be altered in line with this. In addition, determining an appropriate dosing regimen in children with CD is made all the more complicated by the diversity seen in patients. As demonstrated by the patients in our study, disease activity varies considerably not only between patients, but also within the same patient at different times (Table 6.1), and so rhIGF-1 requirements will also vary due to this.

In order to use our data to recommend a dose, we used a modelling approach to kinetics and dosing. In this study, we fitted a structural model that simplified the system without losing the biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability, as well as residual variability (differences between predicted and observed values). This enabled simultaneous model fit for all patients and exploration of covariate relationships. Least-squares regression was used to minimise deviations from the target SDS. We also demonstrated the robustness of the model fit by extrapolating the model to hypothetical patients with real Crohn's disease demographics. The goodness of fit plots also corroborated our findings by showing unbiased representation of the simulated and observed data. Additionally increasing PCDAI score was found to reduce endogenous IGF-1 production rather than its clearance and its inclusion further improved the model. Overall this provided a sophisticated approach for individualising dosing regimes.

6.6 Study strengths and limitations

This study has again demonstrated that active CD and growth failure is accompanied by reduced circulating IGF-1. In addition, we have shown that it is possible to increase IGF-1 concentrations by exogenous rhIGF-1 administration. We performed a detailed PK study, from which we were able to form a mathematical model to determine an IGF-1 dosing regimen that restores IGF-1 concentrations to the normal range, and not in excess. As our patients had two separate admissions, this allowed for us to perform mixed effects modelling adding inter-occasion, as well as inter-individual variability. We were able to use this model to determine the effect of covariates, such as inflammatory markers and PLE. From this, we have been able to suggest a specific dosing schedule based upon a child's age, weight and, importantly, PCDAI also. A child with CD will have variable amounts of inflammation during their course of disease. This dosing regimen allows for this fact. This study has demonstrated how pharmacokinetic mathematical modelling can be used to good effect in a clinical trial. We found that the dosing regimen used in children with growth hormone insensitivity syndrome was not appropriate for all children with CD. The formation of a mathematical model has allowed us to determine an appropriate dosing regimen for use in further studies.

95% of IGF-1 circulates bound to binding proteins, with the remaining 5% being the biologically active free IGF-1. Our patients all had low circulating IGFBP-3 concentrations, and therefore rhIGF-1 may have increased the proportion of free IGF-1. However, there is no assay for free IGF-1, and so we are unable to test this. It

should be noted, though, that patients with GHIS have IBGBP-3 SDS ≤-3 SDS, and have no increased oncology risks as a result of rhIGF-1 therapy [277, 321].

All of the children in our study were prepubertal, as determined by Tanner staging. However, we determined target IGF-1 SDS according to the patient's age rather than pubertal status. Even though we found that recalculating IGF-1 SDS based upon bone age rather than chronological age made little difference (Figures 6.7), normative data for circulating IGF-1 against pubertal stage do not exist. Therefore, we were not able to fully account for the impact of puberty in this model.

Although a detailed PK study was performed, we only had 8 children in our study. A robust mathematical model was formed from our results, which showed a good response to simulation testing. However, more patient data would further increase the accuracy of the mathematical model. This is the advantage of creating a population PK mathematical model: new data can be added to be model as it is accrued without having to reform a new model.

6.7 Future Directions

We have shown that exogenous rhIGF-1 raises circulating IGF-1 levels in children with active CD and growth retardation back to within the normal range, and used a mathematical model to determine an individualised dosing regimen that restores circulating IGF-1 to within the normal range without entering an unphysiological high range. However, it remains to be seen as to whether restoring IGF-1 levels will result in improvements in linear growth in children with CD. In addition, the benefits of rhGH

therapy to growth in children with CD have been studied, with some encouraging results [234, 235]. It remains to be seen as to which of rhGH and rhIGF-1 is the more efficacious. A multicentre randomised controlled trial comparing rhIGF-1 and rhGH would ascertain this. This trial would also give us the opportunity to test the dosing regimen derived from the model presented in this thesis on a wide sample of patients

6.8 Conclusion

Children with active CD and growth retardation have low circulating concentrations of IGF-1. These concentrations can be restored into the normal range by exogenous injections of rhIGF-1, which is well tolerated. There are concerns about exogenous administration of rhIGF-1 as high IGF-1 concentrations may predispose to colon cancer. However, from our pharmacokinetic studies we were able to formulate a mathematical model of IGF-1, which allows doses based on weight, age and disease activity, that will restore IGF-1 levels into the normal range, but not above this. This provides a safe dosing regimen for rhIGF-1 that can be used in further clinical trials.

Discussion and Future Directions

Growth retardation has been a recognised feature of children with CD for over 50 years [322], and is reported wherever CD has been described [96, 323-325]. The recognition and treatment of growth failure is a fundamental part of the management of a child with CD [49, 55, 191]. Initially thought to be solely the result of undernutrition [107, 326], we now understand that the aetiology of growth failure in paediatric CD is multifactorial in origin. Animal studies have demonstrated the contribution of inflammation *per se*, separate from its anorexigenic effects [123], and numerous studies have described the deleterious effect of pro-inflammatory cytokines upon the GH/IGF-1 axis [89, 124, 125]. However, despite these increases in our knowledge of its pathogenesis, growth retardation remains a common problem for children with CD [220]. With the increasing prevalence of paediatric CD [24, 34, 35], and the question as to whether the age of onset is getting younger [32, 35], this is likely to be an increasing problem, and therefore one that needs appropriate management.

