Effect of Therapeutic Interventions on Skeletal Growth & Development in Paediatric Inflammatory Bowel Disease

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Effect of therapeutic interventions on skeletal growth & development in paediatric Inflammatory Bowel Disease

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AUTHOR'S DECLARATION

I hereby declare that all the work in this thesis is unless otherwise indicated is all done by myself and is a record of work performed by me in the department child health between April 2008-2012. This work has not been submitted previously for a higher degree and was performed under the supervision of Professor Syed Faisal Ahmed and Dr Richard Kay Russell.

Umm-ie-Salma Malik

I certify that the work reported in this thesis has been performed by Miss Malik and that during the period of study she has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy, University of Glasgow.

Prof Syed Faisal Ahmed

DISCLOSURE STATEMENT

I hereby declare that all the research work for this thesis (ethical approval, designing case report forms, study design, patient recruitment, data collection and data analysis) was undertaken by myself with the exception of:

1. Bone scans (DXA, pQCT) which were kindly performed by Dr Sheila Khanna
2. Serum ELISA for IGF-1, IGFBP-2, IGFBP-3, BALP, CTX-1, ALS and Infliximab pharmacokinetics which were performed by Martin McMillan
3. Data collection for chapter 4 which were collected and provided by Dr David Wilson and Michelle Wilson at the University of Edinburgh.

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In The Name Of Allah, The Most Merciful, The Most Beneficent

"Read, in the name of your Lord who created, created man from a clot. Read, for your Lord is most Generous, Who teaches by means of the pen, teaches man what he does not know."

Quran Al-Alaq (96: 1-5)

ABSTRACT

Crohn's disease (CD) is a chronic inflammatory bowel disease. Once considered rare in the paediatric population, it is recognized with increasing frequency among children of all ages. Approximately 20-30% of all patients with CD present when they are younger than 20 years. With its increasing recognition, CD has become one of the most important chronic diseases that affect children and adolescents. In addition to the common gastrointestinal (GI) symptoms (diarrhoea, rectal bleeding, and abdominal pain) children often experience growth retardation, pubertal delay, and bone demineralization. In these children, maintenance of skeletal health is a complex process that is influenced by a number of different mechanisms including steroid therapy, the disease process, nutritional status, endocrine status and the response of the body to inflammatory mediators. The recent introduction of biologic therapy that targets specific mediators of the proinflammatory process is a promising adjunct in the therapeutic management of the child with chronic inflammation. These drugs may also exert beneficial effects on the adverse effects of inflammation on growth and skeletal development. It is unclear whether these beneficial effects are due to improvement in overall disease or due to a direct 'anti-cytokine' effect at the level of the target tissue involved in growth and skeletal development.

The hypothesis of this study was that the biologic therapy improves linear growth, puberty, bone health, body composition and muscle function in children with CD and this is associated with changes in the IGF-1 axis and markers of bone formation and bone resorption.

Chapter 1 is an extensive literature review about the effects of biologic therapy on growth and skeletal development in paediatric patients with chronic inflammatory conditions particularly inflammatory bowel disease (IBD). The main aim of this review was to summarize and evaluate effects of inflammation and biologic therapy on growth and skeletal development in children with chronic inflammatory conditions and to explore the areas of interest for further research.

Chapter 2 is the study about the growth in children receiving contemporary disease specific therapy in children with CD. The aim of this study was to assess the frequency of short stature and poor growth and their relationship to disease course and therapy in children with CD. Clinical records of all children with a confirmed diagnosis of CD, who were between 2yrs and 18yrs at the Royal Hospital for Sick Children, Glasgow were examined retrospectively. Data were collected at diagnosis, 1-yr, 2-yr and 3-yr after diagnosis and at maximum follow-up. The relationship of a number of factors including therapeutic modalities to two commonly

used anthropometric markers of growth height velocity standard deviation scores (HVSDS) and change in height standard deviation scores (ΔHtSDS) was examined. This study suggested that ΔHtSDS may be a more valid method of assessing and reporting longitudinal growth in children with chronic disease, particularly when there is a high prevalence of children of a peri-pubertal age. This study provides clear evidence that despite advances in therapy, short stature and slow growth continue to be encountered in a sub-group of children with CD.

Chapter 3 is about the effect of Infliximab therapy on growth, puberty and disease activity in children with CD. The aim of this study was to assess growth, puberty, markers of disease and concomitant therapy over the six months prior to starting Infliximab and for the 6 and 12 months following treatment. Clinical records of all children with IBD who were started on Infliximab therapy between 2003 and 2008 at the Royal Hospital for Sick Children were examined retrospectively. This study has shown an average improvement of approximately 50% in HV in the 6 months after the initiation of Infliximab therapy which was further sustained for a further 6 months. Improvement in growth was found to be better in those children who were responders as compared to non-responders suggests that growth improved as a result of disease control. Improvement in growth was also observed in children who remained pre-pubertal and those who had never been on glucocorticoids (GC) compared to those who had been on GC. This study suggests that increase in height may not be simply due to progress in pubertal status or reduction in glucocorticoid dose.

Chapter 4 is about the effect of Adalimumab therapy on growth in paediatric patients with CD. This is the one and only world wide multicentre study that adequately assess the effect of Adalimumab on linear growth in children with CD. The aim of this study was to assess the effect of Adalimumab therapy on growth, puberty and disease activity over the 6 months prior to and 6 months after starting Adalimumab treatment in children with CD. This study provides evidence that Adalimumab is associated with improvement in short term linear growth in children with CD who enter remission but not in those who do not. It is also more likely to happen in children who are on immunosuppression and those in early puberty but seems to be relatively independent of steroid use. These findings suggest that growth improves as a result of several interrelated factors, including improved disease control. It was also interesting to note that the growth response to Adalimumab varied dependent on the reason for discontinuing Infliximab; those who had an allergic reaction to Infliximab fared best.

Chapter 5 is Longitudinal observational prospective study of changes in physical growth, IGF-1 axis, bone health, body composition, muscle function and disease activity at baseline (BL), 2 weeks (2wk), 6 weeks (6wk), 6 and 12 months (6M & 12M) following biologic therapy in paediatric patients with CD. The aim of this longitudinal observational prospective study was to assess changes in physical growth, puberty, IGF-1 axis, bone health; body composition and muscle function following biologic therapy in paediatric patients with CD. Patients either newly diagnosed or patients with long-lasting disease in clinical relapse, who started treatment with biologic therapy as part of their standard clinical management, were recruited. A non significant improvement was observed in both ΔHtSDS and HVcms/yr at 12M as compared to BL. Individually, the majority of the children experienced improvement in clinical activity and improvement of the systemic inflammatory markers. A significant increase in biomarker of bone formation bone specific alkaline phosphatase (BALP) and a non-significant increase in a biomarker of bone resorption cross-linked c-terminal telopeptides (CTX-1) was observed from BL to 12M. This observation suggests the beneficial effect of biologic therapy on bone formation.

This study showed a significant change in fat mass (FM (kg) in paediatric patients with CD following biologic therapy an effect that has not been reported extensively in previously published studies. A significant change in both fat free mass (FFM (kg) and fat free mass index (FFMI(kg/m^2) shows that the treatment with anti-TNF-α therapy also had a significant impact on fat mass accrual. This is the first study that charts the effect of biologic therapy on changes in lower limb muscle function using jumping mechanography in paediatric patients with CD. A non significant change was observed in jump height (m), V-max (m/s), EFI (%), efficiency % from BL to 12M following biologic therapy and a significant increase in both F-max (kN), and P-max (kW) at 12M. Despite the fact that the increase in efficiency % of the movement was not significant but however, the change was likely to be through improvements in jump height and velocity thereby indicating higher muscular flexibility. These data are suggestive of an effect of biologic therapy on lower limb muscle function through improvements in the mechanical efficiency of the muscle. Thus, it is possible that the better muscle function is mediated through the effect of biologic therapy on muscle mass.

Peripheral quantitative computed tomography (PQCT) for tibia and radius both indicated significant increase in fat and muscle cross sectional area (Fat-CSA (mm^2) and Mus-CSA (mm^2). Moreover, tibia pQCT also indicated significant change in stress strain index (SSI (mm^3) a surrogate marker of bone strength, at 12M of therapy. No significant changes were observed in total body, lumbar spine (L2-L4), proximal femur and femoral neck bone mineral density (BMD (g/cm^2), insulin like growth factor-1 (IGF-1(ng/ml), insulin like growth factor

binding proteins (IGFBP-3 (ng/ml), IGFBP-2 (ng/ml) and acid labile subunits (ALS (ng/ml) following biologic therapy.

In conclusion, these prospective studies of growth, bone health, body composition and muscle function have suggested that biologic therapy in children with CD has a beneficial effect on muscle mass and muscle function and which can be observed over the first year of therapy for conclusive explanation of these changes these data needs to be adjusted for body size. These positive changes are also associated with an increase in bone turnover where the change in bone formation is much greater than bone resorption. These favourable effects on bone health were not accompanied by marked changes in BMD as assessed by DXA but did show some beneficial effects on pQCT assessed SSI, a surrogate marker of bone strength. It is possible that controlling disease activity with biologic therapy may positively outweigh the effects of CD on growth, body composition, muscle function and bone health. The results of this preliminary study need to be confirmed in a larger group of children. An improved understanding of the effect of biologic therapy may improve future therapy directed at promoting growth and skeletal development in a diverse group of children. Further studies are required to understand the duration of the window of opportunity during which linear growth, bone and muscle mass in children with CD can be optimised.

Chapter 6 focuses on the main findings of this thesis and discusses potential limitations of this methodology, and outlines some important and interesting extensions for future research in paediatric patients with CD following biologic therapy.

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DEDICATIONS

This thesis is dedicated to my mother Mumtaz Akhtar and my brother and sisters who have all supported me and given me the strength to complete this thesis.

This thesis is also dedicated to the living memory of my father Malik Muhammad Idrees may Almighty ALLAH bless him.

PUBLICATIONS

Full papers

1. **S Malik** & SF Ahmed. Biologic therapy and its effect on growth and skeletal development in children with chronic inflammatory conditions. Exp Rev Endocrinol Metab, 2010;733-740

2. **S Malik**, SC Wong, J Bishop, K Hassan, P McGrogan, RK Russell, SF Ahmed. Improvement in growth of children with Crohn's disease following anti-TNF-α therapy can be independent of pubertal progress and glucocorticoid reduction. J Pediatr Gastroenterol Nutr. 2011 Jan;52(1):31-7.

3. Avril Mason, **Salma Malik**, Paraic McGrogan, Jonathan Bishop, Richard Russell, Faisal Ahmed. The impact of inflammatory bowel disease on pubertal growth. Horm Res Paediatr. 2011;76(5):293-9.

4. **S Malik,** SF Ahmed, ML Wilson, N Shah, S Loganathan, S Naik, B Bourke, A Thomas ,AK. Akobeng, A Fagbemi, DC Wilson, RK Russell. The effects of anti-TNF-α treatment with Adalimumab on growth in children with Crohn's disease (CD). J Crohns Colitis. 2012 Apr;6(3):337-44.

5. **S Malik**, A Mason, A Bakhshi, J Bishop, V Garrick, P McGrogan, RK Russell, SF Ahmed. Growth in children receiving contemporary disease specific therapy for Crohn's disease. Arch Dis Child. 2012 Aug;97(8):698-703.

Published Abstracts

1. **S Malik**, SC Wong, RK Russell, P McGrogan, SF Ahmed Lack of short term catch up growth in children with Crohn's disease treated with Infliximab in the UK. *Endocrine Abstracts* 2008;17:P8

2. **S Malik**, SC Wong, J Bishop, K Hassan, P McGrogan, RK Russell, SF Ahmed. Improvement in growth of children with Crohn's disease following anti-TNF α therapy can be independent of pubertal progress and glucocorticoid reduction UK. *Endocrine Abstracts* 2009;19:P9

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4. Avril Mason[1], Paraic McGrogan[2], Richard Russell[2], Jonathan Bishop[2], **Salma Malik[1]** & Faisal Ahmed[1]. The impact of IBD on pubertal growth is most marked in boys with Crohn's disease. Endocrine Abstracts, Society for Endocrinology (BES) 2010 P21 P230

5. **S Malik**, SF Ahmed, ML Wilson, N Shah, S Loganathan, S Naik, B Bourke, A Thomas ,AK. Akobeng, A Fagbemi, DC Wilson, RK Russell. The effects of anti-TNF-α treatment with Adalimumab on growth in children with Crohn's Disease (CD).Scottish Medical Journal. DOI: 10.1258/smj.2011.011150

Oral presentations

1. Biologics in children with inflammatory bowel disease (IBD) Research Workshop in Developmental Endocrinology, The Medical School, University of Glasgow, UK Dec 2010

2. The effect of biologicals on growth in children with inflammatory bowel disease (IBD), BSPGHAN Annual Winter Meeting Liverpool, UK, Jan 2010

3. Growth in children receiving contemporary therapy for crohn's disease. Yorkhill research day, Glasgow UK, Nov 2010

4. The effects of anti-TNF-α treatment with Adalimumab on growth in children with Crohn's Disease (CD). Scottish Paediatric Society Summer Meeting Dundee Scotland, UK, June 2011

5. Biologic therapies in children with Crohn's disease, SSPGHAN (Scottish Society for paediatric gastroenterology, Hepatology and nutrition) meeting at Crieff hydro Scotland ,UK November 2011

Poster presentations

1. Lack of short term catch up growth in children with Crohn's disease treated with Infliximab. British Society for Paediatric Endocrinology and Diabetes (BSPED),Nov 2008 Swansea, UK.

2. Improvement in growth of children with Crohn's disease following anti-TNF α therapy can be independent of pubertal progress and glucocorticoid reduction" British Society for Paediatric Endocrinology and Diabetes (BSPED),Nov 2009 Reading, UK.

3. Improvement in growth of children with Crohn's disease following anti-TNF α therapy can be independent of pubertal progress and glucocorticoid reduction. 8th joint ESPE (European Society for Paediatric endocrinology) Sep 2009 New York, USA.

4. The effect of biologicals on growth in children with inflammatory bowel disease (IBD).BSPGHAN Annual Winter Meeting 27th to 29th Jan 2010, Liverpool, UK.

5. Growth in children with Crohn's disease receiving disease specific contemporary. BSPGHAN 25th Annual Winter Meeting Jan 2011, Edinburgh, UK.

6. The effects of anti-TNF-α treatment with Adalimumab on growth in children with Crohn's Disease (CD)". ESPE 50th annual meeting 26-29th Sep 2011 Glasgow, UK.

7. Growth in children with Crohn's disease receiving disease specific contemporary therapy. ESPE 50th annual meeting 26-29th Sep 2011, Glasgow, UK.

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9. Effect of biologic therapy on growth and skeletal development in children with Crohn's disease (CD). Yorkhill research day, Glasgow UK, 9th Nov 2012.

Achievements

Crohn's and Colitis Foundation of America (CCFA) Travel Grant towards attending "The growth of paediatrics and skeletal health in inflammatory bowel disease workshop" 11-13th Nov, 2011 New York, USA.

Best poster prize "Yorkhill Research Day" 9th Nov, 2012 Glasgow, UK

ABBREVIATIONS

ALS	Acid labile subunits
ADA	Adalimumab
ALT	Alanine transaminase
ALP	Alkaline phosphatase
aBMD	Areal bone mineral density
BL	Baseline
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BA	Bone age
BALP	Bone alkaline phosphatase
BA	Bone area
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMPs	Bone morphogenic proteins
BSP	Bone sialoprotein
ΔHtSDS	Change in height standard deviation scores
Crt-BA	Cortical bone area
CrtBMD	Cortical bone mineral density
CRP	C-reactive protein
CD	Crohn's disease
CSA	Cross sectional area
CTX	Cross-linked C-terminal telopeptides
NTX	Cross-linked N-terminal telopeptides
PICP	C-terminal pro-peptides of type I collagen
CF	Cystic fibrosis
DPD	Deoxypyridinoline
D	Dominant
DXA	Dual energy X-ray absorptiometry
EEN	Exclusive enteral nutrition
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EFI	Esslinger fitness index

Fat-CSA	Fat cross-sectional area
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FMI	Fat mass index
FN	Femoral neck
FGFs	Fibroblast growth factors
FSH	Follicle-stimulating hormone
FDA	Food and Drug Administration
INF-γ	Gamma interferon
GLM	General linear models
GC	Glucocorticoids
GnRH	Gonadotropin releasing hormone
GM-CSF	Granulocyte macrophage colony stimulating factor
GRFP	Ground Reaction Force Platform
GH	Growth hormone
GH/IGF-1	Growth hormone/insulin-like growth factor-1
HBI	Harvey-Bradshaw index
HAHA	Human anti-humira antibodies
HACA	Human anti-chimeric antibodies
Ht	Height
HtSDS	Height standard deviation scores
HV	Height velocity
HVSDS	Height velocity standard deviation scores
HUMIRA	Human Monoclonal Antibody in Rheumatoid Arthritis
IBDU	IBD unspecified
IgG-1	Immunoglobulin-1
IBD	Inflammatory bowel disease
IFX	Infliximab
IGFBPs	Insulin-like growth factor -binding proteins
IGF-1	Insulin-like growth factor-I
IGFs	Insulin-like growth factors
IL-1ra	Interleukin-1 receptor antagonist
IL-2	Interleukin-2
IL-1	Interleukins-1

IL-6	Interleukins-6
IC	Intermediate colitis
JHt	Jump height
JIA	Juvenile idiopathic arthritis
LS	Lumbar spine
LHRH	Luteinizing hormone releasing hormone
MIGF	Maximal isometric grip force
F-max	Maximum - force
MF	Maximum follow-up
P-max	Maximum-power
V-max	Maximum-velocity
Mus-CSA	Muscle cross-sectional area
PINP	N- terminal pro-peptides of type I collagen
ND	Non-dominant
OC	Osteocalcin
ON	Osteonectin
OPG	Osteoprotegrin
PTH	Parathyroid hormone
pQCT	Peripheral quantitative computed tomography
PGA	Physicians Global Assessment
PDGF	Platelet-derived growth factors
CRP	Platelets, C-reactive protein
PEG	Polyethylene glycol
PF	Proximal femur
PYD	Pyridinoline
QUS	Quantitative Ultrasound
RUS	Radius ulna short bones
RANKL	Receptor activator of nuclear factor kappa B ligand
Alb	Serum albumin
S2LJ	Single Two-legged Jump
SH	Sitting height
SDS	Standard deviation scores
SSI	Strength strain index
SLE	Systemic lupus erythematosus
TS	Tanner stage

TW2	Tanner-Whitehouse
TRAP5b	Tartrate-resistant acid phosphatase
PCDAI	The Paediatric Crohn's Disease Activity Index
PUCAI	The Paediatric Ulcerative Colitis Activity Index
TB	Total body
TotBMD	Total bone mineral density
TrbBMD	Trabecular bone mineral density
TGFβ	Transforming growth factor-beta
TNF-α	Tumor Necrosis Factor -Alpha
Th1	Type I helper T-cells
UC	Ulcerative colitis
vBMD	Volumetric bone mineral density
Wt	Weight
bCL	β-Cross Laps
12M	12 months
2wk	2 weeks
5-ASA	5-Aminosalicylates
6M	6 months
6wk	6 weeks
6-MP	6-mercaptopurine

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CHAPTER 1

Introduction and literature review

1.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of disorders of the gastrointestinal tract characterized by intestinal inflammation and a chronic relapsing course. IBD has traditionally been categorized as either ulcerative colitis (UC) or Crohn's disease (CD) on the basis of clinical, radiological, endoscopic and histological criteria. About 10% of colitis cases show overlapping features of the two major forms and are called intermediate colitis (IC) or IBD unclassified (IBDU).