For all IBD sufferers, timely recognition and management is very important for clinical remission and improved quality of life, in addition to managing growth failure. As has been described in previous reports [42, 101], we found that the duration of time between the onset of symptoms and diagnosis correlated negatively with height SDS at diagnosis ($p=0.02$). This is often thought to be due to the fact that children with CD commonly present with insidious non-specific symptoms, such as abdominal pain and weight loss, especially where there is no associated colitis [42]. Therefore the degree

of growth retardation in these patients might be due to unchecked disease suppressing growth, or related to the site of the inflammation [53]. As first described by Crohn himself [327], jejunal disease is commonly associated with poor growth [99, 101]. However, in our study, although we found that patients with isolated small bowel disease were most likely to experience a longer duration of symptoms to the time of diagnosis, multivariate analysis showed no correlation between disease location and height SDS. Indeed, the only factor that correlated with height SDS at diagnosis was the symptom duration. This again highlights the need for early recognition and treatment. Reassuringly, the patients in our study had both reduced duration of time between symptom onset and diagnosis, and better height SDS than those reported in a previous study from our centre [101], which perhaps reflects the increased public awareness of paediatric CD [281-283]. However, the fact remains that 25% of children in our study had a height SDS <-1, and 7% <-2 at diagnosis. Therefore, it is important to initiate a treatment at diagnosis that also helps support linear growth.

Patients diagnosed with CD in childhood are likely to require many years of treatment. Therefore, the long-term effects of any medication, both beneficial and adverse, need to be taken into consideration when managing these patients. EEN is the induction of remission agent of choice in Europe due to its excellent safety profile in addition to its proven efficacy [55]. Although the short-term beneficial effects of EEN to patients, both in terms of disease activity and in relation to linear growth, have been well described [164, 174, 182, 183], we were interested to see whether response to EEN resulted in any sustained beneficial effects to patients. We hypothesised that as EEN

results in mucosal healing [188, 328], patients who were put into full clinical remission following EEN induction therapy would have a sustained CS-free clinical remission when compared to non-responders, and that this would result in better linear growth. However, even though we found that responders to EEN did show better linear growth across a 5 year follow up period, this occurred despite no differences in time to relapse, or in treatment escalation between the two groups. This could not be attributed to CS use either, as there was both no significant difference in linear growth between patients who had been treated with CS and those who remained CS free, and also no correlation between the period of time a child was CS free and their change in height SDS. Therefore, response to EEN alone resulted in sustained benefits to linear growth in our patients. One hypothesis is that this is due to the promotion of mucosal healing with EEN use [188], and that this results in continuous beneficial effects to growth. Another explanation may relate to differential changes in intestinal microflora and subsequent changes in local inflammatory immune responses and antigenic potency. Patients with CD exhibit altered intestinal microbiota as compared to the general population [4, 15, 16], and children who respond to EEN show an alteration in their microbiota after treatment [329-331]. However, it is not known whether this differs between patients who enter remission with EEN and those whose disease remains active. Studies examining the above factors would help us to understand why some children respond to EEN whereas others do not, and from this, we can progress to determine the implications of this on linear growth.

Even though no studies have thus far demonstrated any beneficial effects of thiopurine use upon growth in children with CD [209, 220], these studies have all been

from North America, where CS are preferentially used over EEN as the induction of remission agent of choice [170]. Interestingly, in our unit, where we use EEN as first line therapy, patients who showed complete response to thiopurine treatment after 12 months had better growth than those who did not. However, these results are very difficult to interpret given the fact that 50% of patients also received anti-TNF therapy, or underwent a bowel resection within those 12 months. Indeed, overall, our children still did not show improved linear growth in following thiopurine therapy, despite having clinical and biochemical improvement of disease activity. However, none of the patients in our study initiated thiopurine therapy at the time of diagnosis. Therefore, it is still possible that starting thiopurine therapy at the same instance as EEN may support growth. EEN promotes mucosal healing [188], and as we report in this thesis, response to EEN in our patients is associated with sustained beneficial effects upon linear growth. Adult studies comparing the efficacy of infliximab as compared to azathioprine [215] have reported that patients receiving combined treatment had the highest rates of mucosal healing and the longest duration of CS-free remission. Therefore, it is possible that the early introduction of an immunomodulator alongside a treatment that promotes mucosal healing will have long-term beneficial effects upon growth. However, a randomised controlled trial comparing outcomes in children treated at diagnosis with EEN alone to those treated with a combination of EEN and thiopurine is needed in order to answer this question. In addition, although we did not demonstrate any beneficial effects of anti-TNF therapy to growth, the numbers involved in our study were too small to draw any conclusions from.

Nevertheless, until we can find a cure for CD, there will always be children whose inflammation cannot be completely controlled. We need a strategy to promote growth in these patients. There have been several studies investigating the effects of rhGH [231, 234, 235], often with encouraging results. However, despite the fact that children with CD have been shown to have low circulating IGF-1 levels [133], the only study investigating the beneficial effects of rhIGF-1 therapy has been an animal study [123]. This report demonstrated how subcutaneous IGF-1 injections helped to restore growth in rats with experimentally induced colitis and resultant low circulating IGF-1. However, the first step to using IGF-1 as a treatment option for growth failure in CD is to establish if it is possible to restore plasma levels to normal levels in this cohort of children. In this thesis, we have demonstrated that it is possible to increase circulating IGF-1 concentrations in children with CD following exogenous dosing. We have also demonstrated a way in which it is possible to restore IGF-1 levels into the normal range without entering the high ranges that are associated with increased colonic cancer risk in patients with acromegaly [238]. In order to do this, we utilised a mathematical modelling approach to kinetics to decide on an appropriate dosing regimen. This has also demonstrated how PK modelling can be used in paediatrics to beneficial effects, by individualising dosing strategies and reducing the need for repeated trials [248]. Our PK model helped us formulate a very specific dose that would have been highly unlikely to be determined from conventional PK studies.

However, it remains to be seen whether increasing circulating IGF-1 levels will result in improved linear growth in children with active CD. A clinical trial is needed in order to

determine this, preferably one that also compares the efficacy of rhGH. Our study has provided a safe dosing regimen that can be utilised in such a trial.