Ulcerative Colitis (UC) is a condition in which the inflammatory response and morphologic changes remain confined to the colon. The rectum is involved in 95% of patients, with variable degrees of proximal extension. Inflammation is limited primarily to the mucosa and consists of continuous involvement of variable severity with ulceration, oedema, and haemorrhage along the length of the colon. The characteristic histologic findings are acute and chronic inflammation of the mucosa by polymorphnuclear leukocytes and mononuclear cells, crypt abscesses, distortion of the mucosal glands, and goblet cell depletion (1).

Crohn's disease (CD), in contrast to UC, can involve any part of the gastrointestinal tract from the oropharynx to the perianal area. Diseased segments frequently are separated by intervening normal bowel, leading to the term "skip areas." Inflammation can be transmural, often extending through to the serosa, resulting in sinus tracts or fistula formation. Histologic findings include small superficial ulcerations over a Peyer's patch (aphthoid ulcer) and focal chronic inflammation extending to the submucosa, sometimes accompanied by noncaseating granuloma formation. The most common location is the ileo-cecal region, followed by the terminal ileum alone; diffuse small bowel, or isolated colonic disease in decreasing order of frequency (1).

In around 10% of cases of IBD in which the inflammation is limited to the large bowel it may be impossible, at least at presentation, to differentiate between CD and UC. These cases are referred to as IBDU (2).

1.1.1 Phenotypic classification of IBD

There are a great number of phenotypic classifications of IBD; mostly commonly used phenotypic classifications are Vienna, Montreal and the recently developed Paris classification (3-5). Vienna classification of CD considered age of onset, disease location and disease behaviour as the predominant phenotypic elements (3). Although great efforts were made by working group to develop a reproducible classification, but still this classification has limitations.

This classification is unable to clearly distinguish disease location when it is present in both the upper GI tract and the intestinal regions. Moreover, in this classification perianal disease is not considered as a separate category (3). A working party of investigators was established in 2005 which modified the Vienna phenotypic classification of IBD and named it Montreal classification. The Montreal modification of the Vienna classification has not changed the three predominant parameters of age at diagnosis, location, and behaviour, but modifications within each of these categories have been made. Montreal revision to Vienna classification included (1) an additional category to classify children diagnosed at ≤ 16 years of age (2) allowing for upper GI tract disease to be classified

independently of ileocolonic and colonic involvement and (3) addition of perianal disease under separate category. For UC, the group proposed that patients be classified according to maximal extent of inflammation at any time during follow-up. The Montreal working group has recommended that the term ‘‘indeterminate colitis’’ should be reserved only for those cases where colectomy has been performed and pathologists are unable to make a definitive diagnosis of either CD or UC after full examination (5;6) (Table 1.1.1). Montreal classification has several weaknesses with respect to the classification of young patients. It lacks the dynamic features of the paediatric disease phenotype i.e change in disease location and behaviour over time. Moreover, linear growth is not considered at all in Montreal classification. More recently, additional modifications to the existing Montreal classification have been made to capture features of the disease that are pertinent to paediatric IBD patients by a working party of paediatric IBD experts, in conjunction with the 2nd international symposium on paediatric IBD held in Paris France in 2009 (4) (Table 1.1.1) . Paris revision to Montreal classification included (1) the subdivision to age so as to ensure that the younger patients are appropriately classified (2) an additional behaviour category, combining both stricturing and penetrating disease (3) a category denoting the growth status (4).

1.1.2 Clinical indices in paediatric IBD

Longitudinal assessment of disease activity is necessary for the estimation of disease activity and for studies of therapeutic interventions in children with IBD. The Pediatric Crohn's Disease Activity Index (PCDAI) currently the only validated and most widely used tool for assessing disease activity in children with CD. It was developed and validated in April 1990 by a group of senior paediatric gastroenterologists with extensive experience in the clinical management of children with IBD (7). The PCDAI consists of subjective patient reporting of symptoms (abdominal pain, number off liquid stools, general well being), physical examination (weight, height, abdominal exam, perianal disease and extra-intestinal

manifestations) and common laboratory tests (hematocrit, erythrocyte sedimentation rate (ESR) and albumin.

Table 1.1.1: The Montreal and Paris classification of IBD (adapted from Levine et al (4)

Phenotype classification	Montreal	Phenotype classification	Paris
Crohn's Disease			
Age at Diagnosis			
A1	Below 17 years	A1a	0-<10years
A2	17-40 years	A1b	10-<17years
A3	Above 40 years	A2	17-40 years
		A3	>40 years
Location			
L1	Terminal Ileal ± limited caecal disease	L1	Distal 1/3 Ileal ± limited caecal disease
L2	Colonic	L2	Colonic
L3	Ileocolonic	L3	Ileocolonic
L4	Isolated upper disease	L4a	Upper disease proximal to ligament of Treitz
		L4a	Upper disease distal to ligament of Treitz and proximal to distal 1/3 Ileal
Behavior			
B1	Non stricturing, non penetrating	B1	Non stricturing, non penetrating
B2	Stricturing	B2	Stricturing
B3	Penetrating	B3	Penetrating
		B2B3	Both penetrating and stricturing disease, either at the same or different times
p*	Perianal disease modifier	p*	Perianal disease modifier
Growth			
n/a		G0	No evidence of growth delay
		G1	Growth delay
Ulcerative Colitis			
Extent			
E1	Ulcerative proctitis	E1	Ulcerative
E2	Left-side UC (distal to the splenic flexure)	E2	Left-side UC (distal to the splenic flexure)
E3	Extensive Colitis (proximal to the splenic flexure)	E3	Extensive Colitis (hepatic flexure distally)
		E4	Pancolitis (proximal to hepatic flexure
Severity			
S0	Clinical remission (Asymptomatic)	S0	Never Severe
S1	Mild UC (Passage of four or fewer stools/day (with or without blood	S1	Ever Severe
S2	Moderate UC		
S3	Severe UC		
Inflammatory bowel disease unspecified			
IBDU	Reserved only for those cases where colectomy has been performed and pathologists are unable to make a definitive diagnosis of either CD or UC after full examination		

It is a valid and reliable instrument that has been further validated in a multicenter prospective study by Hyams et al in 2005 (8) and is commonly used in paediatric CD studies. All of the components were obtained at the clinic visit. Disease activity was categorized as: inactive (<10 points), mild (11 to 29 points), moderate (30 to 44 points), and severe (≥45 points) (7;8) . An increase in score of 15 points was considered clinically significant (7;8). The Paediatric Ulcerative Colitis Activity Index (PUCAI) is a non-invasive disease activity index used to measure disease activity in children with ulcerative colitis (9). PUCAI was established by item selection by a Delphi group of 36 experts in paediatric inflammatory bowel disease. Validation was assessed on a separate prospective cohort of 48 children with ulcerative colitis undergoing complete colonoscopy. Responsiveness was evaluated at a follow-up visit of 75 children using effect size statistics and diagnostic utility approaches. The rigorously developed PUCAI is a non-invasive, valid, highly reliable, and responsive index with which to assess disease activity in paediatric ulcerative colitis. Each of the components was assessed at the clinic visit. Disease activity was categorized as: inactive (<10 points), mild (10 to 34 points), moderate (35 to 64 points), and severe (≥65 points)(9). An increase in score of 20 points was considered clinically significant (9).

A clinical suspicion of IBD is raised in children with persistent (≥4 weeks) or recurrent (≥2 episodes in 6 months) symptoms such as abdominal pain, diarrhoea, rectal bleeding and weight loss. For these children height and weight must be recorded at diagnosis and at all subsequent visits. Historical data of height and weight are essential to assess deceleration of growth velocity. Parental height and weight are required to identify the target height of the patient. Pubertal development should be staged according to Tanner (10). The mouth should be examined for lip swelling, gingival hyperplasia or aphthous ulcerations. Skin abnormalities such as vitiligo or extraintestinal manifestations (erythema nodosum, pyoderma gangrenosum) must be recorded. The abdomen should be examined for palpable tenderness and abdominal mass (suggestive of ileo-cecal infiltration or abscess). The anal region should be inspected for skin tags, fissures, fistulae or abscesses. In case of arthralgia, the joints should be inspected for arthritis. Screening blood tests should include full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum levels of urea and creatinine, serum albumin. Reduced level of haemoglobin, raised markers of inflammation (ESR,CRP), elevated platelet count and reduced serum albumin are suggestive of IBD (11). Measuring faecal calprotectin can also be highly useful in the diagnosis of patients with IBD as it has been approved to be a surrogate marker of intestinal inflammation. Every child suspected of IBD should undergo a complete diagnostic program consisting of colonoscopy with ileal intubation, upper gastrointestinal endoscopy and radiologic contrast imaging of the

small bowel. Multiple biopsies from all segments of the gastrointestinal tract are needed for a complete histologic evaluation.

1.1.3 Epidemiology of IBD

The incidence of IBD varies around the world, although there is a rising rate of paediatric IBD in both developing and developed countries (12). CD and UC are most commonly diagnosed in early adulthood but the diagnosis may occur at any age (13) with approximately 15%-25% of IBD being diagnosed by 20 years of age (14). Pediatric studies from the United States, Scandinavia, and the United Kingdom have shown a definite increase in overall mean annual incidence rates and overall disease prevalence over the past 4 decades in both paediatric CD and UC. Worldwide, the overall incidence of paediatric IBD currently ranges from 4.0 to 7.0 cases per 100,000. Kugathasan et al (15) reported 199 new paediatric IBD cases over 24 months starting from January 2000. The overall incidence of IBD was 7.05 per 100,000, the incidence of CD as 4.56 per 100,000, and the incidence of UC was 2.14 per 100,000. A Swedish paediatric study looked at incidence and prevalence rates between 1984 and 1998 and covered more than 56% of the country's paediatric population, finding a more than 3-fold increase in the mean incidence of paediatric UC (16) CD cases, however, remained static. Overall, incidence increased from 4.6 to 5.8 per 100,000. European studies have shown slightly lower rates of incidence but do reflect trends of steadily increasing numbers of cases reported. Studies in the United Kingdom, including Scotland and Wales, have shown an increase in overall incidence from 1-2 to approximately 4 cases per 100,000 between the 1960s and the 1990s (17;18). Scotland presents the highest prevalence for CD and is second after Ireland for UC. The highest incidence occurs between 12-16 years (19;20) with almost 2/3 of the patients diagnosed with CD.

There is a historical body of evidence with regard to paediatric IBD incidence in Scotland. The first report by Barton et al (21) showed the incidence of CD to be 0.7/100,000/yr and UC 1.9/100,000/yr in 1968, demonstrating the exceptional rise in CD. Armitage et al (17) later reported that between 1981-1995 there was an overall incidence of 2.6/100,000/ yr for CD and a marginal fall in UC to 1.2/100,000/yr. Another prospective study carried out by the British paediatric surveillance unit which recorded all instances of new IBD diagnoses in those less than 16 years over a 13-month period during 1998-1999 showed that the overall incidence of paediatric IBD UK wide was 5.2/100,000/yr, with Scotland producing the highest rate of 6.5/100,000/yr (20).The recent data by Henderson et al (22) have compared the current incidence and other demographic attributes of paediatric IBD in Scottish population to previous data.

According to Henderson's study the incidence of CD was 4.75/100,000/year, UC 2.06/100,1000/year and IBDU 1.01/100,1000/year. This study has shown significant rise in the incidence of IBD as compared to 1990-1995. This study also reported a significant reduction in median age at IBD diagnosis from 12.7 years to 11.9 years between the periods, with a continued male preponderance. Other geographic regions, including Australia and Asia, also report an increase in paediatric CD over the past 2 decades, especially in urban regions (23;24).

1.1.4 Pathogenesis of IBD

Although the exact cause of IBD is still unknown, clinical and laboratory studies indicate that these diseases are multi-factorial and involves both the genetic and environmental factors (25). Several theories about the pathogenesis of IBD have been revealed, including an autoimmune response to a luminal or mucosal antigen, a dysfunction of immune response to a commensal bacterium and an infection with a pathogenic organism which remains in the intestinal tissue and results in a chronic inflammatory response (1;26).

1.1.4.1 Genetic factors

There is strong evidence to suggest a genetic basis for IBD, including familial clustering and racial and ethnic differences in risk for IBD. Ten to twenty percent of affected individuals have family history of IBD; with the highest risk among first-degree relatives of an affected IBD patient (15;27-29). Increased rates of IBD among identical twins compared to fraternal twins, and among siblings compared to spouses of affected individuals, suggest that genetic rather than environmental factors are primarily responsible for the observed familial aggregation or concentration of incidence for the disease (30-33). A large number of candidate genes have been held responsible for the pathogenesis of IBD (30). In 1996, French researchers for the very first time identified the region on chromosome 16 known as (IBD1) (34). Detailed mapping of this chromosome resulted in the identification of the NOD2/CARD15 gene that encodes a cytoplasmic protein designated NOD2 or CARD15 which serves as a pattern recognition receptor for bacterial lipopolysaccharide, and regulates activation of nuclear factor-kβ and secretion of a-defensins by ileal paneth cells (35). People who are homozygous for variant NOD2 may have 20-fold or more increased susceptibility to CD (36;37). However, fewer than 20% of patients with CD are homozygous for NOD2 variants so it does not fully explain the disease pathogenesis. Since these first observations a number of other candidate genes have been identified with the strongest evidence for the HLA region, MDR-1, as well as the recently implicated IL23R and ATG16L1 genes (38). Few published studies

have investigated association between genetic variants and growth impairment in patients with CD. Russell et al (39) reported that patients with an OCTN1/2 now known as SLC224/A5 haplotype had a lower height at diagnosis. Sawczenko et al (40) reported that patients with IL6-174 GG genotype had lower height z-scores as compared to those with GC or CC genotypes. Levine et al (41) have reported that patients carrying TNF-α promoter polymorphism with loss of function had higher mean height z-score. However, the association of these genetic variants has not yet been confirmed. Lee et al (42) investigated genetic variants associated with linear growth in paediatric-onset CD and reported significant association between growth impairment in CD and a stature related polymorphism in dymeclin gene DYM. This study indicated that the genetic influences due to stature-associated and possibly CD risk alleles may predispose CD patients to alterations in linear growth.

1.1.4.2 Environmental factors

Environmental factors are essential components of the pathogenesis of IBD and primarily responsible for its growing incidence around the world. Epidemiological, clinical and experimental evidence support an association between IBD and a large number of seemingly unrelated environmental factors, which include smoking, diet, drugs, geographical and social status, stress, microbial agents, intestinal permeability and appendectomy (13).

1.1.4.3 Microbial factors (Intestinal commensal flora)

Over the past decade, there has been an exponential increase in interest about commensal bacteria as aetiological agents of IBD. Based on fairly solid data , a substantial body of evidence has accumulated suggesting that the normal enteric flora plays a key role in the development of IBD (43). Additionally, the following clinical observations support this hypothesis: 1) that the beneficial effect of antibiotics in the treatment of CD, and to a lesser extent UC, has been appreciated for years, 2) diversion of the faecal stream from the inflamed bowel loops has been known to induce symptomatic improvement in CD patients, while relapse often occurs upon restoration of intestinal continuity and 3) pouchitis, a chronic inflammation of a surgically constructed ileo-anal pouch, develops in a considerable proportion of UC patients after procto-colectomy and is associated with the dysbiosis caused by the contact of the once near sterile small bowel mucosa with a rich colon-like flora repopulating the pouch after surgery (44). Further more recent data demonstrating that probiotics (primarily lactic acid bacteria), defined as live microbial feeds, beneficially affect the host by modulating gut microbial balance, and improve both human IBD and experimental

colitis, adds an important dimension to the role of gut flora in IBD. In addition, it has also been observed that much larger numbers and concentrations of bacteria make up the bio film covering the intestinal epithelium of IBD patients compared to the epithelium of healthy subjects (45). And loss of immune tolerance against the autologous enteric aerobic and anaerobic flora has been reported (46). Finally, and probably most convincingly, the majority of animal models of IBD fail to develop intestinal inflammation when kept in a germ-free environment (47).

1.1.5 Clinical presentation of IBD

Inflammatory bowel disease manifests during childhood or adolescence in up to 25% of cases (48). The presentation of IBD in children and adults depends on the disease location and the extent of inflammation (49). The most commonly encountered gastrointestinal symptoms are abdominal pain and diarrhoea. Abdominal pain can be located anywhere in the abdomen, although in patients with CD it occurs frequently in the right mid quadrant, whereas in patients with UC it is located in the lower abdomen. Diarrhoea may be associated with blood in the stool, more so in UC. Other gastrointestinal symptoms include loss of appetite, weight loss, nausea, vomiting, and perianal disease (50). Approximately 25 to 35% of individuals with IBD develop extraintestinal manifestations (51). The most common extraintestinal manifestations include erythema nodosum, stomatitis, aphtae, hepatitis, uveitis, conjunctivitis, pulmonary vasculitis, fibrosing alveolitis, arthralgia, arthritis, ankylosing spondylitis, iron deficiency anaemia, thrombocytosis, autoimmune hemolytic anaemia, vitamin B12 deficiency, osteoporosis (50;51). Twenty-five to thirty-five percent of adult patients with IBD have at least one extraintestinal manifestation (52). Up to 35% of paediatric IBD patients in some series have at least one extraintestinal manifestation as a presenting sign (51;53). Patients with colonic involvement are more likely to have extraintestinal manifestations than patients with isolated small intestinal lesions (51;52).

Growth failure, delayed sexual maturation, perianal lesions including fistulae, tags and frequent abscesses, abdominal pain, diarrhoea, weight loss and osteoporosis occasionally may be the initial presentation of IBD in children especially with CD (Table 1.1.2). The impact of IBD on growth and pubertal development of the patients is presented in detail in Section 1.6 & 1.7. Beyond the health related consequences of IBD, acute active disease may require recurrent hospital admission, causing major interruptions in social, family life, professional career, and academic attendance in children that consequently lead to low quality of life (54) and psychological concerns (55). Psychotherapy is now considered an essential part of the IBD management.

Table 1.1.2: Clinical presentation of IBD in children and adolescence (adapted from Kim & Ferry 2004 (56)

	Signs and symptoms
Systemic	Growth Failure (decreased growth velocity; short stature delayed bone age), weight loss Anorexia Malaise Fever of unknown aetiology
Gastrointestinal	Abdominal pain Diarrhoea (with or without blood and mucus) Gastrointestinal bleeding Nausea/vomiting Early satiety/bloating Oral ulcerations Jaundice
Endocrine	Delayed sexual maturation (late-onset of puberty) Osteopenia/osteoporosis

1.1.6 Management of IBD

Currently, the main therapeutic objectives in IBD are to induce symptomatic remission and to decrease mucosal inflammation. Though IBD has long been considered a disease characterized by exacerbations and remissions, evidence now reveals that endoscopic and histologic lesions may persist despite resolution of symptoms and biochemical abnormalities (57;58). Therefore, control of intestinal inflammation is included as an important therapeutic goal. Goals for the therapy in paediatric IBD are the induction and maintenance of remission, prevention of cancer of the affected bowel in adult life, facilitation of normal growth and development and improvement of the quality of life (59). The choice of treatment depends on disease subtype, localization and associating presenting features such as weight loss and short stature (60). In moderate to severe IBD, the current therapeutic strategy may involve 5-aminosalicylate (5-ASA) preparations, Glucocorticoids, immunomodulators (e.g. 6-mercaptopurine, Azathioprine, Methotrexate and Cyclosporine), nutritional interventions, biologic therapy (Infliximab, Adalimumab) and surgery (61). The common treatment options and their impact on growth and skeletal development along with their indications of use in paediatric IBD are presented in (Table 1.1.3).

1.1.6.1 5-Aminosalicylates (5-ASA)

5-ASA preparations sulfasalazine and mesalazine are a widely used to induce and maintain remission in mild to moderate UC. Mainly due to transmural inflammation efficiency of 5-ASA in CD remained controversial, especially for preventing relapse (62). Sulphasalazine consists of a sulphapyridine linked to a 5-aminosalicylate, which is the active moiety, released as a

result of bacterial cleavage in the colon (63) . The precise mechanism of action of these drugs is as yet unclear; however, one of the most important mechanistic effects is most likely the inhibition of cyclo-oxygenase and 5-lipoxygenase which blocks the production and pro-inflammatory activity of prostaglandin E2 and leukotrienes (64). High level evidence supports the use of 5-ASAs for induction of remission in UC (65) and for a moderate benefit in maintenance of remission in UC (65). Modest benefits have been demonstrated with 5-ASAs in inducing remission in patients with mild to moderate colonic CD, but, the evidence does not support their use in maintaining remission in CD (66). 5-ASAs also have a well documented adverse effect profile, including rash, headache and pruritus and, more rarely, toxic hepatitis, leucopoenia, pancreatitis and neurotoxicity (66). Because of the limitations of both steroids and 5-ASA in maintaining remission in many IBD patients often require additional therapy with immunomodulating agents is required. There are very few trials on 5-ASA use in children with IBD and no data could be found regarding their growth.

1.1.6.2 Nutritional management

Medical therapy usually alleviates the symptoms associated with intestinal inflammation (abdominal pain, diarrhoea, and bleeding), but chronic issues of malnutrition and mineral deficiencies often persist. Enteral therapy, defined as a specific liquid formula diet administered either orally or by nasogastric tube as the primary or sole means of nutrition, has been used both for nutritional support and as a method of treatment in place of corticosteroids. Elemental enteral therapy has included elemental formulas (consisting of free amino acids) or either oligomeric (protein hydrolysates) or polymeric (whole proteins) formulas. Enteral nutrition is usually administered exclusively for and initial 6-8 weeks course, followed by progressive reintroduction of normal feeding over several weeks. Studies have demonstrated additional beneficial impact of EEN which include mucosal healing, improvement in anthropometric measurements and bone turnover (67-72). The exact mechanism for catch-up growth with EEN is still unclear. However, it has been shown that besides restoring multiple nutritional deficits and providing required caloric intakes, EEN induces a significant mucosal healing and reduces the production of mucosal inflammatory cytokines (73;74). It has also been reported that EEN causes bowel rest and modification of intestinal flora (75).

Studies have examined serum markers of nutrition such as IGF-1, as well as anthropometric markers as outcome measures indicating the nutritional impact of this form of therapy. EEN can lead to prompt improvement in weight (76) along with rapid increases in IGF-1(67;68). Combination of EEN and Infliximab might be an attractive approach to ensure optimal growth in children. Indeed prolonged EN, even administered intermittently, has clear positive effects

on anthropometric parameters, with significant increase in weight and height velocity (69;70;77). An early study in children showed that elemental formula was as effective as steroids in controlling disease and was associated with better linear growth compared to steroid treated patients (78).

Furthermore, children treated with EEN have better growth velocity than those treated with GC (79). In fact, after EEN initiation, a rapid increase in growth factors IGF-1 and IGFBP-3 level is observed (67;68;70), which is probably due to the reduction in inflammation rather than an improvement in nutritional status. Whitten, at al (72) demonstrated changes in markers of bone turnover in children managed with EEN. In this prospective cohort, EEN led to a reduction of a marker of bone breakdown and increased levels of a marker of bone formation. Together these changes suggest that EEN may have beneficial effects on bone metabolism as well, which is likely consequent to altered levels of pro-inflammatory cytokines. Interesting, these changes do not always correspond directly with changes in serum or mucosal inflammatory markers, suggesting that EEN may act via multiple coincident mechanisms.

1.1.6.3 Corticosteroids

Corticosteroids have a strong anti-inflammatory effect and remain widely used to induce remission in moderate and severe acute flares in IBD patients (80). A Cochrane systematic review has demonstrated that they are effective in achieving clinical remission in CD in comparison to placebo (81). Corticosteroids are also effective in inducing remission in patients with active UC (82). Corticosteroids show their effects by binding to a specific intracellular receptors to effect the transcription of steroid responsive genes (83). It is also recognised that activated steroids receptors interact with NF-κβ in regulating the inflammatory response (83). Pro-inflammatory proteins suppressed by corticosteroids include IL-1, IL-2, IL-8, IL-6, γ-interferon, and TNF-α. These in turn result in impaired inflammatory signalling in terms of antigen presentation, and inflammatory cells activation (84).

However the adverse effects of oral corticosteroids are high with cushingoid appearance, hypertension and increased infections being commonly encountered in the short-term and osteoporosis, adrenal suppression and growth retardation as long-term concerns (84). The mechanism by which Corticosteroids suppress growth and bone metabolism are multiple and complex. It has been shown that Corticosteroids show their impact on growth and bone metabolism by (i) by decreasing calcium absorption from gut and increase calcium urinary excretion, with subsequent secondary hyperparathyroidism, further promoting bone resorption (ii) by decreasing the expression of hepatic GH receptors leading to decreased

IGF-1 production (iii) impair GH receptor expression, reduce IGF-1 production and activity and inhibit proliferation of chondrocytes at the level of growth plate (85;86;86-88).

1.1.6.4 Immunomodulators

The 6-mercaptopurine (6-MP) and its precursor, Azathioprine, have been used in clinical practice for the treatment of IBD for over four decades. The majority of clinical experience, in the UK, is with Azathioprine, which was developed in 1957 as a slower release formulation of 6-mercaptopurine (89). Azathioprine is rapidly metabolised to 6-MP in the body which exerts its effect by inhibiting purine synthesis in DNA and RNA and thus reducing cell proliferation and thereby causing immunosuppression (89). Azathioprine has been shown to be effective in both inducing remission and for maintenance therapy for CD, with significant steroid-sparing effects (90). Azathioprine may reduce the requirements for steroids in inducing remission in UC (91) and appears to reduce maintenance relapse rates long term (92). Azathioprine/6-MP is being used in paediatric IBD since 1970s (93). Immunosuppression induced by Azathioprine/6-MP allows maintenance of clinical and biological remission and leads to the disappearance of mucosal ulceration in 50% of patients with CD (94). Therefore these drugs should enable normal growth and puberty in paediatric IBD patients. Due to the steroid sparing effect of Azathioprine (95) it could improve growth. Only two studies have reported the impact of Azathioprine of growth (96;97).

Methotrexate also causes immunosuppression by inhibiting purine synthesis in DNA and reducing T-cell proliferation. Methotrexate at higher dose (25mg weekly) appears to induce remission in CD (98). Methotrexate has also been shown to be superior to placebo for the maintenance of remission in CD and is now commonly used as an adjunct to therapy in patients who relapse on or are intolerant to thiopurines (99). Although the experience of Methotrexate on paediatric setting is limited however; Methotrexate appears to be well-tolerated and good therapeutic alternative for children unresponsive or intolerant to Azathioprine/6-MP (100;101). The impact of low dose Methotrexate on growth have been observed by Turner D et al (100) in 60 children with CD unresponsive or intolerant to Azathioprine/6-MP and have been reported to improve height velocity z-scores.

Adverse events with immunosuppressants are significant with nausea and vomiting both common. Over-immunosuppression leading to leucopoenia is a potential risk with both Azathioprine and Methotrexate, which requires frequent white cell level monitoring (91). In addition Methotrexate is also associated with the development of hepatitis and hypersensitivity pneumonitis (99).

Fatal sepsis and malignancy risk are low (102). A significant proportion of patients are intolerant of, or do not achieve long-term remission of symptoms, with immunosuppressive agents.

1.1.6.5 Biological therapies

Biologic therapies have been developed to target more specific aspects of the inflammatory cascade because of the limited success of immunosuppressive agents to treat moderate and severe IBD, and because of their adverse effect profile. Biological therapy refers to the use of medication that is tailored to specifically target an immune or genetic mediator of disease (103). The biological agents that have been evaluated for the treatment of CD include: the anti-tumor necrosis factor alpha (TNF-α) Infliximab, Adalimumab, certolizumab pegol and anti-adhesion molecule (Natalizumab) drugs. Biologic therapies are discussed in detail in Section 1.8.

Table 1.1.3: Therapeutic interventions in paediatric IBD and their impact on growth and skeletal development (compiled from (104-106)

Drug	Examples	Mechanism of action	Indication of use in IBD	Effect on growth	Effect on bone
Aminosalicylates (5ASA)	Mesalazine Sulfasalazine Balsalazide Olsalazine	Inhibition of the cyclo-oxygenase that impedes the production of pro-inflammatory prostaglandins and leukotrienes	Induction and maintenance of remission for mild/moderate UC & CD	No	No
Corticosteroids	Prednisolone Prednisone Hydrocortisone Budesonide	Inhibit many inflammation-associated molecules such as cytokines, chemokines, arachidonic acid metabolites, and adhesion molecules	Maintenance of remission for mild/moderate UC & CD	Inhibitory	Yes (suppression of osteblastogenesis, inhibition of chondrocyte proliferation and collagen synthesis)
Immunomodulators	Azathioprine 6-mercaptopurine	Inhibiting purine synthesis in DNA and RNA and thus reducing cell proliferation and thereby causing immunosuppression	Maintenance of remission for moderate/severe UC & CD, steroid refractory/dependency, fistulae, concomitant therapy with Infliximab	Stimulatory	No known
Antibiotics	Metronidazole Ciprofloxacin	Changes in bacterial flora	Induction of remission	No	No
Immunosuppressants	Methotrexate	Methotrexate competitively inhibits dihydrofolate reductase an enzyme that participates in the tetrahydrofolate synthesis	Maintenance of remission for moderate/severe UC & CD, steroid refractory/dependency, fistulae, concomitant therapy with Infliximab	Stimulatory	Not known
Biologic agents	Infliximab Adalimumab	Inhibit the activity of the pro-inflammatory cytokine TNF-α	Maintenance of remission for moderate/severe UC & CD Unresponsive to conventional therapy and for severe fistulizing disease	Stimulatory	Indirectly by removing suppression of chondrocytes proliferation and differentiation induced by proinflammatory cytokines
Exclusive enteral nutrition	Polymeric feeds Elemental feeds	Reduction in inflammatory markers Direct mucosal healing, bowel rest with a decreased intestinal metabolic activity, secretions and altered motility	Induction of remission Mild/Moderate CD	Stimulatory	Enhancing bone metabolism

1.2 Bone biology

Bone is a specialized, dynamic, highly vascular and mineralized connective tissue that makes up together with cartilage the skeletal system. Bone performs several functions in the body: (1) mechanical, as support and site of muscle attachment for locomotion; (2) protective, for vital organs and bone marrow; and (3) metabolic, for the maintenance of serum homeostasis, which is essential to the life. Furthermore, it is capable of maintaining an optimal shape and structure throughout life.

Two types of bones can be distinguished in the skeleton: flat bone (skull ones, scapula, mandible and ileum) and long bones (tibia, femur, humerus, etc). External-examination of long bone shows two wider extremities (Figure 1.2.1) the epiphysis, diaphysis and a metaphysis. The external part of the bone is formed by a thick and dense layer of calcified tissue, the cortex (compact bone), which in the diaphysis, encloses the medullary cavity where the hematopoietic bone marrow is housed. Toward the metaphysis and the epiphysis, the cortex becomes progressively thinner, and the internal space is filled with a network of thin, calcified trabeculae; this is the cancellous bone also named as spongy or trabecular bone. The spaces enclosed by these thin trabeculae are also filled with hematopoietic bone marrow and are in continuity with the medullary cavity of the diaphysis. The bone surface at the epiphysis take part in the joint, covered with a layer of articular cartilage that does not calcify. There are consequently two bone surface at which the bone is in contact with soft tissue, external surface (the periosteum surface) and an internal surface (the endosteal surface).

1.2.1 Bone tissue

Bone is classified in to two different types of tissues, one of which is relatively dense, known as cortical bone (compact bone), while the other consists of a network of struts or trabeculae surrounding interconnected spaces known as trabecular bone (cancellous bone). The microscopic difference between compact and cancellous bone is that the compact bone consists of haversian sites and osteons, while cancellous bones do not. Also, bone surrounds blood in compact bone, while blood surrounds bone in the cancellous bone.

1.2.1.1 Cortical bone

The hard outer layer of the bone is composed is composed of a thick and a dense layer of calcified tissue (compact bone tissue), so-called due to its minimal gaps and spaces. Its porosity is 5-30%.