In conclusion, despite increases in our knowledge of the pathogenesis of CD-related growth failure, and increased treatment options available to us to help control inflammation, a significant percentage of children with CD remain growth retarded. Isolated growth retardation is associated with low self-esteem and reduced QOL in otherwise healthy children [305-307], and provides an extra burden to children with CD, whose lives are already affected by chronic disease. The inclusion of assessments of growth in both the PCDAI score [56] and the Paris classification [55] highlights its recognition by treating clinicians. Crucially, its inclusion in patient forums shows us its importance to our patients and their families [332, 333]. Therefore it is important that we continue to develop strategies to help support children with CD achieve their growth potential.

Summary

We have found that growth retardation is still a very common finding in children with CD, with 25% of patients having a height SDS <-1 at diagnosis. In this thesis, we have evaluated current therapeutic strategies for the management of paediatric CD. We have found that response to EEN at induction of remission predicts improved growth over a 5 year follow-up period, when compared to non-response, and that this occurs independently of CS-free time spent in remission. Patients who completely responded to thiopurine therapy at 12 months had better growth than patients who did not respond. However, we found no beneficial effects on growth from anti-TNF therapy, although the numbers involved in this analysis was very small.

Children with active CD and growth retardation have low circulating IGF-1 levels. We have found that it is possible to restore these levels into the normal range following exogenous dosing of rhIGF-1, and have utilised a mathematical model to determine a dosing regimen that keeps IGF-1 concentrations to within the normal range, but not in excess. This both provides a dosage of rhIGF-1 to be used in future trials, and also demonstrates the benefits of using pharmacokinetic mathematical modelling in paediatrics.

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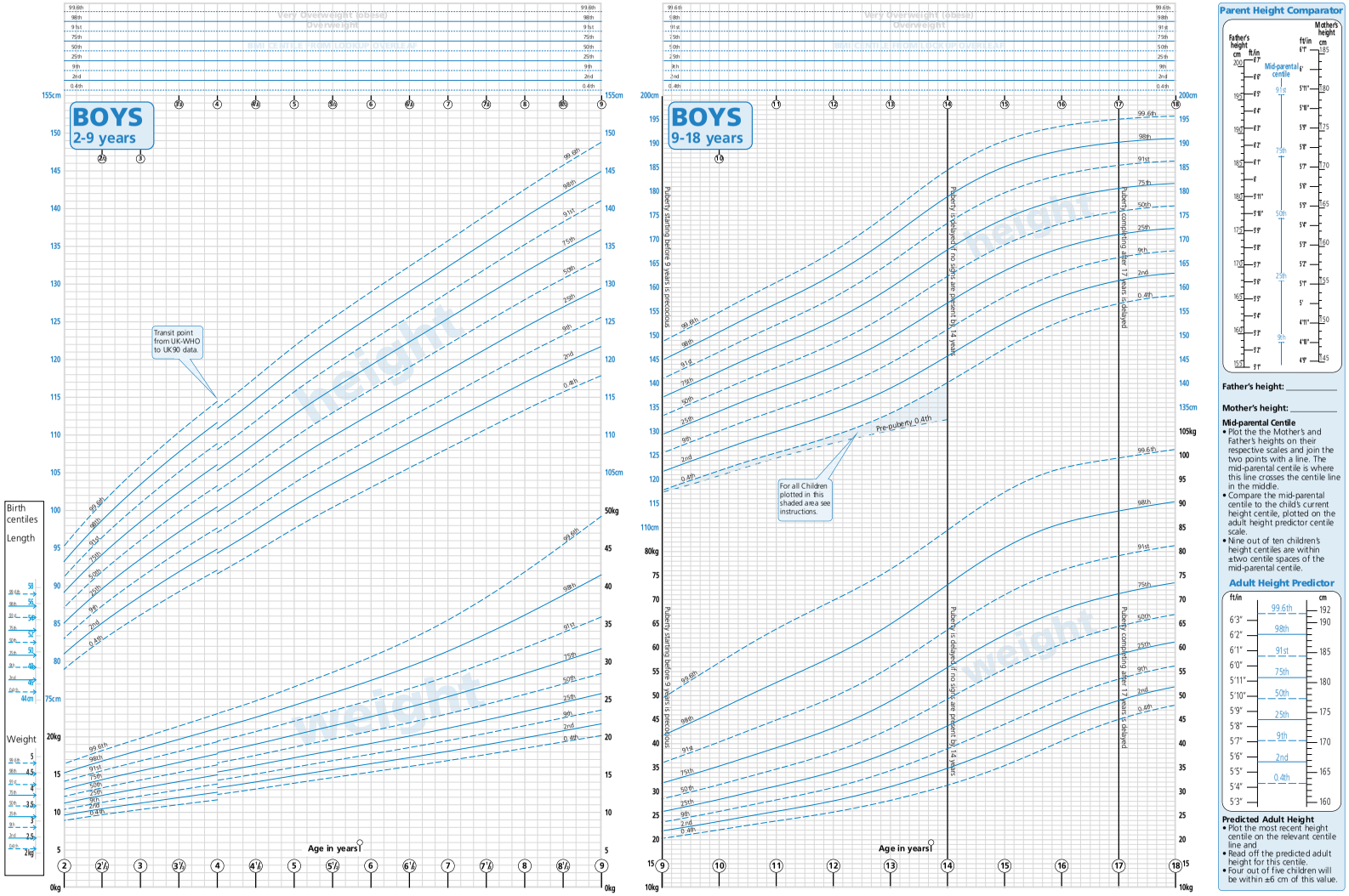
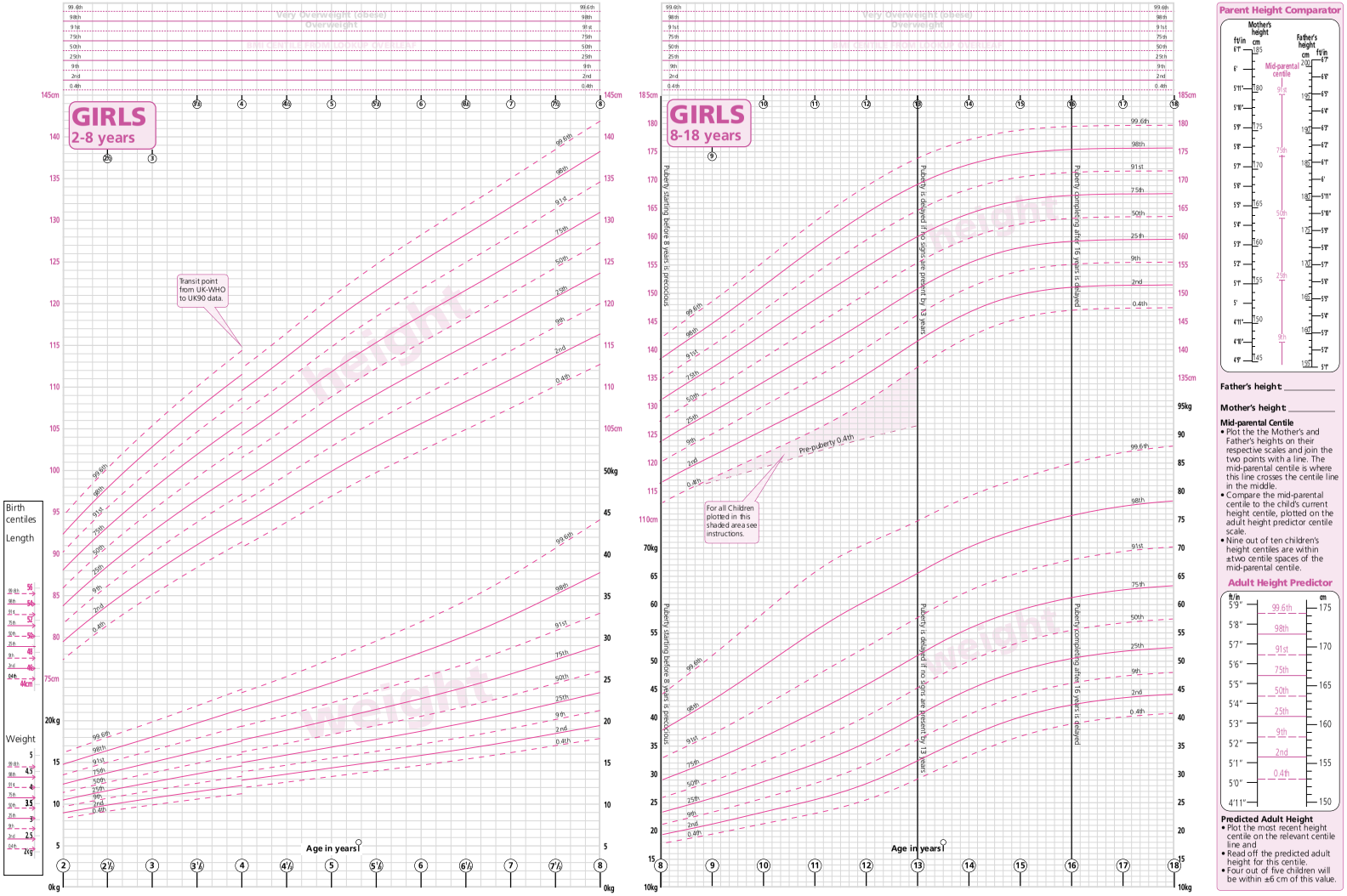
Appendix 1: Standardised UK growth charts