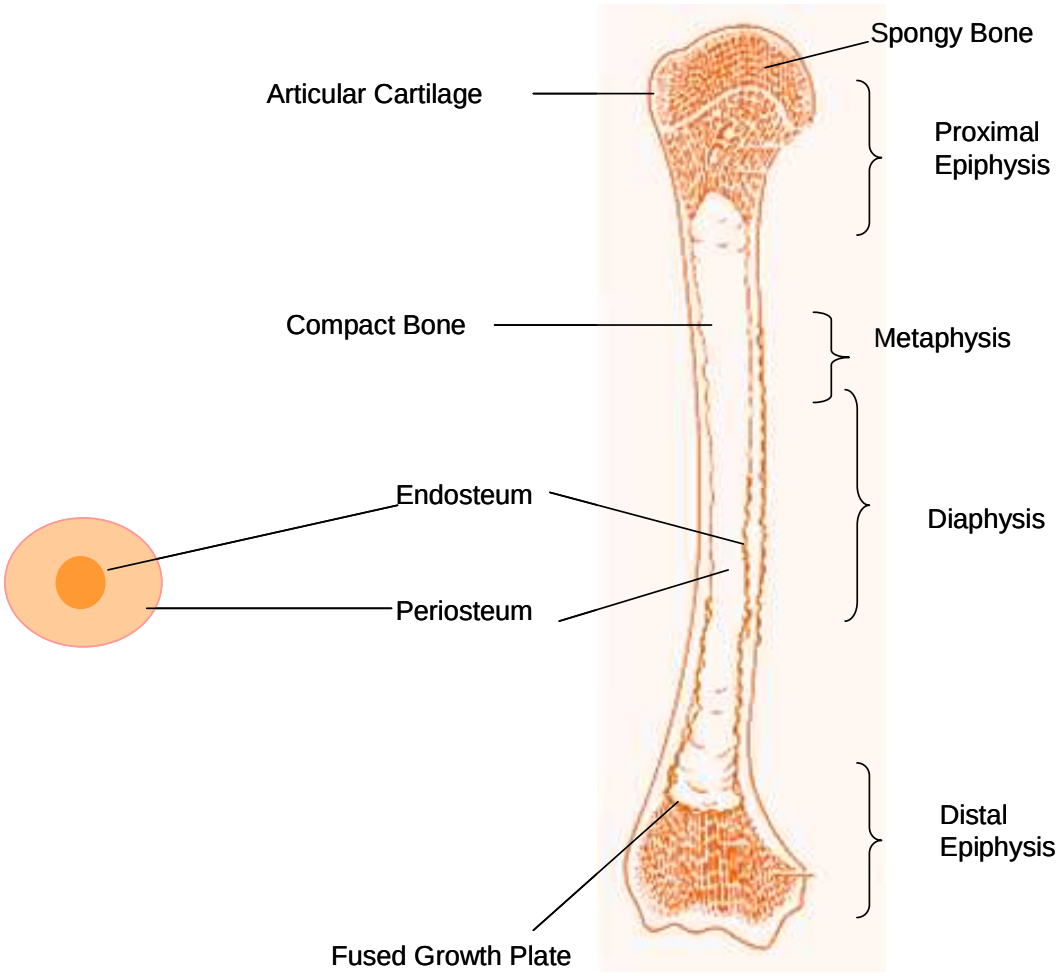


Figure 1.2.1: Structure and components of long bone. Long bones are longer than they are wide, consisting of a long shaft (the diaphysis) and two articular (joint) surfaces, called epiphysis. They are comprised mostly of compact bone, but are generally thick enough to contain considerable spongy bone and marrow in the hollow centre.

This tissue gives bones their smooth, white, and solid appearance, accounts for 80% of the total bone mass of an adult skeleton (107). It provides protection and support by helping the long bones resist the stress of weight placed upon them (95). Blood vessels, lymphatic vessels, and nerves from the periosteum penetrate the cortical bone through perforating (Volkmann's) canals. The blood vessels and nerves of these canals connect with those of the medullary cavity, periosteum, and central (Haversian) canals of the osteons. The central canals run longitudinally through the bone, and around the canals are concentric lamellae-rings of hard, calcified matrix. Between the lamellae are small spaces, or lacunae, which contain osteocytes. Radiating in all directions from the lacunae are minute canals known as canaliculi, which are filled with extracellular fluid. Inside these canaliculi are slender finger-like processes of osteocytes. The canaliculi connect lacunae with one another and, eventually, with the central canals. Thus, there is an intricate branching network of canals which provide a route for nutrients and oxygen to reach the osteocytes and for wastes to diffuse away. Osteocytes from neighbouring lacunae form gap junctions with one another, facilitating easy movement of materials from cell to cell. Each central canal, with its surrounding lamellae, lacunae, osteocytes and canaliculi, forms an osteon (Haversian system).

1.2.1.2 Trabecular bone

In contrast to cortical bone, trabecular (cancellous) bone does not contain true osteons, but instead consists of lamellae arranged in an irregular latticework of thin columns of bone called trabeculae. The macroscopic spaces between the trabeculae of some bones are filled with red bone marrow, which produces blood cells. Within the trabeculae are osteocytes that lie in lacunae, and radiating from the lacunae are canaliculi. Blood vessels from the periosteum penetrate through to the trabecular bone, and osteocytes in the trabeculae receive nourishment directly from the blood circulating through the marrow cavities. Osteons are not necessary in spongy bone as osteocytes are not deeply buried as they are in cortical bone, and so have access to nutrients directly from the blood. Trabecular bone constitutes the majority of bone tissue of short, flat and irregularly shaped bones and most of the epiphyses of long bones. Trabecular bone tissue in the ribs, sternum, vertebrae and in the ends of some long bones is the only site of red bone marrow storage, and hence haemopoiesis in adults.

1.2.2 Bone cells

1.2.2.1 Osteoblasts

Osteoblasts arise from multipotent precursor cells of mesenchymal origin (Figure 1.2.2). Osteoblasts are responsible for bone formation and secrete collagen and other bone matrix proteins. The organic bone matrix is called the osteoid. Osteoblasts also contribute to mineralization of the osteoid by secreting several proteins, such as alkaline phosphatase, osteocalcin, and osteopontin (bone sialoprotein) that are essential in the mineralization process. Osteoblast activity is regulated by numerous hormones and cytokines. Osteoblast lineage cells regulate osteoclasts by secreting osteoprotegrin (OPG), which is an important inhibitor of osteoclastogenesis. Osteoblast lineage cells have receptors for e.g. 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] and parathyroid hormone (PTH) (108).

1.2.2.2 Osteoclast

Osteoclasts originate from the hematopoietic lineage (Figure 1.2.2) and are responsible for bone resorption. Osteoclasts function on the surface of calcified bone. The key regulators of osteoclastogenesis are receptor activator of nuclear factor kappa B ligand (RANKL) and OPG. RANKL and OPG are produced by osteoblasts. Mature osteoclasts secrete acid and proteolytic enzymes causing bone resorption. Degradation products, including collagen fragments, phosphate, and calcium, are released into the circulation. In an optimal/normal situation, bone formation and resorption are coupled (109).

1.2.2.3 Osteocytes

Osteocytes originate from osteoblasts (Figure 1.2.2). Osteocytes comprise the largest proportion of cells in mineralized bone and give support to bone structure by forming numerous cytoplasmic connections with adjacent cells. Remodeling is thought to be mediated by osteocytes. Further, osteocytes respond to mechanical strain on bones and mediate signals for bone formation and resorption. Osteocytes may undergo apoptosis, which is important for skeletal development, but also contributes to bone loss in osteoporosis (110).

1.2.3 Bone modeling and remodeling

During growth the bones maintain their normal shape by bone modeling. The process of bone maintenance, in which old bone is removed and then replaced by new bone, is called remodeling (Figure 1.2.3). Modeling and remodeling mechanisms are influenced by a vast variety of systemic and local factors, and they respond rapidly to the body's metabolic homeostasis. Bone formation is mediated by osteoblasts and bone resorption by osteoclasts

(108). Bone is continuously turned over in the remodeling process, which consists of bone resorption and formation in a lifelong process with successive (111). In this process, osteoclasts resorb old bone tissue. This part is soon replaced by new bone made by osteoblasts.

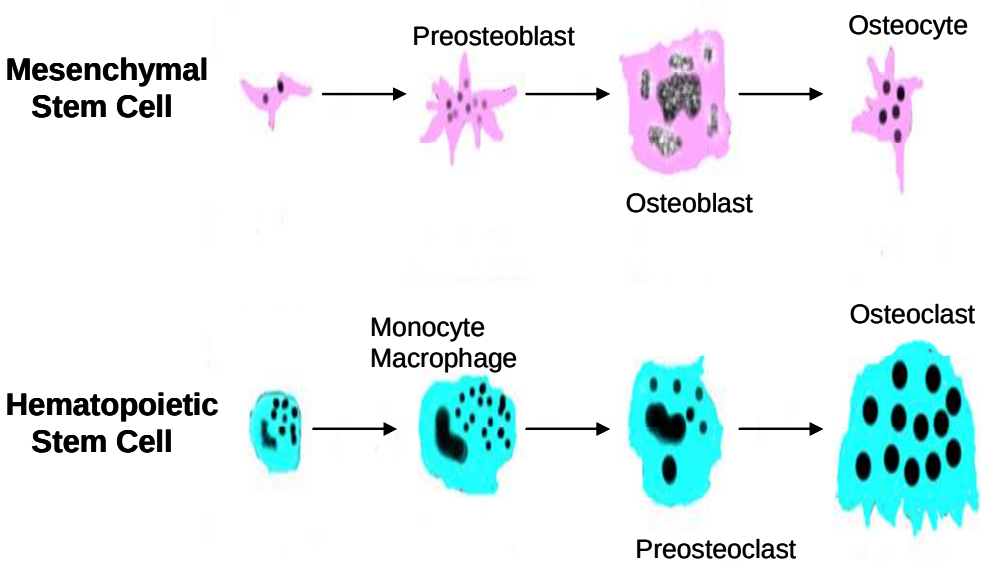


Figure 1.2.2: Origin of osteoblasts, osteocytes, and osteoclasts.

Remodeling maintains the normal shape of bones and renews bone tissue. Further, through remodeling, damaged bone tissue can be removed and replaced by new bone. Normally, the activities of osteoblasts and osteoclasts are balanced in adults, and remodeling has no effect on the amount of bone. Bone loss occurs, as in menopausal osteoporosis, when the amount of bone resorption is higher than the amount of bone formation. Trabecular bone is more active in remodeling than cortical bone (111). In the modeling process, osteoblasts form new bone without prior bone resorption, and consequently, an increase in the amount of bone tissue is possible (111). Further, osteoblasts form more bone than osteoclasts remove, and this leads to net increase in bone tissue. During childhood there is more modeling than remodeling. Modeling maintains the normal shape of bones during growth and is responsible for the increase in bone circumference during growth (111). Both bone modeling and remodeling are influenced by parathyroid hormone (PTH), sex, growth, and thyroid hormones; glucocorticoids; growth factors, cytokines, and prostaglandins; hereditary and nutritional factors; and physical activity (112;113). Longitudinal bone growth is called endochondral bone formation and occurs mainly in growth plate cartilage (114). Growth plates are present in the ends of long bones. In the growth plates, mesenchymal cells condense and turn into chondrocytes. Chondrocytes synthesize collagens and other matrix molecules. This spongiosa is subsequently remodeled into mature trabecular bone.

1.2.4 Bone development and growth

1.2.4.1 Ossification

There are two types of processes involved in the bone ossification (development); intramembranous ossification (flat bones) and endochondral ossification (long bones). The main difference between them is the presence of the cartilaginous phase in the latter.

1.2.4.1.1 Intramembranous ossification

In intramembranous ossification, a group of mesenchymal cells, under the influence of the local growth factors, forms a condensation within the highly vascularised area of the embryonic connective tissue by proliferating and differentiating directly into pre-osteoblasts and then on osteoblasts. Osteoblasts cluster together to form an ossification centre and secrete osteoid element. The subsequent mineralisation of the osteoid matrix begins working outward from the ossification centre. The early trabeculae forms and the periosteum develop resulting in the formation of woven bone.

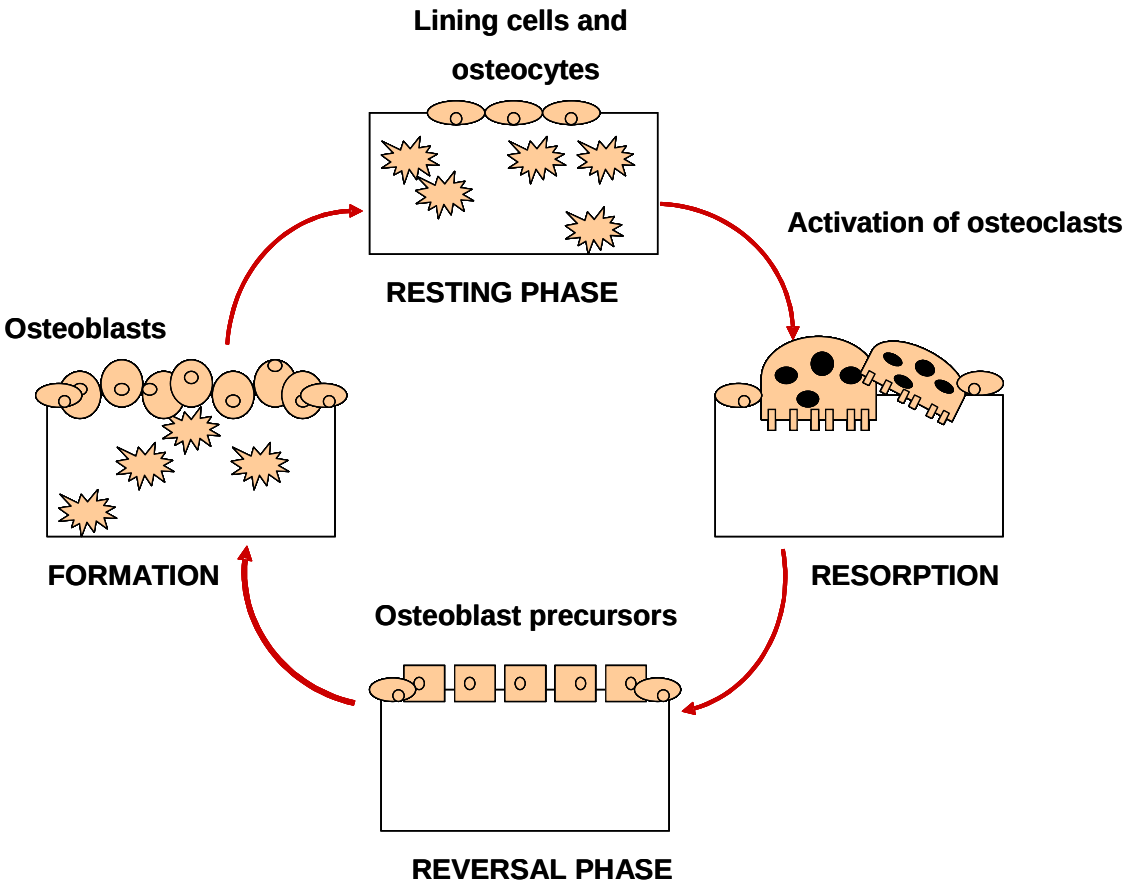


Figure 1.2.3:Bone Remodeling Cycle: Resortptive phase: activated multinucleated osteoclasts derived from bone marrow monocytes resorb a discrete area of mineralized bone matrix. Reversal phase: subsequently osteoprogenitor cells (osteoblast precursor) cells, which can locally proliferate and differentiate into osteoblasts migrates into the lacuna and disclose the former osteoclastic activity. Formative phase: the osteoblast deposits new bone matrix which is initially unmineralized and called osteoid, and in this way fill the resorption lacuna. Resting phase: once embedded in the osteoid, the osteoblasts mature into terminally differentiated osteocytes. The osteoblasts lying on the surface of the newly formed bone packet are quiescent lining cells until activated.

1.2.4.1.2 Endochondral ossification

In endochondral ossification the hyaline cartilage model grows and develops. In this process the mesenchymal cells proliferate and differentiate in to pre-chondroblasts and then into chondroblasts instead of osteoblasts.

Chondroblasts begin to secrete collagen and other proteins forming the cartilage matrix. Some of the chondroblasts that become caught in the matrix are subsequently known as chondrocytes. A membrane (perichondrium) surrounds the cartilage model. Formation of blood vessels takes place in and around the cartilage matrix to bring nutrients and allow the removal of waste. A bone collar and the periosteum form around the diaphysis of the cartilage precursor when the perichondrium develops osteogenic cells. Enlargement of the hypertrophic chondrocytes located at the edges of the primary ossification centres occur. The cells eventually degenerate, the matrix becomes compressed and subsequently mineralises. This area is known as the proliferative zone. A representation of the growth plate and adjacent metaphysis during growth is illustrated in (Figure 1.2.4). The original cartilage model is then gradually replaced by woven bone in a complex system of bone apposition, resorption and elongation. This process occurs in the zone of cartilage transformation and the resulting trabeculae form the primary ossification centre. Osteoclasts then begin to resorb the woven bone and cartilage remnants, osteoblasts begin to deposit bone matrix and the crystals produced inside the matrix vesicles become organised into the matrix, producing lamellar bone. This process occurs in the zone of ossification and the resulting mature trabeculae. More blood vessels form during growth because of the constant need to bring nutrients to the newly developing bone.