Parent Height Comparator

Father's height: _____

Mother's height: _____

Mid-parental Centile

- Plot the the Mother's and Father's heights on their respective scales and join the two points with a line. The mid-parental centile is where this line crosses the centile line in the middle.
- Compare the mid-parental centile to the child's current height centile, plotted on the adult height predictor centile scale.
- Nine out of ten children's height centiles are within ±two centile spaces of the mid-parental centile.

Adult Height Predictor

Predicted Adult Height

- Plot the most recent height centile on the relevant centile line and
- Read off the predicted adult height for this centile.
- Four out of five children will be within ±6 cm of this value.

Appendix 2: Pediatric Crohn's Disease Activity Index

Age- years Sex: Male Female

			Score
Subtotal			
Abdominal pain	no abdominal pain	0	
	Mild; no interference with activities of daily living(ADL)	5	
	Moderate,severe;daily nocturnal, interferes with ADL	10	
Stools/day	0-1 liquid no blood	0	
	≤ 2 semi formed + small blood or 2-5 liquid	5	
	≥ liquid stools, gross blood , or nocturnal diarrhoea	10	
General function	Well, no limitation of activities	0	
	Below par, occasional difficulty with activities	5	
	Very poor , frequent limitation of activities	10	
Examination			
Weight	Weight gain (or voluntarily stable/reduction)	0	
	Weight loss ≤ 10% (or involuntarily stable)	5	
	Weight loss≥ 10%	10	
Height[+] (at diagnosis)	< 1 channel decrease from previous percentile	0	
	1 to < 2 channel decrease from previous percentile	5	
	≥ 2 channel decrease from previous percentile	10	
or			
Height velocity[++]	≤ -1 standard deviation from normal	0	
	-1 to < -2 standard deviation from normal	5	
	≥ -2 standard deviation from normal	10	
Abdomen	No tenderness or mass	0	
	Tenderness or mass without tenderness	5	
	Tenderness, involuntary guarding, definite mass	10	
Peri-rectal disease	None, asymptomatic tags	0	
	1-2 indolent fistula, scant drainage, non-tender	5	
	Active fistula, drainage, tenderness, or abscess	10	
Extra-intestinal[+++]	None	0	
	1 manifestation	5	
	≥ 2 manifestations	10	
Laboratory			
Haematocrit(%) M = Male F = Female	M/F 6-10 years: ≥ 33	0	
	M 11-14 years: ≥ 35		
	F 11-19 years: ≥ 34		
	M 15-19 years: ≥ 37		
	M/F 6-10 years: 28-32	2.5	
	M 11-14 years: 30-34		
	F 11-19 years: 29-33		
	M 15-19 years: 32-36		
	M/F 6-10 years: < 28	5	
	M 11-14 years: < 30		
	F 11-19 years: < 29		
	M 15-19 years: < 32		
ESR (mm/hr)	< 20	0	
	20-50	2.5	
	>50	5	
Albumin (g/L)	≥ 35	0	
	31-34	5	
	≤ 30	10	
+ Height-channel represents lines on the standard percentile		**Total PCDAI score**	