Appearance of the secondary ossification centres at the epiphyses of long bones allows formation of more blood vessels, to bring nutrients to these areas. The development of a medullary cavity occurs through osteoclastic resorption and the endosteum begins to form. Then bone marrow begins to develop in the newly created cavity. The epiphyses do not have separate medullary cavities. The smaller bones do not develop medullary cavities but all bones have some degree of marrow within their trabecular structure. The articular cartilage forms and remains to form the joint margins. When the epiphysis and diaphysis of a growing long bone become adjacent to one another, the diaphysis will gradually fuse with the epiphysis via the creation of mineralised bridges until the hyaline-cartilaginous growth plate has been completely replaced by bone and bone marrow (115) making a complete bone. The articular cartilage remains at joint surfaces to form the joints and prevent attrition. Long bones must also have a mechanism for increasing their diaphyseal circumference and their length.

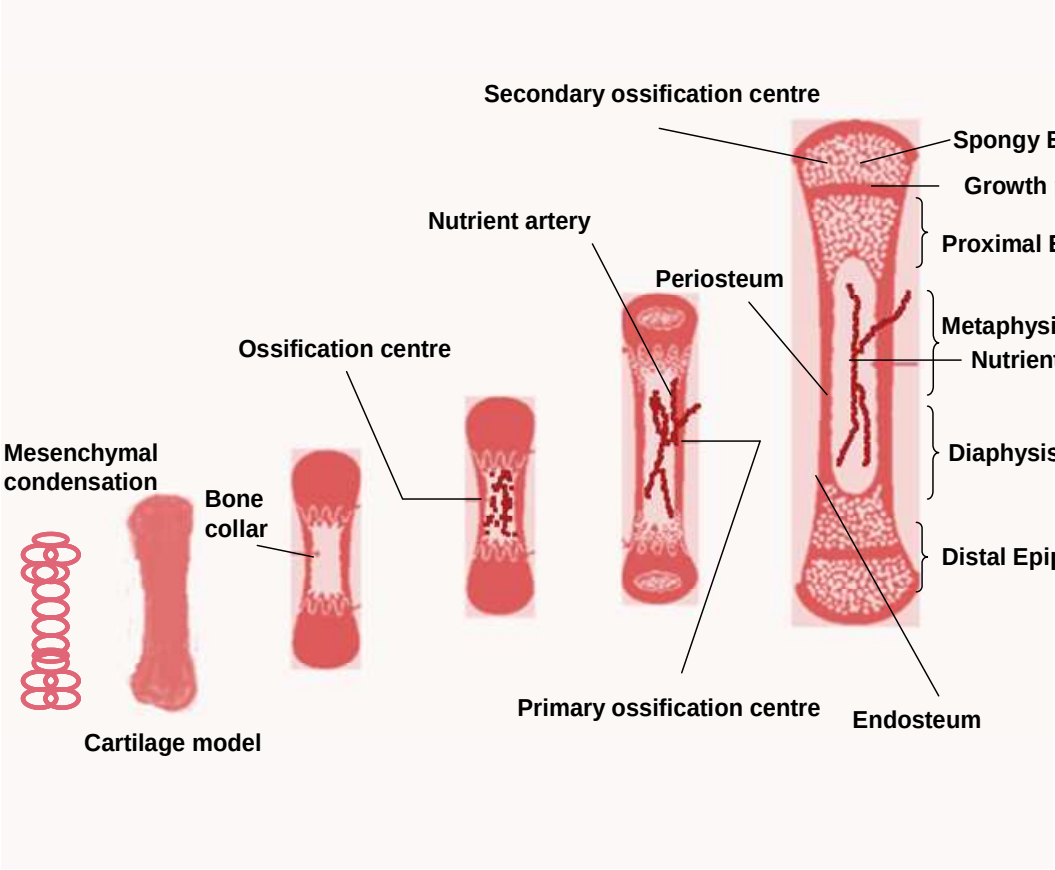


Figure 1.2.4: Bone Development. The schematic diagram shows the initial stages of endochondral ossification

They do this by sub periosteal addition of bone combined with endosteal resorption. This mechanism ensures that the cortical bone layer does not become too thick, which would therefore make the bone much heavier and mechanically disadvantaged. The size, shape and density of the cortical and trabecular bone will generally develop to a stage at which they are sufficiently strong and light to accommodate loading and activity to which the skeleton is normally exposed

1.2.5 Growth plate

1.2.5.1 Structural organization of growth plate

The process of bone growth relies upon chondrocytes produced at the epiphyseal growth plate, which are progressively synthesized and replaced by bone with accompanying longitudinal (endochondral) bone growth. Growth plate (epiphyseal plate) is a layer of hyaline cartilage in growing bone located in the metaphysis between the epiphysis and diaphysis. It is left over cartilage from the endochondral ossification. The epiphyseal plate consists of four zones (Figure 1.2.5).

1.2.5.1.1 The zone of resting cartilage

The zone of resting cartilage is near the epiphyses and consists of a small, scattered chondrocytes. These cells do not function in bone growth therefore; these are termed as “resting”. They attach the epiphyseal growth plate to the bone of the epiphysis.

1.2.5.1.2 The zone of proliferative cartilage

The zone of proliferating cartilage consists of slightly larger chondrocytes arranged like stack of coins. Chondrocytes divide to replace those that die at the diaphyseal surface of the epiphyseal plate.

1.2.5.1.3 The zone of hypertrophic cartilage

The zone of hypertrophic cartilage is also called as maturing cartilage. It consists of even larger chondrocytes that are also arranged in columns. The lengthwise expansion of the epiphyseal plate is the result of cell division in the zone of proliferating cartilage and maturation of the cells in the zone of hypertrophic cartilage.

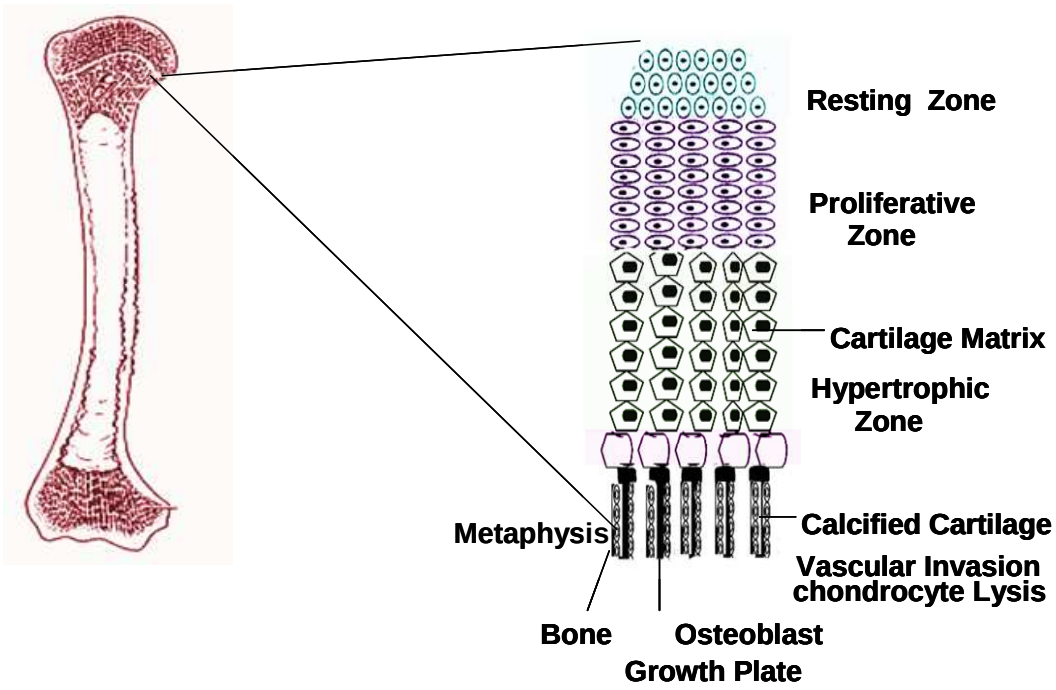


Figure 1.2.5: Structure of growth plate

1.2.5.1.4 The zone of calcified cartilage

The zone of calcified cartilage is only a few cells thick and consists mostly of dead cells because the matrix around them has calcified. The calcified matrix is taken up by the osteoclasts, and the area is invaded by osteoblasts and capillaries from the bones in the diaphysis.

These cells lay down bone on the calcified cartilage that persists. As a result, the diaphyseal border of the epiphyseal plate is firmly cemented to the bone of diaphysis. The activity of the epiphyseal plate is the only mechanism by which the diaphysis can increase in length.

The epiphyseal growth plate allows the diaphysis of the bone to increase in length until early adulthood. It also shapes the articular surfaces. As the child grows cartilage cells are produced on the growth plate. They are then destroyed and the cartilage is replaced by the bone on the diaphyseal side of the plate. In this way the thickness of the growth plate remains almost constant, but the bone on the diaphyseal side increases in length. Eventually, the epiphyseal cartilage cells stop dividing, and the bone replaces the cartilage. The newly formed bony structure is called epiphyseal line, a trace of the once active growth plate. With the appearance of the epiphyseal line, bone stops growing in length. In general, lengthwise growth in bones is in females is completed before that in males

1.2.6 Bone matrix and minerals

Bone extracellular matrix has two main components: the organic collagen fibers and the inorganic bone mineral crystals. Together they make up approximately 95% of the dry weight of bone, the remainder being composed of other organic molecules, collectively known as non-collagenous proteins. Collagen accounts for 70-90% of non-mineralized components of bone matrix; it consists of carefully arranged arrays of tropocollagen molecules, which are long rigid molecules, composed of three left handed helices of peptide, known as α-chains, which are bound together in a right-handed triple helix. The organic part of matrix is mainly composed of type-I collagen which is composed of tropocollagen molecules containing two identical and one dissimilar α-chains ($\alpha 1\ (I)_2\ \alpha\ 2$).

The inorganic composition of bone (bone mineral is formed from hydroxyl apatite, a hydrated calcium phosphate ceramic, with a similar crystallographic structure to normal bone mineral, which has a chemical formula of ca $(PO4)_6(OH)_2$; however bone-apatite is characterized by calcium, phosphate and hydroxyl bone-apatite is characterized by calcium, phosphate and hydroxyl deficiency, internal crystal disorder, and ionic substitutions, thus resulting in the presence of significant levels of additional trace elements within bone mineral: it is not a

direct analogue of hydroxyl apatite, but more closely a carbonate-substituted apatite. All these factors contribute to an apatite that is insoluble enough for stability, yet sufficiently reactive to allow the in vivo crystallites to be constantly resorbed and reformed as required by the body.

The most important non-collagenous organic constituents of bone matrix are four proteins: osteocalcin (OC), bone sialoprotein (BSP), osteopontin (OP) and osteonectin (ON). They are produced by bone cells and their relative composition within the bone matrix appears to be self-regulating through a feedback effect on their expression by osteoblasts. They all appear to be multi-functional, and are all involved in regulating bone mineralization and remodeling. Bone matrix also contains a great number of growth factors, including fibroblast growth factors (FGFs), insuline-like growth factors (IGFs), platelet-derived growth factors (PDGF), transforming growth factor-beta (TGFβ) superfamily, and bone morphogenic proteins (BMPs): they play several critical roles in regulating cell proliferation and differentiation, inducing the complete sequence of endochondral bone formation, when cartilage forms first and is subsequently replaced by bone.

1.2.7 Markers of bone metabolism

Biochemical markers of bone turnover are bone tissue proteins or their fragments, or enzymes released from bone cells during bone turnover. Proteins can be by-products of collagen formation or products of collagen degradation, or non-collagenous proteins such as osteocalcin and bone sialoprotein. Enzymes such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b can also be used as markers of bone turnover. Bone turnover markers can be detected in serum or urine. Ideally, they should reflect only the activity of osteoblasts or osteoclasts.

The bone turnover markers that are mainly released during bone formation or resorption are known as bone formation or resorption markers, respectively (Table 1.2.1). Formation and resorption are usually tightly coupled in time and space; thus, any such marker reflects the overall rate of bone turnover. Certain bone turnover markers may reflect different stages of formation and resorption, but they cannot reflect disease-specific processes and cannot distinguish between the activities at cortical or trabecular bone.

Assessment of bone turnover using bone turnover markers has the advantages of relatively low cost and non-invasive sample collection compared to the evaluation of bone turnover rate by histomorphometry in bone biopsies from the iliac crest. Although bone biopsy may give direct evidence concerning the aetiology, pathogenesis and progress of metabolic bone diseases, it has the disadvantage of being invasive and of giving information on bone turnover only concerning that specific skeletal region.

1.2.7.1 Bone formation markers

1.2.7.1.1 Bone specific alkaline phosphatase

Alkaline phosphatase (ALP) is an enzyme located on the cell surface. Three different tissue-specific genes encode the intestinal, placental and germ-line enzymes, and the tissue-unspecific gene is expressed in numerous tissues, including bone and the liver.

Table 1.2.1: Markers of bone turnover (adapted from (116)

Markers of bone formation	Tissue of origin	Markers of bone resorption	Tissue of origin
Alkaline phosphatase	Liver, bone, placenta, intestine, germ cells	Tartrate-resistant acid phosphatase 5b	bone (osteoblasts)
Bone-specific alkaline phosphatse	bone (osteoblasts), platelets	C-terminal cross-linking telopeptide of type I collagen	bone, soft tissue, skin
Osteocalcin (intact, total, carboxylated)	bone (osteoblasts)	N-terminal cross-linking telopeptide of type I collagen	bone, soft tissue, skin
Procollagen I C-terminal extension peptide	bone, soft tissue, skin	C-terminal cross-linking telopeptide of type I collagen, generated by metaproteinases	bone, skin
Procollagen I N-terminal extension peptide	bone, soft tissue, skin	Deoxypyridinoline	Bone, dentine
		Pyridinoline	Bone cartilage, tendon, blood vessels
		Osteocalcin	bone

Tissue-unspecific ALPs are produced by the same gene but there are tissue-specific differences in their post-translational modification of the carbohydrate chains (117). The most common sources of elevated serum ALP levels are liver and bone. In bone, ALP is present on the cell surface of the osteoblasts, and it is probably cleaved off from the membrane and released into circulation. In healthy individuals, about half of the serum alkaline phosphatase (S-ALP) is derived from bone. Thus, measurement of S-ALP can be used as a marker of bone turnover, but it lacks sensitivity and specificity, especially under conditions in which there is only a small increase in bone turnover. Measurement of the bone-specific isoform S-bone ALP has better sensitivity for detecting changes in bone turnover. However, the S-bone ALP assays that are currently available still have cross-reactivity with the liver isoenzyme of 15–20% (118).

1.2.7.1.2 Osteocalcin

Osteocalcin (or bone Gla protein) is the most abundant non-collagenous matrix protein in bone. It forms about 1% of the organic component of bone. It is a low-molecular-weight protein consisting of 49 amino acids and is expressed by osteoblasts, osteocytes, odontoblasts and hypertrophic chondrocytes. Osteocalcin has a high affinity for Ca^{2+} in bone hydroxyapatite, due to the three γ-carboxy glutamic acid residues at positions 17, 21 and 24 (119). Part of the newly synthesized osteocalcin is incorporated into new bone matrix and part of it enters the circulation, where it can be detected. Serum osteocalcin (S-OC) is considered to be a specific marker of osteoblast activity, and its serum levels thus reflect the rate of bone formation. Circulating osteocalcin consists of different immune-reactive forms. Approximately one third of serum osteocalcin is intact, one third consists of the mid-molecule fragment 1-43 and one third is smaller fragments (120). It is not clear whether these fragments are by-products during the biosynthesis of OC, from proteolysis of osteocalcin in the circulation or whether they are released directly from bone during bone resorption. Different osteocalcin assays can detect different fragments of osteocalcin in serum. The presence of multiple isoforms of OC in serum and the differences between assays in detection of these isoforms limit the clinical usefulness of S-OC (121). Osteocalcin may also be released from the bone matrix during bone resorption (122). Osteocalcin that enters the circulation is rapidly degraded (123). Breakdown fragments are cleared via the liver and the kidneys, and immune-reactive osteocalcin fragments can also be detected in the urine (124). Urinary osteocalcin (U-OC) appears to be more related to bone resorption than bone formation (125;126).

1.2.7.1.3 Procollagen type-1propetide

During the extracellular processing of newly-synthesized type I collagen, the amino-terminal and carboxy-terminal extension peptides are cleaved before fibril formation (127). These extension peptides guide the helical folding of the collagen molecule and the released N- and C-terminal pro-peptides of type I collagen (PINP and PICP, respectively) can be detected in the circulation. PICP and PINP are considered to be quantitative measures of the newly formed type I collagen. Type I collagen is also a component of several soft tissues; thus, there is a possible contribution from sources other than bone. However, the rate of collagen turnover in bone is faster than in other tissues, and therefore the changes in S-PINP and S-PICP are assumed to primarily reflect changes in collagen synthesis in bone (127).

1.2.7.2 Bone resorption markers

1.2.7.2.1 Tartrate-resistant acid phosphatase (TRAP5b)

Acid phosphatases are catalytic enzymes that act on phosphoesters in an acidic environment. Six isoenzymes of acid phosphatase have been identified in humans. Type 5 is expressed by osteoclasts and by alveolar and monocyte-derived macrophages, and is resistant to tartrate inhibition. Two isoforms of TRAP5 can be found in the human circulation. TRAP5a is sialylated and originates from macrophages and dendritic cells, whereas TRAP5b lacks sialic acid and is derived from osteoclasts.

The two isoforms also have different pH optima. The biological function of S-TRAP 5b in osteoclasts remains elusive. It is believed to destroy the endocytosed bone matrix degradation products during trans-cytosis through the osteoclast. TRAP-containing vesicles are added to the trans-cytotic vesicles transporting matrix degradation products, and TRAP is believed to assist matrix degradation in vesicles by producing reactive oxygen species (ROS) (128). TRAP has been reported to reflect the bone resorption rate, but more recent data have shown that it more accurately reflects the number of osteoclasts rather than their activity (129). Circulating TRAP5b levels are not affected by the renal function, and the effect of food intake is negligible (130). Furthermore, the level of TRAP5b is relatively stable in serum samples (131).

1.2.7.2.2 Collagen cross-links and cross-linked telopeptides

Collagen structure is stabilised by intra- and intermolecular cross-links. In bone, the predominant cross-links are pyridinoline (PYD) and deoxypyridinoline (DPD). Pyridinoline and deoxypyridinoline cross-links are released during bone resorption when type I collagen is degraded. PYD is more predominant in collagen while DPD is the minor component, but since DPD is most abundant in bone and dentin, it is considered to be a more bone-specific cross-link (132). Cross-links are cleared by the kidney, and they can be measured in serum or urine either as free cross-links or when bound to short collagen peptides.

Cross-linked telopeptides of type I collagen include the cross-linked N-terminal telopeptides (NTX) and cross-linked C-terminal telopeptides (CTX and ICTP). Fragments are generated by different collagenolytic pathways. NTX and CTX are released by cathepsin K cleavage and ICTP is a larger fragment produced by matrix metalloproteinases (133). CTX exists in an isomerised beta-CTX form and a non-isomerised alpha-CTX form. Isomerisation is associated with the aging of bone, and the assay for beta-CTX is therefore considered to measure the degradation of relatively old bone (134). Currently, beta-CTX-I is perhaps the most commonly used cross-link assay.

1.2.7.3 Use of bone turnover markers

Bone turnover markers assessed in serum or urine can be used in three main clinical areas, although individual patient management guidelines are still to come. The clinical areas are: (i) prediction of bone loss and the risk of developing osteoporosis, (ii) identification of individuals with a high risk of fracture, and (iii) monitoring of anabolic or anti-resorptive therapy.

1.2.8 Bone health assessment

Bone can be quantitatively assessed in vivo using imaging techniques. Clinically, the techniques available for measuring bone mineral in children are the same as those used in adults, but due to the reduced availability of normal values for children, selecting an imaging technique and interpreting the results (135) becomes more difficult. This is because growth is non-linear and children of the same age can have different levels of skeletal maturity. Also, children of different ages, heights, weights and ethnicities cannot necessarily be compared to one another easily. Techniques which are commonly used for assessment of bone health are peripheral quantitative computed tomography (pQCT), dual energy X-ray absorptiometry (DXA), Quantitative Ultrasound (QUS).

1.2.8.1 Dual energy X-ray absorptiometry (DXA)

Dual energy X-ray absorptiometry (DXA) has been made available since the late 1980s. It is widely used in adult medicine as the current gold standard. Its use in paediatric is rising rapidly as bone health in children is becoming an area of growing concern.

The fundamental principle of DXA is to measure the transmission of X-rays through the body at high and low energies. The use of two energies is to allow discrimination between soft tissue and bone. X-ray attenuation values are converted to pixel-by-pixel measurement of 'areal' bone mineral density (aBMD in g/cm^2). Software algorithms detect the bone edges and bone area (BA in cm^2) is calculated by summing the pixels within the bone edges. The reported value of the aBMD is the mean bone density overall the pixels within the bone area and the bone mineral content (BMC in g) are calculated by multiplying the mean aBMD by BA. The most commonly measured sites are spine, then hip and total body; peripheral measurements can also be made, for example the distal forearm. Radiation dose at all the sites is appreciably less than that which we are exposed to from the natural environment (background radiation). Since the ionizing radiation dose that is used is relatively low, at only a tenth of that of a chest radiograph, and since the scan time is less than five minutes, this technique is suitable for examining children. DXA may be applied to the whole body or skeletal regions of interest, for example the spine, proximal femur and radius. In pre-pubertal

children the lumbar spine is the most useful site to scan in clinical practice. In older children the spine and the hip are generally scanned.

The most significant limitation of DXA is the size dependence of the measurement. DXA provides a bone mineral density (BMD) based on two-dimensional projection of a three dimensional structure. By doing this, it does not account for the depth of the bone measured. The net result of this is that the BMD is of small bone is underestimated and the large bones BMD is overestimated. This may result in underestimation of BMD in paediatric subjects with growth retardation, such as some of our IBD patients. It is possible to compensate for these inaccuracies by correcting BMD values for bone age. Several methods have been proposed to adjust for the size dependence of the measurement (136-142).

In addition to the size dependence of DXA measurements, longitudinal studies may also be influenced by changes in body composition, i.e. the amount of fat/lean mass overlying the scanned region of interest. DXA corrects for the tissue around the bone by assuming a homogenous distribution; in a growing child the soft tissue will be undoubtedly change and may cause some inaccuracies in measurement. Another limitation of DXA is that it cannot make distinctions between cortical and trabecular bone.

1.2.8.2 Peripheral quantitative computed tomography (pQCT)

Peripheral Quantitative computed tomography (pQCT) first became available in early 1990s (143-145). This method uses a traditional rotate-translate CT technology, and only single slice can be obtained (1 to 2mm thick). Peripheral QCT offers the same advantages as axial QCT (volumetric VMD [vBMD, mg/cm^3] so size dependent; separate measures of trabecular and cortical bone). The technique is only applicable to the peripheral skeleton (the radius, tibia and femur) so is obtained at a lower cost and radiation exposure than axial QCT. Furthermore, pQCT distinguishes between cortical and trabecular bone. This procedure has so far only been put to limited use for paediatric patients (146;147).

1.2.8.3 Quantitative ultrasound (QUS)

Quantitative ultrasound (QUS) was first introduced in 1984 (148), when calcaneal ultrasound scanner was developed for assessment of bone status in adults. Measurements obtained from QUS are based upon the attenuation of the ultrasound beam when it passes through the investigated region of interest. QUS can only be applied to the peripheral skeleton and sites for the measurements are the phalanges, radius, calcaneus, patella, and tibia. Axial sites cannot be measured by QUS due to the large amount of the soft tissue and muscle that overlie these sites and attenuate the ultrasonic beam. Since QUS does not use ionizing radiation, it is especially appropriate for examining children. Paediatric studies of both healthy

and diseased children have been carried out to assess the method. A study of paediatric patients with CD showed that by comparison with the DXA method, the investigation of the radius and tibia with QUS was not sufficiently sensitive to detect lower BMD (149). QUS should therefore only be used to complement clinical practice.

1.3 Cytokines in paediatric IBD

Cytokines are the key signals in the intestinal immune system, and are known to participate in the distribution of the so-called normal state of controlled inflammation (physiological inflammation of the gut) (150). Cytokines are small peptide proteins produced mainly by immune cells that facilitate communication between cells and mediate the local and systemic inflammation in an autocrine, paracrine and endocrine pathways (151). In IBD the innate immune response plays a critical role. Active dendritic cells and macrophages secrete cytokines that actively regulate the inflammatory response in CD and UC. Once secreted by these antigens presenting cells cytokine trigger and differentiate many T-cell activating the adaptive immune response. IBD also has a T-cell dysregulation where clearance of overeactive and autoreactive cells is distributed. The lack of appropriate regulation from T-cells, participate in the exacerbation of IBD (152). CD is associated with Type I helper T-cells (Th1) mediated response and their cytokines: interleukin-2 (IL-2) and gamma interferon (INF-γ). Theses Th-1 products promote a self-sustaining cycle of activation with macrophages that includes interleukin-12 (IL-12), which further increased Th-1 activity, and interleukins-1 and 6 (IL-1,IL-6) and tumor necrosis factor alpha (TNF-α), which create a broader inflammatory response. Although macrophage derived IL-6 and TNF-α are also important for the pathophysiology of UC demonstrate an atypical type 2 helper-T-cell (Th2) phenotype, with interleukine-5 (IL-5) as a distinctive cytokine mediator (153). The anti-inflammatory cytokine, interleukin-1 receptor antagonist (IL-1ra), is decreased (154).

1.3.1 Role of proinflammatory cytokines in IBD

Pro-inflammatory cytokines are produced by various cell types and have actions as diverse as T-cell activation, induction of an acute phase proteins and inflammatory chemical pathways, stimulation of cell growth and differentiation and control of apoptosis. Evidence of an imbalance of proinflammatory cytokines in patient with IBD includes the positive correlation of serum cytokines concentrations with IBD disease activity and the effectiveness of IBD therapies that involve cytokine modulation (155;156). The major proinflammatory cytokine influential in IBD include Interleukine-1β (IL-1β), TNF-α, and IL-6 (157;158). Data also suggest that the normal IL-1 receptor antagonist IL-1ra/IL-1β ratio in which IL-1ra predominates is decreased systemically and in the mucosa of the patients with IBD especially

those with CD. The imbalance of IL-1ra and IL-1β ratio appears to be associated with increasing mucosal inflammation but it is still unclear about the direct effect of this imbalance on growth and puberty in children with IBD (159;160).

1.3.1.1 Tumor necrosis factor alpha (TNF-α)

Tumor necrosis factor alpha is the most recognised cytokine due to the increasing use of TNF-α blockers for the treatment of CD and UC. TNF-α is secreted by the macrophages, monocytes, neutrophils, T-cells, natural killer cells (NK), following their stimulation by bacterial lipopolysaccharides. $CD^{+}4$ T lymphocyte secrete TNF-α while, $CD^{+}8$ T-cells do not. The synthesis of TNF-α is mainly induced by different stimuli including interferons, IL-2 and granulocyte macrophage colony stimulating factor (GM-CSF). The production of TNF-α is inhibited by IL-6 and transforming growth factor β (TGFβ). It is a potent proinflammatory cytokine that exerts its stimulatory effects on cells which produce INF-γ. In resting, macrophages, TNF-α induces the synthesis of IL-1 and prostaglandin E2 which can act in concert to potentiate the inflammatory cascade. TNF-α can also enhance the proliferation of T-cells induced by various stimuli in the absence of IL-2. Beyond its effect on immune response, TNF-α activates osteoclasts and thus induce bone resorption. The cytokine possesses both growth stimulating properties and growth inhibiting properties, and appears to have self regulatory properties as well. The signalling of TNF-α starts with serum soluble TNF receptor I and II (sTNF-RI, II) levels correlate with disease activity in IBD patients. More, specifically sTNF-RI is up regulated in the serum from active CD patients as compared to IBD patients compared to healthy controls and could be used as a marker of disease activity (161). sTNF-RII levels are significantly more elevated in serum from active CD patients as compared to UC and can be used additional parameter to differentiate both diseases (162).

1.3.1.2 Interleukin-1 (IL-1)

In addition to TNF-α, IL-1 seems to be important in the pathogenesis of IBD because of its immunological up-regulated and proinflammatory activities. The IL-1 system consists of IL-1α and IL-1β. IL-1 system can be also highly regulated by IL-1 receptor antagonist (IL-1Ra), as supported by the findings of high plasma and tissue levels of IL-1Ra in patients with IBD, indicating that IL-1Ra may be a part of the host mechanism for down regulation of inflammation (163). The IL-1Ra/IL-1 ratio decreases with increasing IBD activity, while remaining constant in uninvolved CD and inflammatory control specimens that may contribute to the pathogenesis of chronic gut inflammation (164). Increase levels of IL-1 in IBD may be the result of stimulation of colonic macrophages that can activate IL-1 converting enzyme and hence release mature IL-1β into the colonic mucosa (165).

1.3.1.3 Interleukin-6 (IL-6)

In contrast to the other cytokines, IL-6 is a pleiotropic cytokine that exerts its proinflammatory effects largely by means of its soluble IL-6 receptor (sIL-6R). The combination of sIL-6R and IL-6 stimulates cells that only express glycoprotein 13 (gp130) and IL-6R, a process known as trans-signalling. IL-6 signalling through signal transducers and activator of transcription 3 (STAT3) plays a central role in several immunologic reactions during the development of IBD and circulating levels of IL-6 and sIL-6R correlates with many clinical features of CD and UC (166;167). Blockade of IL-6 trans-signalling causes T-cell apoptosis, indicating that IL-6- sIL-6R system mediates the resistance of T-cell to apoptosis in CD (168).

1.3.2 Proinflammatory cytokines and IGF-binding proteins interactions

Studies have reported potential interactions between proinflammatory cytokines and insulin like growth factor binding proteins (IGFBPs) (169-174). These studies have shown that the interactions were cell specific, and studies in adult rats have shown that the effects of cytokines on IGFBPs could also be organ specific (175). Modifications in mucosal IGFBPs could account for the intestinal mucosal damage that occurs in patients with IBD (174). Endogenous production of IL-1 mediates the changes in IGF-I and IGFBPs induced by chronic abdominal sepsis in rats (176). The in vivo IL-1β administration in rats decreases IGF-I, increases IGFBP-1 and IGFBP-2 levels in plasma, liver and skeletal muscle and suppresses GH induced acid-labile subunit (ALS) mRNA levels and secretion in primary hepatocytes (177). There is also evidence suggesting that IL-6 is associated with reduced levels of IGFBP-3 which then leads to an increased clearance of circulating free IGF-1 (178). Moreover, the addition of IL-1β and IL-6 to cell cultures of the intestinal line Caco-2 decreased IGFBP-2 and IGFBP-4 secretion in both undifferentiated and differentiated cells (174). In vivo study, in rats, has shown that the infusion of TNF-α may decrease total and free IGF-1 and IGFBP-3 concentration (179).

1.4 Insulin-like growth factor system (IGFs)

The IGFs were initially discovered as factors in human serum that mediate the growth promoting actions of growth hormone (GH) on the skeleton (180).The IGFs are proteins with high sequence similarity to insulin. IGFs are a part of complex system which cells use to communicate with their physiologic environment. This complex system often referred to as the IGF "axis" consists of two cell-surface receptors (IGF1R and IGF2R), two ligands (IGF-1 and IGF-2), a family of six high-affinity IGF-binding proteins(IGFBP 1-6), as well as associated IGFBP degrading enzymes , referred to collectively as proteases.

1.4.1 Insulin like growth factor-1 (IGF-1)

IGF-1 is a single polypeptide chain produced mainly from liver under stimulation of GH. IGF-1 is the predominant post-natal IGF that mediates many of the growth promoting actions of the GH between birth and puberty (180). IGF-1 promotes longitudinal bone growth by increasing both size of the hypertrophic zone and the chondrocyte proliferation rate during the early stage of bone growth (181). The effect of IGF-1 at the proliferative phase is induced by shortening the time cycle rather than by clonal expansion (182). Not only is IGF-1 abundant in the bone, but it appears to affect bone health through a myriad of pathways (183). In vitro studies have shown that IGF-1 stimulated proliferation of bone cells and collagen synthesis and contributed to up to 50% of the proliferation in bone cell cultures (184). IGF-1 also stimulated bone growth in vivo studies, and increased osteoclast differentiation (184). IGF-1 increases both longitudinal bone growth (185) and circumferential bone growth (186). An in vitro study shows that the IGF-1 signalling pathway plays a vital role in regulating endochondral bone growth independently through the p44/42 mitogen activated protein kinase (Erk1/2) and phosphor-inositide 3-kinase (PI3K) pathways (87). The cellular actions of IGF-1 are activated by a receptor tyrosine kinase (IGF-1R) and this receptor is expressed in the chondrocytes of growth plate. Binding IGF-1 to its receptors in the chondrocytes initiates a number of autophosphorylation reactions. The IGF-1 signaling pathway can be interrupted by different expressions such as TNF-α and IL-1 (187). IGF-1R null mice die shortly after birth and show disorganisation in chondrocyte pattern and abnormalities in vascularisation and mineralization (188). Furthermore, the GHR/IGF-1R knocked out mice show a greater reduction in bone growth compared with individual gene mutation only in the IGF-1R and missing the GHR. In fact, the functional correlation between the GH and IGF-1 is still unclear. Nevertheless, IGF-1 can stimulate bone growth in the lack of GH. IGF-1 influences on chondrocyte functions is regulated, in part, by IGF-binding proteins (IGFBP) (189).

1.4.2 IGF binding proteins (IGFBPs)

There are six IGFBP that bind IGFs with high affinity and specificity. The major functions of these binding proteins are to 1) prolong the half-life of IGFs in circulation 2) prevent IGF induced hypo-glycemia 3) regulate the passage of IGFs from the vasculature to the extra vascular space 4) limit the bioavailability of the IGFs to interact with cell surface receptors 5) enhance IGF actions and 6) affect cellular proliferation and death via IGFBP receptors (190). The most abundant IGFBP in serum is IGFBP-3 (190-192). IGFBP-3 predominates in the circulation (90-95%)(193). In serum, most of the IGF-I and IGF-II are found in a complex formed by IGFs, IGFBP-3 and a non-IGF binding glycoprotein known as the acid labile subunit (ALS) (190-192). The molecular weight of IGFBP-3 in its non-glycosolated form is 29

kDa. However, IGFBP-3 is found in circulation in its glycosylated form with a molecular weight between 40 and 44 kDa (191). Its primary function in the circulation is thought to be the prolongment of the half-life of IGF-I. Mukherjee and Rotwein (194) demonstrated that IGFBP-4 and IGFBP-5 predominate in bone. IGFBP-4 has ability to block the action of IGFs and inhibits bone formation. On the other hand, the exact function of IGFBP-5 remains controversial. IGFBP-5 is a polypeptide chain and the amino terminal is attached with IGF1. This protein is released during endochondral formation and it deposits in adult bone. Global knockout of IGFBP-5 in mice causes low changes in bone minerals or whole animal physiology. However, its over expression has a significant detrimental effect on mineralisation. In contrast, other studies show that IGFBP-5 in combination with IGF-1 has both stimulatory and inhibitory effects on bone. However, it has been found that the bone morphogenetic proteins -2 induced osteoblasts differentiation can be blocked by IGFBP-5 and also suppress longitudinal growth and bone mineralisation in mice. Therefore, this study supports the inhibitory effect of IGFBP-5 (194).