- chart,e.g. 10 - > 25 - > 50 percentile is 2 channels difference
- ++ Height velocity is calculated from measurements over last 6-12 months in cm/year compared to standard deviation below (minus to) normal
- +++ oral ulcers, arthritis, uveitis, erythema nodosum, pyoderma gangrenosum

Appendix 3: 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

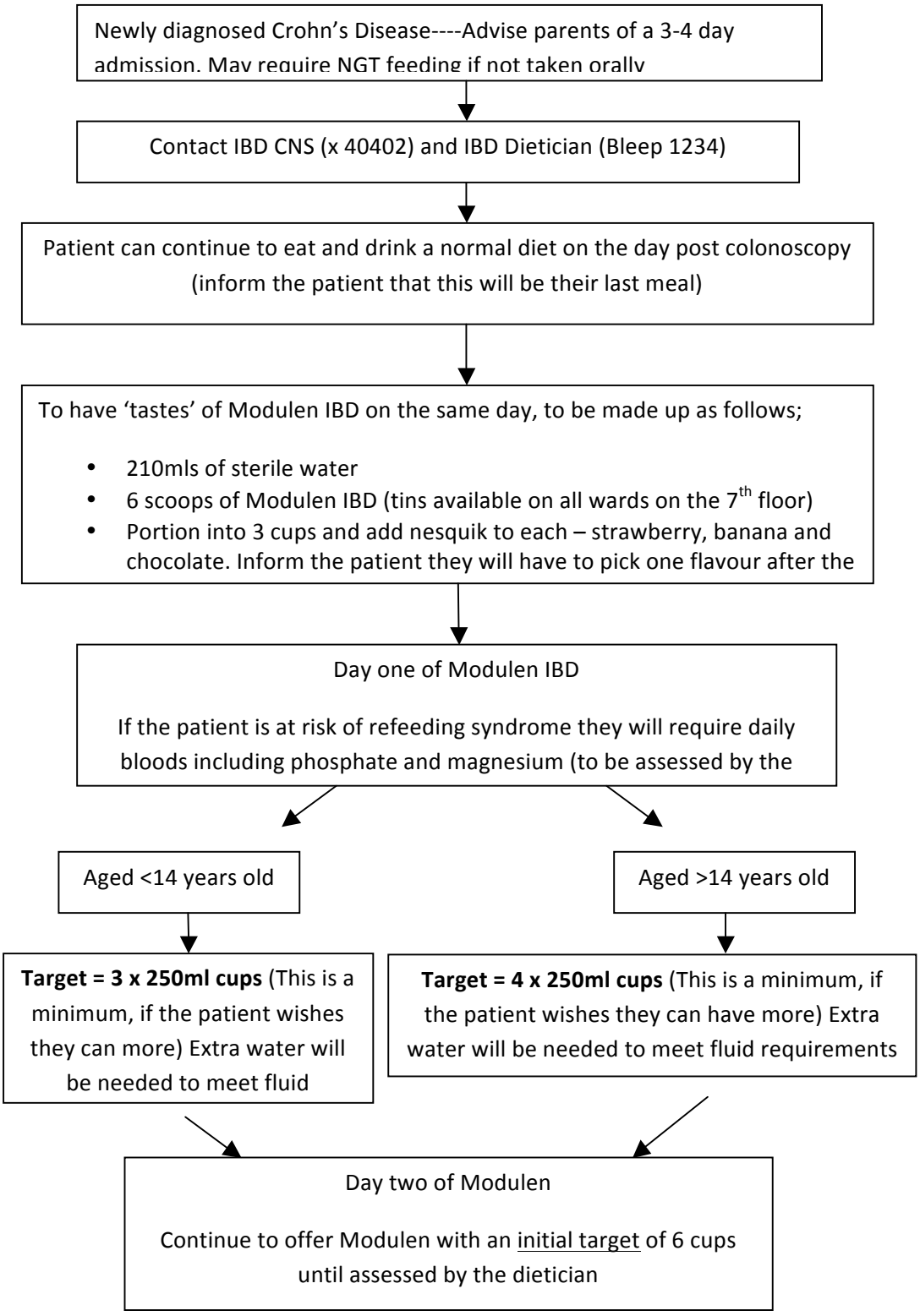
Add 10 scoops to the shaker cup and then add 160ml cool, boiled water

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.

Modulen flowchart:



- This pathway should not be used for children under the age of 6 years.
- In addition to Modulen IBD patients are only allowed to take plain water and sugar free chewing gum or mints orally.

Appendix 4: Composition of Modulen® IBD (Nestle UK)