1.5 Bone health in paediatric IBD

IBD in children leads to a profound disturbance in skeletal development. Poor bone health and reduced bone mineral density has been increasingly reported in children and adults with IBD (195;196).

1.5.1 Mechanism of bone impairment in paediatric IBD

The pathophysiology of bone impairment in paediatric IBD is complex and is associated with multiple risk factors, including growth retardation, pubertal delay, malnutrition, malabsorption, micronutrient deficiencies, alteration in growth hormone/IGF axis, decreased muscle mass and weight-bearing activity, corticosteroid therapy and increased circulatory cytokines (Figure 1.5.1). Inflammation from CD may compromise bone accrual directly through bone active cytokines and indirectly through delayed growth and maturation, decreased physical activity, malnutrition and anti-inflammatory medication.

Malnutrition, mainly due to reduced intake of calories and protein is common in children with IBD and can result in suboptimal bone development (197). Inflammatory cytokines promote osteoclastogenesis and accelerated bone resorption. TNF- α induces the expression of receptor activator of nuclear factor-kB ligand (RANKL). RANKL stimulates osteoclast differentiation and activation and inhibits osteoclast apoptosis (198;199) thereby dramatically prolonging osteoclast survival and increasing bone resorption.

Additionally, TNF-α decreases expression of osteoprotegrin (OPG), a decoy receptor that blocks RANKL (200;201). Inflammatory mediators, including interleukin (IL)-1 and IL-6, also increase RANKL secretion and contribute to bone loss (202). TNF-α has direct effects on bone formation by inhibiting osteoblast differentiation and osteoblast collagen secretion, causing increased resorption by inducing osteoblasts secretion of IL-6, and inducing osteoblast apoptosis (203;204). These effects on bone formation are strikingly similar to the effects of glucocorticoids (205). Control of inflammation improves inflammatory cytokine profiles, nutrition status, muscle mass, and BMD. Use of Infliximab in adults has been shown to improve not only bone formation as indicated by bone biomarkers, but also BMD (206). In children with CD, Infliximab therapy is associated with improved bone formation biomarker (207) .The effect of Infliximab on bone is discussed in detail in Section (1.10).

Hormonal disturbances associated with IBD can affect bone development and maintenance. Delayed puberty is often a feature of IBD (208) and is associated with a relative oestrogen deficiency, which may affect bone mass accrual. Hypo-gonadism can be seen in adults and may similarly impair bone remodeling (209). Serum insulin like growth factor-1 (IGF-1), a potent growth factor for bone, is commonly reduced in children with active inflammation. This is due to a combination of malnutrition and growth hormone signalling blockade in the liver and peripheral tissues (210). Low IGF-1 may impair longitudinal growth by chondrocytes, expansion of the outer cortical layer by periosteal osteoblasts, and recruitment of undifferentiated stroma cells into the osteoblast linage (211). Normal bone development is stimulated by weight-bearing exercise. Patients with IBD may have limited endurance and energy and prefer more sedentary activities. Potentially this can decrease mechanical stress on bone that is important to maintain its strength. Crohn's disease is associated with persistent deficits in lean body mass and musculature (212;213), which result in reduced mechanical strain and decreased bone formation (214).

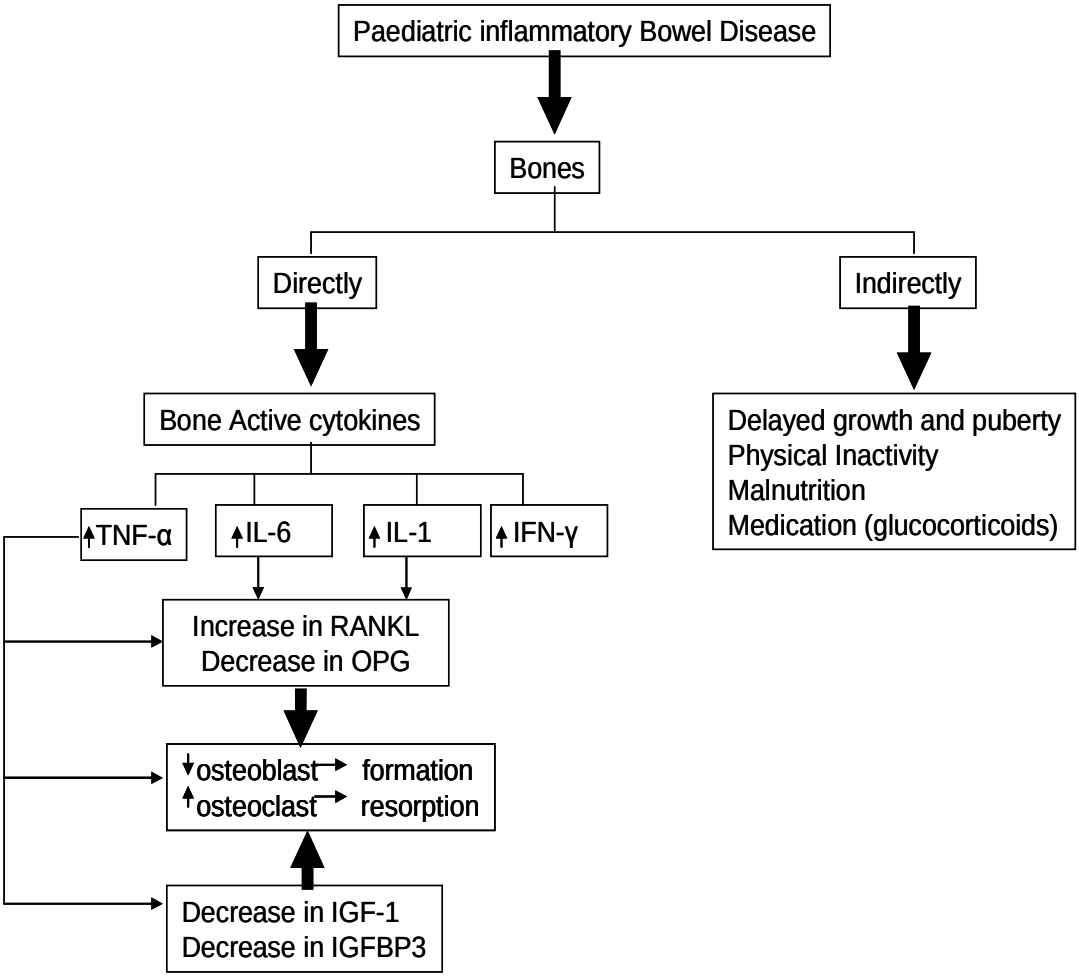


Figure 1.5.1: Mechanism of bone impairment in paediatric IBD

1.6 Growth in children with chronic inflammation

1.6.1 Normal growth in children

Normal children grow at very different rates. A child's growth is the result of both genes and environment; it appears principally mediated by hormones and nutrition (215). Linear growth can be represented by stature (attained height) or by the rate of growth (height velocity). A child's attained height represents the culmination of growth in all preceding years; height velocity reflects growth status over a particular period in time. Growth can be conceptualized as the product of three overlapping biological phases: infancy, childhood, and puberty. Final height represents the sum of each of the individual components. The growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis plays a pivotal role in normal postnatal growth. IGF-1 stimulates mitosis of epiphyseal chondrocytes resulting in linear bone growth (216). Thyroxine, cortisol and sex steroids are also implicated in the maintenance of normal linear growth. Linear growth velocity decreases from birth onwards, punctuated by a short period of growth acceleration (the "adolescent growth spurt") just prior to completion of growth. As the rapid growth of infancy tails off, the steady growth of childhood predominates. Healthy children grow at a consistent rate in the range of 4 to 6 centimetres annually from six years of age until the onset of puberty (217). The age at which children enter puberty and the speed with which they progress through puberty is variable and may be influenced by a number of factors including genetics and the environment.

1.6.2 Growth in children with chronic inflammatory conditions

Chronic inflammatory disease such as juvenile idiopathic arthritis (JIA), inflammatory bowel disease (IBD), cystic fibrosis (CF) and systemic lupus erythematosus (SLE) may impair growth and lead to significant deficits in bone mass and alterations in bone microarchitecture. Impaired linear growth and bone mass are commonly encountered in children suffering from chronic inflammatory diseases both at disease presentation and following treatment. In these children, maintenance of skeletal health is a complex process that is influenced by a number of different mechanisms, including not only the steroid therapy, but also other factors such as the disease process, nutritional status, endocrine status and the response of the body to inflammatory mediators. Inflammation has a profound effect on a number of key mediators of skeletal development and it is likely that improvement in disease and reduction of inflammation will lead to improvement. The recent introduction of biologic therapy that targets specific mediators of the proinflammatory process is proving to be a useful adjunct in the therapeutic management of the child with chronic inflammation.

These drugs may also exert beneficial effects on the adverse effects of inflammation on growth and skeletal development. It is currently unclear whether these beneficial effects may be simply due to improvement in overall disease or due to a direct effect of the 'anti-cytokine' at the level of the target tissue involved in growth and skeletal development.

1.6.2.1 Mechanism of growth impairment in chronic inflammation

Chronic inflammation in children can disturb skeletal development and impair growth. This can be caused by a combination of factors relating both to the disease itself and to its secondary effects, and also to the maintenance therapy, mainly glucocorticoids, employed to control disease (Figure 1.6.1). Growth retardation can be variably defined as "height below third percentile", "height velocity or weight gain below the third percentile", "bone age retarded by two years" (69) or "height velocity below third percentile for age and bone age greater than two standard deviation below chronological age" (70) and "no signs of puberty", and out with the target range (218;219). In JIA, linear growth impairment is reported in 11% to 41% of patients with systemic forms of JIA (220;221). In CD final height may be substantially reduced in 15-30% of patients (219). Growth failure is frequently seen in cystic fibrosis (CF), and has been attributed to both malnutrition and to chronic inflammation (222). One study in 70 patients with childhood systemic lupus ertythematosus found that the average height was less than age- and sex-matched healthy controls (223). Nutrition is another important factor that determines bone mass and growth. Protein intake is important for muscle development, calcium intake for skeletal development and, in JIA; these factors have been shown to be associated with bone mineral density (224). The association between body weight and bone mass (225), may be attributed to the effect of leptin, the GH/IGF-1 axis (226) as well as the mechanical effect of muscle on bone (227).

The effect of under nutrition on skeletal development has been studied primarily in children affected by IBD who may have low lean mass and low bone mineral density (228). Besides low IGF-1 levels, impaired nutrition state may also be associated to reduced levels of thyroid hormone (229). In CD prospective studies show that growth failure correlates strongly with disease activity (230). In children with systemic JIA, childhood CD and perinatal HIV infections, an inverse correlation has been found between circulating IL-6 and both IGF-1 and IGFBP-3 levels. Furthermore, a direct link between IL-6 and adverse bone health has been found in CF, where raised IL-6 levels were associated to reduced bone mineral content gain and raised markers of bone resorption (231;232).

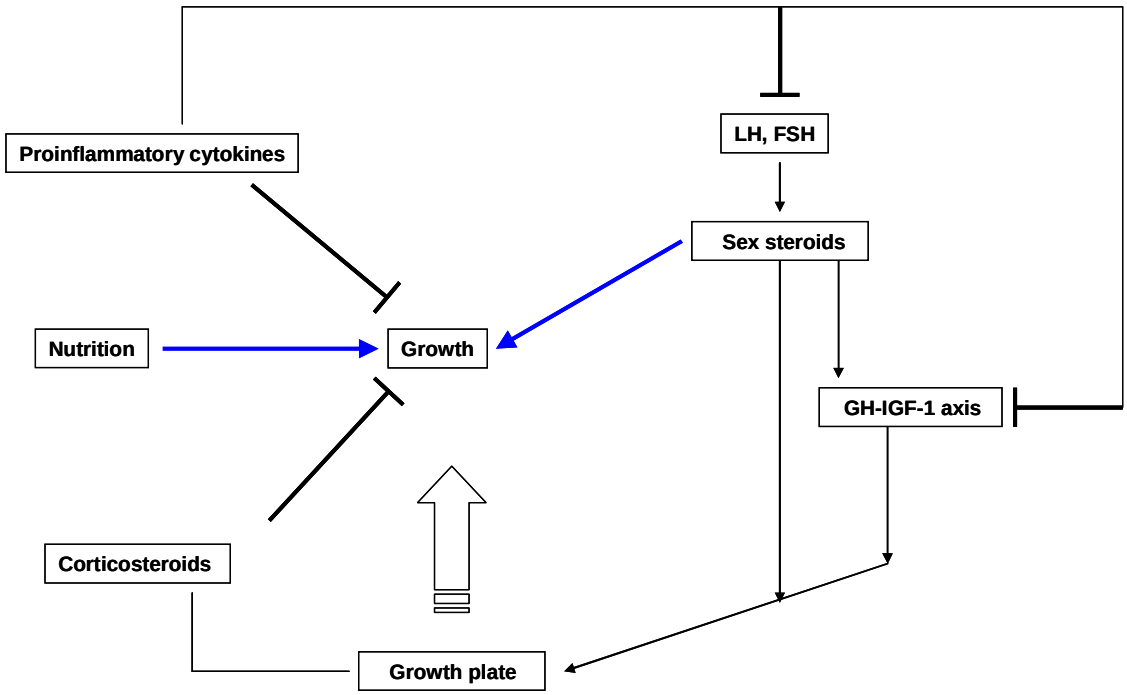


Figure 1.6.1: **Mechanism of growth failure in children with chronic inflammatory conditions**

In CD, serum IL-6 has been reported as an important inhibitory mediator and has been shown to inhibit bone mineralization in vitro (233). Increased IL-6 may therefore represent a general mechanism by which chronic inflammation affects the developing skeleton. Most studies that have studied final adult height in those with childhood-onset CD show a variable degree of persistent short stature on completion of growth (221;234). Some studies have suggested that up to 25% of patient may not be able to achieve full growth potential (235;236).

1.7 Puberty development in children with IBD

1.7.1 The pubertal processes in healthy children and adolescents

Puberty is defined as the sequential biologic process that ultimately leads to reproductive capacity (237). Serum levels of dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) begin to rise at approximately 6-8 years of age before the physical changes of puberty. This is called adrenarche and typically antedates the onset of gonadal puberty, i.e. gonadarche, by a couple of years. In adrenarche a slight increase in linear growth rate (mid-childhood growth spurt) and possibly the appearance of some pubic and/or axillary hair, predominantly in girls, can take place (238).

The onset of gonadarche is initiated following the synthesis and secretion of luteinizing hormone releasing hormone (LHRH, also called as gonadotropin releasing hormone GnRH), in the hypothalamus and its transport to ganadotrophs inside the pituitary. Once stimulated by LHRH, the ganadotrophs secretes the gonadotropin luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which regulate ovarian and testicular function. Pituitary sensitivity to LHRH varies throughout life but increases prior to the onset of puberty. During this time the LH begins to be secreted in a pulsatile manner during sleep but subsequently changes to pulsatile pattern throughout the day as puberty progresses (239). This pulsatile secretion of gonadotropin is responsible for the enlargement of the testicles and the ovaries and the secretion of sex steroids, testosterone and estradiol. The sex steroids then cause the development of the secondary sex characteristics. These can be classified in 5 stages according to Tanner (10) evaluating breast development and pubic hair in girls, and genital organ development and pubic hair in boys. Serum levels of testosterone and estradiol, as well as DHEAS, continue to increase throughout puberty.

1.7.1.1 Pubertal assessment

The Tanner Pubertal Stages are commonly used to assess the progression through adolescence and puberty for both males and females (240). The system rates, over five stages, the development of genitalia and pubic hair in males and pubic hair and breast development in females (10). Adolescents are often asked to rate themselves, or self-report, using the Tanner stages for their sex. Black and white drawings of the stages of genital, breast and pubic hair development for females, or genital and pubic hair development combined with an orchidometer (a string of 12 wooden beads approximating the size, shape and volume of the testes) for males are used. It is important to note that there will always be a certain amount of error in every self-reporting system, however self-assessment may sometimes be preferable to being examined by a study investigator (241). Puberty and skeletal maturity are closely related. In fact, puberty is much more related to skeletal maturation than it is to chronological age (10). In normal circumstances, children who reach maturity faster and begin puberty earlier are often shorter in stature than those who mature at a slower rate (10). This is because sex-steroids or hormones, which play an essential role in puberty, also play an essential role in determining the timing of the cessation of bone growth, e.g. epiphyseal fusion (242).