Typical values	Per 100g	Per 100ml at 1.0 kcal/ml	Per 100ml at 1.25 kcal/ml	Per 100ml at 1.5
General				
Energy kJ/kcal	2070/500	420/100	525/125	630/150
Protein (15% kcal) g	18	3.6	4.5	5.4
Carbohydrates (43% kcal) g	54	11	13.8	16.5
of which sugars g	15	3.0	3.8	4.5
of which lactose mg	90	18	23	27
Fat (42% kcal) g	23	4.7	5.87	7.05
of which saturates g	13	2.70	3.38	4.05
of which	0.8	3.9	4.88	5.85
of which	0.49	2.4	3	3.6
of which MCT g	6.0	1.2	1.5	1.8
Vitamins				
A µg RE	420	84	105	126
D µg	4.9	1.0	1.2	1.5
E mg α-TE	6.5	1.3	1.6	1.9
K µg	27	5.5	6.9	8.3
C mg	47	9.7	12.1	14.5
B1 (Thiamin) mg	0.59	0.12	0.15	0.18
B2 (Riboflavin) mg	0.64	0.13	0.16	0.19
Pantothenic acid mg	2.4	0.50	0.62	0.75
B6 mg	0.83	0.17	0.21	0.25
B12 µg	1.6	0.32	0.40	0.48
Niacin mg	5.8	1.2	1.5	1.8
Folic acid µg	120	24	30	36
Biotin µg	16	3.2	4.0	4.8
Minerals				
Sodium mg/mmol	170/7.3	35/1.5	44/1.9	53/2.3
Potassium mg/mmol	600/15.4	120/3.1	150/3.8	180/4.6
Calcium mg/mmol	450/11.3	91/2.3	114/2.8	136/3.4
Phosphorus mg/mmol	300/9.6	61/2.0	76/2.5	92/3.0
Magnesium mg/mmol	100/4.1	20/0.83	25/1.04	30/1.25
Chloride mg/mmol	370/10.4	75/2.1	93.7/2.6	111/3.1
Iron mg	5.4	1.1	1.4	1.6
Zinc mg	4.7	0.96	1.20	1.44
Copper mg	0.49	0.10	0.12	0.15
Manganese mg	0.98	0.20	0.25	0.30
Fluoride µg	<10	<2.0	<2.5	<3.0
Chromium µg	25	5.1	6.3	7.6
Molybdenum µg	37	7.5	9.3	11.3
Selenium µg	17	3.5	4.4	5.3
Iodine µg	49	10	12	15
Other Nutrients				
Choline mg	35	7.2	9.0	10.8
Water g		3		
Osmolarity mOsm/l		290		
Osmolality mOsm/kg		340	426	539

Ingredients:

Glucose syrup, casein, sucrose, milk fat, medium chain triglycerides, corn oil, emulsifier: soya lecithin, potassium citrate, calcium phosphate, sodium citrate, calcium carbonate, magnesium chloride, acidity regulator: potassium hydroxide, potassium chloride, vitamins: C, E, niacin, pantothenic acid, B6, thiamin (B1), A, riboflavin (B2), D, folic acid, K, biotin, B12; choline bitartrate, ferrous sulphate, zinc sulphate, magnesium oxide, manganese sulphate, copper sulphate, sodium molybdate, potassium iodide, chromium chloride, sodium selenate. Clinically nil lactose. Gluten free.

Appendix 5. The normal range, mean and standard deviation scores of A) Insulin like growth factor-1, B) Insulin like growth factor binding protein-3 and C) Acid labile subunit according to age and gender

Data provided from Esoterix Laboratory Services Inc, Calabasas Hills, CA, USA

A

Age (yrs)	Male (ng/ml)	Mean	SD	Female (ng/ml)	Mean	SDS
1-2	30-122	76	23	56-144	100	22
3-4	54-178	116	31	74-202	138	32
5-6	60-228	144	42	82-262	172	45
7-8	113-261	187	37	112-276	194	41
9-10	123-275	199	38	140-308	224	42
11-12	139-395	267	64	132-376	254	61
13-14	152-540	346	97	192-640	416	112
15-16	257-601	429	86	217-589	403	93
17-18	236-524	380	72	176-452	314	69
19-20	281-510	371	76	217-475	323	75
21-30	155-432	289	73	87-368	237	74
31-40	132-333	226	62	106-368	225	71
41-50	121-237	160	42	118-298	205	60
51-60	68-245	153	48	53-287	172	55
61-70	60-220	132	34	75-263	180	51
71-80	36-215	131	46	54-205	156	46

B)

Age (yrs)	Range (mg/l)	Mean
1-4	0.8 - 3.0	2.1
5-6	1.5 - 3.4	2.4
7-8	2.1 - 4.2	3.0
9-11	2.0 - 4.8	3.3
12-13	2.1 - 6.2	3.8
14-15	2.2 - 5.9	4.2
16-18	2.5 - 4.8	3.8
19-30	2.0 - 4.2	3.0
31-70	1.9 - 3.6	2.7

C)

Age	Range (mg/l)	Mean	SD
0-2m	0.2 - 5.1	2.1	1.7
3-6m	0.7 - 5.6	3.4	1.3
7-11m	0.7 - 7.9	4.0	2.3
12m-2y	0.9 - 9.3	5.5	2.5
3-4y	1.9 - 10	6.8	2.7
5-7y	2.3 - 11	7.2	2.5
8-10y	4.2 - 13	8.9	2.5
11-13y	5.6 -16	12	3.6
14-18y	5.6 - 16	12	4.2
19-25y	7.0 - 16	12	2.5
26-35y	7.0 - 16	12	2.1
36-45y	7.0 - 16	11	1.7
46-55y	7.0 - 16	11	2.1
56-65y	7.0 -16	10	3.0

Appendix 6. Consent and assent forms for Pharmacokinetic studies of recombinant human insulin-like growth factor-1 (rhIGF-1) in children with Crohn's disease-induced growth retardation.

PI: Professor Ian Sanderson. Trial reference: 07/H0705/77

PARENT/CARER'S INVITATION TO PARTICIPATE IN A RESEARCH STUDY:

(VERSION 1; 14 AUGUST 2007)

PHARMACOKINETIC STUDIES OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR I (rhIGF-I) IN CHILDREN WITH CROHN'S DISEASE-INDUCED GROWTH RETARDATION.

Dept of Paediatric Gastroenterology

Barts and the London NHS Trust

We invite your child to participate in research which we think may be important. The information which follows tells you about it. It is important that you understand what is in this leaflet. It says what will happen if your child takes part and what the risks might be. Try to make sure you know what will happen to your child if you decide to take part. Whether or not you do take part is entirely your and your child's choice. Please ask any questions you want to about the research and we will try our best to answer them.

The Department of Paediatric Gastroenterology at Barts and the London has a major role in leading research into diseases of the bowel in children. Diseases which we see often in the clinics and have on-going research interest include stomach ulcers and inflammation, inflammatory bowel disease (Crohn's disease and ulcerative colitis), coeliac disease, food allergy, abdominal pain and ulcers, gut involvement of cystic fibrosis, gut problems in children undergoing treatment for cancers, and reflux disease.. We are able to do a lot of work using special cells bought from companies. However, to test medicines designed for children we need to test them in children before they can be prescribed by doctors.

As you know, the inflammation in Crohn's disease has reduced your child's growth. We have been using medicines to settle down this inflammation as way of treating this. But, at the moment, there is no treatment that directly improves growth. To develop such a treatment, we would need to test a medicine that has the possibility of improving growth. There is a medicine called human insulin-like growth factor-I (IGF-I), which is used in children who, because of their genes, do not produce sufficient IGF-I Because of this low IGF-I, they do not grow well. (Your child's genes are fine regarding IGF-I, but the inflammation in Crohn's disease reduces the production of IGF-I).

We would like to test the IGF-I medicine in your child, using it as it is used in children that do not produce IGF-I because of their genes. However, we cannot just give him/her the medicine and see if he/she grows, because the way that the medicine is broken down by his/her body may be different from these other children. We need to first see if giving the medicine increases the amount of IGF-I in your child's blood, and by how much does it do this.

What we are asking

We wish to examine the levels of IGF-I in your child over a period of 7 days while he/she is on the medicine.

The medicine is given under the skin, twice a day. We would therefore like to bring your child into hospital for a week, give him/her the IGF-I medicine twice a day on the first day and measure the blood levels regularly over 24 hours and then each morning after that. In order to avoid pricking him/her regularly with needles, we will put one needle into the back of the hand from which we can sample blood regularly. Over the course of the first day we will take about 5 tablespoon fulls of blood; and then one tablespoonful of blood each morning for the rest of the week. (A tablespoon full of blood is the amount of blood we take from your child when you come to our outpatients.)

We would then like to see if we can build up the IGF-I in your child's blood over time, and so we will give the medicine again twice a day for the last two days of your stay with us.

How will this affect my child ?

Your child will need to come into hospital for a week. It will be in the same hospital as he/she has already stayed in for your endoscopies and other tests.

He/she will have a cannula in the back of your hand from which take blood. Again, this is like the cannula we put into the back of the hand when for the colonoscopy.

It is unlikely that the one-week of treatment will have a measurable effect on height. But once we establish that the IGF-I we give increases the levels of IGF-I in the blood of children with Crohn's disease in this study, we will apply for research permission to test the IGF-I over a longer period and see if it improves height. However, this is not part of the present study and we do not have permission to do this until the present study has been completed.

While you are in hospital your child will be able to eat and drink normally; and he/she will be able to attend the hospital school as normal, if it is school term.

What research is being done ?

Broadly speaking, we are studying how Crohn's disease affects growth.. We have discovered many of the pathways that lead from inflammation to growth retardation. We have also found variations in a gene that this important in pathway. (You may even have consented to us looking at your child's DNA for this if he/she was one of our patients in 2002-2004). We have been successful in getting rats with intestinal inflammation to grow with IGF-I. But this is the first time we have used this treatment in children with Crohn's disease.

The research included within this application will not have any impact on your care (including any direct benefit or harm). All our research is aimed to increase understanding of how and why diseases occur, how treatments work and how we may be able to improve treatments in the future. If any of studies affect the care of individual children then a separate consent form will be produced.

Feedback

As the information found is not of any direct clinical relevance we will not routinely be feeding back any information to you. However it is possible for you to know more about the research and what has happened to any blood collected:

- We have a web page (http://www.icms.qmul.ac.uk/centres/gastroenterology/index.html). You can click onto individual members of staff where you can see the titles of recent publications (including our colleagues in adult gastroenterology) can be seen.
- Dr Croft and Professor Sanderson maintain a file of ongoing projects (which may use specimens from patients) which can be seen on request.
- We will also write to your GP about your coming in for the study.
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Is this anonymous ? While collection of these specimens will not be anonymous only doctors or nurses involved in his/her clinical care will have access to your name. This allows us to go back to the hospital notes if we need to identify important and relevant information later on. Within the laboratory anonymity will be maintained by allocating a number (rather than his/her name) to the specimens. Only Professor Sanderson, Dr Croft or staff from the wards/clinics under his supervision will be able to link his/her name to the specimen..

What happens if I am not keen or change my mind ?

You are entirely at liberty to decide not to participate or drop out at any time and this will not affect your child's care in any way. If after specimens have been collected you change your mind contact us and the specimens will be discarded if they have not already been used.

Is this ethical ?

We have gained approval from the Research Ethics Committee for the collection and storage of these specimens and data and each individual projects.

Will my child's taking part in this study be kept confidential?

Only the doctors and members of the hospital who look after you will be able to identify the medical information we collect as being from your child. The results of the tests we carry out will be shared by other researchers, but no-one will know that the measurements have come from you. The research we do can be inspected by inspectors appointed to safeguard patients' interests. We would like to ask you if they can look at your child's records if they wish.

***What happens if we are worried or if there is an emergency*?** You will always be able to contact someone to discuss your concerns and/or to get help: **via** Grovesnor Ward, Barts and the London NHS Trust. or Professor Sanderson's secretary: Tel. No. 020 7377-6339; (if no response phone 020 7377-7000 requesting the paediatric gastroenterology registrar on call).

Any other Questions ? Any other questions can be addressed by writing to Professor Sanderson, Dr Croft at the Dept of Paediatric Gastroenterology, Barts and the London NHS Trust or contacting his secretary (0207 377 6339). Professor Sanderson and Dr Croft (if no response phone 020 7377-7000 requesting the paediatric gastroenterology registrar on call.)

We will take every care in the course of this study. If through our negligence any harm to you results, you will be compensated. However, a claim may have to be pursued through legal action. Even if the harm is not our fault, Barts and the London NHS Trust will consider any claim sympathetically. If you are not happy with any proposed compensation you may have to pursue your claim through legal action.

Queen Mary University of London has agreed that if your child is harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures you received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 020 7377 6335, minicom 020 7943 1350, or email pals@bartsandthelondon.nhs.uk, you can also visit PALS by asking at any hospital reception.

CHILD (10-16 YEARS)'S INVITATION TO PARTICIPATE IN A RESEARCH STUDY:

(VERSION 2-1ST SECTION; 11 NOVEMBER 2007)

PHARMACOKINETIC STUDIES OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR I (hIGF-I) IN CHILDREN WITH CROHN'S DISEASE-INDUCED GROWTH RETARDATION.

Dept of Paediatric Gastroenterology

Barts and the London NHS Trust

First part of the study: a single dose

What we are asking

We wish to examine the levels of IGF-I in your blood after a single dose.

We would therefore like to bring you into hospital for a night, a day and then a second night. We will give you the medicine (IGF-I) under the skin and measure the blood levels regularly over 24 hours. In order to avoid pricking you regularly with needles, we will put one needle into the back of your hand from which we can sample your blood regularly. Over the course of the day we will take about 3 tablespoons of blood. (2 tablespoons full of blood is the amount of blood we take from when you come to our outpatients.)

How will this affect me?

Why do I need a sampling tube in the back of my hand?

Early in the morning, we will put have a cannula in the back of your hand from which we take blood. This is like the cannula we put into the back of your hand when you had your colonoscopy. We will leave it in for 24 hours. It allows us to take blood regularly without using a needle each time.

We need to measure the levels of the medicine regularly to see if its level has increased in your blood. We also want to measure other chemicals in the blood that are important for growth. Finally, the medicine we give you can (not very often) reduce the sugar level in your blood. We therefore need to measure your sugar levels regularly.

Why do I need to come into hospital?

We need to measure the levels of medicine and the sugar levels for a whole day (24 hours) to see how active the medicine is. We can only do the sampling in hospital. It will be in the same hospital as you have already stayed in for your endoscopies and other tests. While you are in hospital you will be able to eat and drink normally; and you will be able to attend the hospital school as normal, if it is school term.

Will the medicine cause any side-effects?

This medicine is regularly prescribed by doctors to children with IGF-I deficiency; but it has not been used in children with Crohn's disease. From our knowledge of its use in children, it hardly ever causes side effects, but we will describe what has been seen:

Sugar levels in the blood

The medicine makes the sugar level go down in about 1 in 10 children for a few minutes. This is why we need to measure it regularly during the study. If your sugar level falls, you will feel sleepy or that things around you do not seem real. Doctors have not needed to give any treatment for this, as it quickly wears off. However, if we need to, we can give sugar solution into your vein (through the cannula) or give you a sugary drink.

Skin where we give you the medicine

Normally, one can never see where the medicine has been given. But just occasionally, the fat under the skin becomes slightly reduced. This makes the skin less tight over the area about half an inch from where the medicine was given.

What research is being done?

Broadly speaking, we are studying how Crohn's disease affects growth. We have discovered many of the pathways that lead from inflammation to growth retardation. We have also found variations in a gene that this important in pathway. (You may even have consented to us looking at your DNA for this if you were one of our patients in 2002-2004). We have been successful in getting rats with intestinal inflammation to grow with IGF-I. But this is the first time we have used this treatment in children with Crohn's disease.

The research included within this application will not have any impact on your care (including any direct benefit or harm). All our research is aimed to increase understanding of how and why diseases occur, how treatments work and how we may be able to improve treatments in the future. If any of studies affect the care of individual children then a separate consent form will be produced.

Feedback

As the information found is not of any directly affect how we treat your Crohn's disease, we will not routinely be feeding back any information to you. However it is possible for you to know more about the research and what has happened to any blood collected:

- We have a web page (http://www.icms.qmul.ac.uk/centres/gastroenterology/index.html). You can click onto individual members of staff where you can see the titles of recent publications (including our colleagues in adult gastroenterology) can be seen.
- Dr Croft and Professor Sanderson maintain a file of ongoing projects (which may use specimens from patients) which can be seen on request.
- We will also write to your GP about your coming in for the study.

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Is this anonymous? Collection of these specimens will not be anonymous, but only doctors or nurses involved in your clinical care will have access to your name. This allows us to go back to the hospital notes if we need to identify important and relevant information later on. Within the laboratory anonymity will be maintained by allocating a number (rather than your name) to the specimens. Only Professor Sanderson, Dr Croft or staff from the wards/clinics under their supervision will be able to link your name to any of the specimens.

What happens if I am not keen or change my mind ?

You are entirely at liberty to decide not to participate or drop out at any time and this will not affect your care in any way. If after specimens have been collected you change your mind contact us and the specimens will be discarded if they have not already been used.

Is this ethical?

We have gained approval from the Research Ethics Committee for the collection and storage of these specimens and data and each individual projects.

Will my taking part in this study be kept confidential?

Only the doctors and members of the hospital who look after you will be able to identify the medical information we collect as being from you. The results of the tests we carry out will be shared by other researchers, but no-one will know that the measurements have come from you. The research we do can be inspected by inspectors appointed to safeguard patients' interests. We would like to ask you if they can look at your records if they wish.

***What happens if we are worried or if there is an emergency*?** You will always be able to contact someone to discuss your concerns and/or to get help: **via** Grovesnor Ward, Barts and the London NHS Trust. or Professor Sanderson's secretary: Tel. No. 020 7377-6339; (if no response phone 020 7377-7000 requesting the paediatric gastroenterology registrar on call). You will be in hospital for the study itself, and you can ask for a nurse or doctor to answer your questions while you are in hospital.

Any other Questions ? Any other questions can be addressed by writing to Professor Sanderson, Dr Croft at the Dept of Paediatric Gastroenterology, Barts and the London NHS Trust or contacting his secretary (0207 377 6339). Professor Sanderson and Dr Croft (if no response phone 020 7377-7000 requesting the paediatric gastroenterology registrar on call.)

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received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

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