1.7.2 Pubertal delay in children with IBD

The complex interactions between severity of disease, fluctuations in inflammatory cytokines and their effect on nutritional status and hormonal profile make it difficult to determine how individual factors influence the onset and progression of puberty in paediatric patients with IBD. Under nutrition in the absence of disease may cause delay in menarche and sexual maturation. Reduction in calorie intake has been documented in many studies of paediatric-onset IBD, especially CD (79;243;244). Thus, under-nutrition is likely to be one of the contributing factors leading to delay in the onset and progression of puberty. In vitro studies have elucidated ways in which pro-inflammatory cytokines, known to be elevated in patients with IBD such as TNF-α, IL-6 and IL-1β affect endocrine function. Several, of these findings may be applicable to explaining pubertal delay in patients with chronic IBD (219).

1.8 Biologic therapy in paediatric IBD

In paediatric patients with IBD, therapeutic interventions should be aimed to resume weight gain and promoting growth and development as these are the major complications of paediatric IBD, particularly in CD. Therefore, these aims should be achieved within a relatively short period of opportunity, before growth impairment and development deficiencies become permanent. Conventional treatment of CD consists of enteral nutrition, corticosteroids, anti inflammatory agents and immunomodulators. However, these treatments have been largely unsuccessful in altering the natural course of the disease (245). The 1990's have been characterized by the introduction of biological therapies. Biological therapy refers to the use of medication that is tailored to specifically target an immune or genetic mediator of disease (103). Biologics are proteins which selectively block or neutralize the effects of cytokines, such as TNF-α and include fusion proteins and monoclonal antibodies (246;247). Biologic therapy has found a niche in the management of cancer (248;249), autoimmune disease (250) and diseases of unknown cause that result in symptoms due to immune related mechanisms (251;252). The biological agents that have been evaluated for the treatment of CD include: the anti-TNF-α inhibitors Infliximab, Adalimumab, Certolizumab pegol and anti-adhesion molecule (Natalizumab) (Table 1.8.1).

1.8.1 Infliximab

Infliximab (Remicade) is a chimeric IgG-1 monoclonal antibody with a high specificity for TNF-α. It was the first biologic therapy that was approved for IBD by the U.S. Food and Drug Administration (FDA) in 1998. According to the National Institute for Health and Clinical Excellence (NICE), Infliximab was licensed in 2008 for use in paediatric CD and 2012 for UC. Infliximab, within its licensed indication, is recommended for the treatment of paediatric patients aged 6-17 years with severe active CD whose disease has not responded to conventional therapy (including corticosteroids, immunomodulators and primary nutrition therapy), or who are intolerant of or have contraindications to conventional therapy. This drug is 25% murine-derived and 75% human (Figure 1.8.1). It is administered as an IV infusion, and has a half-life of 9.5 days. The binding epitope for TNF-α is of murine origin while the IgG fragment is of human origin. Infliximab neutralizes the biological activity of TNF-α by binding with high affinity to the soluble (free floating in the blood) and transmembrane (located on the outer membranes of T-Cells and similar immune cells) forms of TNF-α and inhibits or prevents the effective binding of TNF-α with its receptors.

Besides neutralization of TNF-α, Infliximab also blocks leucocytes migration and induces apoptosis of T-lymphocytes and monocytes (253-257). The latter is believed to be one of the key mechanisms of action of the drug. A third mechanism involves complement fixation and complement-dependent cytotoxicity and antibody-dependent cytotoxicity (258).

1.8.2 Adalimumab

Adalimumab (HUMIRA (Human Monoclonal Antibody in Rheumatoid Arthritis) produced by the phage-display technology. It was the first humanised monoclonal antibody drug approved by the FDA in 2002. Adalimumab is indicated for the treatment of severe, active CD in patients whose disease has not responded despite full and adequate treatment with an immunosuppressant and/or corticosteroid, or who are intolerant to or have contraindications to such therapies. Adalimumab is not currently licensed for use in paediatric IBD patients in UK. Adalimumab is a fully human IgG1 monoclonal antibody to TNF-α (Figure 1.8.1). It fixes complement, mediates antibody-dependent cytotoxicity, and induces T-cell apoptosis (259;260). It is administered subcutaneously, and has a half-life of 12–14 days. Controlled trials have shown that Adalimumab is effective for the treatment of rheumatoid arthritis (261;262), psoriatic arthritis (263) and ankylosing spondylitis (264). In addition, 4 placebo controlled trials have demonstrated that Adalimumab is effective for the induction and maintenance of remission in adult patients with CD and based on these studies it recently received regulatory approval in the U.S for this indication (265-268).

1.8.3 Certolizumab pegol

Certolizumab is a humanized TNF-α Fab monoclonal antibody fragment linked to polyethylene glycol (PEG) (Figure 1.8.1) that is administered subcutaneously. Its half-life is 2weeks. In vitro, certolizumab pegol has a high affinity for TNF-α, it is devoid of the Fc portion of the antibody and does not induce complement activation or antibody-dependent cellular cytotoxicity, and it does not induce apoptosis in T-cells or macrophages.

1.8.4 Natalizumab

Natalizumab is a humanized IgG4 monoclonal antibody against the adhesion molecule α4 integrin, which is involved in migration of leukocytes across the endothelium, and is up-regulated in sites of inflamed endothelium. It is administered intravenously every 4 weeks. Its half-life is 11 ± 4 days. Natalizumab is used in the treatment of multiple sclerosis (269) and CD (270). It was approved in 2004 by the U.S FDA. It was the first drug developed in the class of selective adhesion molecule inhibitors.

1.8.5 Safety profile and side effects of biologic therapy in IBD

Crohn's disease refractory to conventional therapy, fistulising CD and chronic active UC generally respond well to anti-TNF therapy. However, the use of biologics is associated with significant, but rarely, fatal complications, leading to serious concerns about safety and long-term consequences. Potential side effects of anti-TNF therapy include opportunistic infections, which show a higher incidence when concomitant immunosuppressive therapy, such as steroids or thiopurine is used (271). Serious infections during anti-TNF therapy include the reaction of the latent tuberculosis. Therefore, tuberculosis screening prior to starting anti-TNF therapy is recommended.

The monoclonal antibodies used for anti-TNF therapy frequently induce the formation of antibodies (ATIs), human anti-chimeric antibodies (HACA) and human anti-humira antibodies (HAHA). ATIs are associated neutralising and interfere with the efficacy and safety of the drug. ATIs are associated with acute infusion reactions and loss of response and with delayed hypersensitivity phenomena. Acute infusion reactions are manifested by shortness of breath, chest pain, palpitation, flushing, headache, urticaria and hypotension. The prevalence of acute infusion reactions varies greatly between studies depending on the sample size, dosing, regimen (episodic or maintenance) and concomitant therapies, but ranges in most studies between 15-25% (252;272;273).

1.8.6 Infliximab in paediatric CD

Studies evaluating the safety and efficacy of Infliximab in children were first reported in several non-randomized studies (156;274-277). These initial studies showed that the response and remission rates (both partial and complete) were far superior compared to conventional therapy. Interestingly, its efficacy in children appeared to be higher than in adults (156;275). In a multicentre, open-label, dose-blinded trial (n = 21), Baldassano and co-workers demonstrated the efficacy and safety of a single infusion of Infliximab in the treatment of paediatric CD. During the 12-week duration of the study, 100% achieved a clinical response and 48% achieved clinical remission, with significant improvements in the paediatric CD activity index (PCDAI), modified CDAI, erythrocyte sedimentation rate, and other outcome variables of interest. There were no infusion reactions in any of the patients and it was suggested that Infliximab may be safe and effective as short term therapy of medically refractory moderate to severe CD (278). A prospective study published by Cezard and coworkers also explored the efficacy and toxicity of Infliximab in children with severe CD.

Twenty-one children (median age 15, range 13 to 17) were treated with Infliximab with an induction sequence of 5 mg/kg at 0, 15, and 45 days.

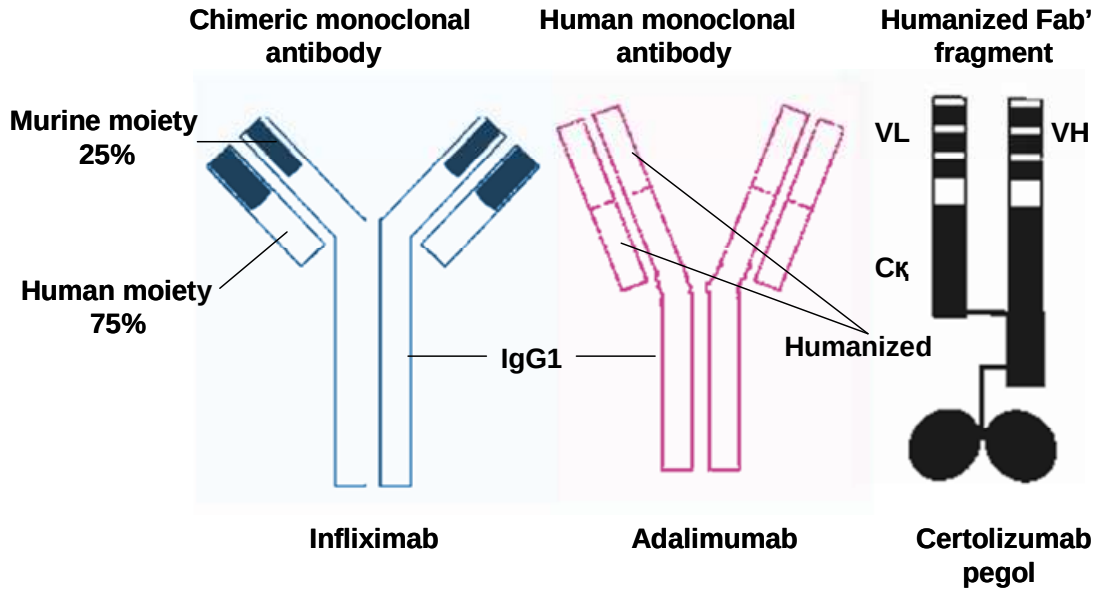


Figure 1.8.1: Structure of biological therapies to treat Crohn's disease

Table 1.8.1:Biological therapies in IBD and their mechanism of action

Drug	Action	Route of administration	Dose	Indications
Infliximab (Remicade)	Chimeric human/mouse monoclonal antibody that binds to the soluble TNF- α and its membrane bound precursor neutralizing its action	IV infusion	5mg/kg 0, 2 and 6 weeks	Rheumatoid arthritis Psoriatic arthritis Ankylosing spondylitis Plaque psoriasis Crohn's disease Ulcerative colitis
Adalimumab (Humira)	A humanized immunoglobulin G1 monoclonal antibody which binds to TNF- α	Sub/cut injection	$24mg/m^2$ Maximum 40mg every other week	Rheumatoid arthritis Psoriatic arthritis Ankylosing spondylitis Plaque psoriasis Crohn's disease
Natalizumab (Tysabri)	Humanized IgG_4 monoclonal antibody against the adhesion molecule α4 integrin	IV infusion	3mg/kg 0, 2 and 6 weeks	Multiple Sclerosis Crohn's Disease
Certolizumab pegol (Cimzia)	Humanized TNF- α Fab monoclonal antibody fragment linked to polyethylene glycol(PEG)	Sub/cut injection	400 mg 0,2 and 4 weeks, then every 4weeks	Crohn's Disease in Adults

Nineteen children were in complete remission (defined as Harvey-Bradshaw index (HBI) <4) on day 45. 14/21 patients had stopped taking steroids at 3 months, and all had stopped parenteral nutrition. All perianal fistulas (n = 12) were also closed by day 90 and the drug appeared to be well tolerated (279). Much evidence at present comes from retrospective analysis of children treated with Infliximab, often as a rescue medication. In a retrospective study in children and adolescents with either corticosteroid dependent or resistant CD, patients were randomized to receive 1 to 3 infusions of Infliximab (5 mg/kg/dose) over a 12-week period. The mean daily prednisone dosages decreased significantly in all the patients ($P<0.01$) studied. A significant initial improvement (as assessed by a significant decline in PCDAI value) was noted in all subjects ($P<0.0001$). Interestingly, over the subsequent 8-week period, 8 of 19 treated subjects had worsening of symptoms (280). Lamireau and coworkers (272) described yet another retrospective study in 88 children and adolescents (median age: 14, range: 3.3 to 17.9) treated with Infliximab for active disease (66%) and/or fistulas (42%) that were refractory to corticosteroids (70%), and/or other immunosuppressive (82%) agents, and/or parenteral nutrition (20%). Patients received a median of 4 (1 to 17) infusions of 5 mg/kg of Infliximab during a median time period of 4 months (1 to 17 months). From day 0 to day 90, the Harvey-Bradshaw score decreased from 7.5 to 2.8 ($P<0.001$), with a significant decrease in both C-reactive protein and ESR ($P< 0.001$). At day 90 after the first infusion of Infliximab, 49% of patients had symptom improvement, 29% were in remission; 53% of patients could be weaned off of corticosteroids and 92% off of parenteral nutrition (272). The authors in both these studies concluded that treatment with Infliximab was well tolerated and effective in most children and adolescents with CD refractory to conventional immunosuppressive therapy. No serious events were noted in any of these studies.

The FDA approval for the use of Infliximab therapy in paediatric CD was based on the results of the much publicized REACH clinical study, a randomized, multicenter, open-label study to evaluate the safety and efficacy of Infliximab in paediatric subjects with moderate to severe CD. A total of 112 paediatric patients (ages 6 to 17 years) with moderate to severe CD who took part in this study received Infliximab at 5 mg/kg at week 0, 2 and 6. Patients, who had symptom improvement or showed response, were then randomized to 2 groups and received Infliximab every 8 or 12 weeks for almost 1 year. A concurrent immunomodulator was also required. At week 10, 88% patients showed response (defined as decrease from baseline in the PCDAI score ≥15 points; total score ≤30) and 58% patients achieved clinical remission (defined as PCDAI score ≤10 points).

At week 54, 63% and 56% patients receiving Infliximab every 8 weeks were in clinical response and clinical remission, respectively, compared with 33% and 23% patients receiving treatment every 12 weeks (P=0.002 and P<0.001, respectively). The data from this important prospective trial thus suggested that Infliximab is not only highly effective in inducing clinical response and remission but also in maintenance of remission, more so with an 8-week dosing compared with every 12-week dosing (273).The same research consortium also found Infliximab to be an effective therapy in children with perianal disease, including patients with perianal fistula (281) and in prolonging the withdrawal of corticosteroids over a 3-year follow-up period (282). Similar observations have also been made in a European study in children with CD. In that study, children on an on-demand treatment schedule were more-likely to experience a relapse (92%) when compared to patients on 2-month infusion schedule (23%) (283).

1.8.7 Adalimumab in paediatric CD

Adalimumab is used primarily in clinical practice for patients who are primary anti-TNF responders, but become intolerant or non responsive to Infliximab (284-286). Adalimumab is not currently licensed for use in paediatric IBD patients. The published experience to date of Adalimumab in children is limited mostly to case reports (287-290) and small clinical case series (291-294) with the exception of one large retrospective RESEAT study by Rosh et al (295) and recently published prospective IMAgINE 1 study by Hyams et al (296)(Table 1.8.2).
RESEAT study reposted Adalimumab to be well-tolerated and effective rescue therapy for moderate-to-severe paediatric CD patients previously treated with Infliximab. Moreover it was also reported that Adalimumab response was steroid sparing as it was not enhanced by the use of concomitant immunomodulators and >70 % of patients achieved rapid response that was sustained through 12 months (295).
IMAgINE 1, is a randomized, multicenter, open-label study which evaluated the safety and efficacy of 2 Adalimumab double blind maintenance dosing regimens in paediatric patients with moderate to severe CD. A total of 192 paediatric patients (ages 6 to 17 years) with moderate to severe CD who took part in this study received open label induction therapy with Adalimumab at week 0 and week 2. Patients who weighed ≥40 kg at baseline received subcutaneous adalimumab 160 mg at week 0 and 80 mg at week 2, and patients who weighed ≤40 kg at baseline received subcutaneous adalimumab 80 mg at week 0 and 40 mg at week 2. At week 4, patients were randomly assigned (1:1) to high-dose or low-dose adalimumab double-blind maintenance therapy for 48 weeks, stratified according to their week 4 responder status and prior exposure to Infliximab.

Clinical response was defined as decrease in the PCDAI score ≥15 points from baseline. Starting at the week 12 study visit, patients who experienced disease flare or nonresponse to treatment were switched from blinded every other week dosing to blinded weekly dosing, continuing with the same dose. Disease flare was defined as an increase in the PCDAI of ≥15 points when compared with week 4 and an absolute PCDAI >30. Nonresponse was defined as 2 consecutive visits at least 2 weeks apart in which a decrease in PCDAI ≥15 points from baseline was not achieved. Patients were assessed at baseline and weeks 2, 4, 8, 12, 16, 20, 26, 32, 40, 48, and 52. A total of 152 patients (80.9%) completed all 26 weeks of the study. At week 26, 63 patients (33.5%) were in clinical remission, with no significant difference between high-dose (38.7%) and low-dose groups (28.4%) (p=0.075). This study did not raise any safety concerns (296). It is now increasingly clear that Adalimumab is used widely in paediatric clinical practice.

1.8.8 Infliximab in paediatric UC

The experience with Infliximab in the treatment of paediatric patients with ulcerative colitis is quite limited, except one all published studies are retrospective, non-controlled, and include a small number of patients (Table 1.8.3) (297-301). Moreover, there is no homogeneity either in the inclusion criteria for treatment (although most patients had a steroid-refractory or steroid-dependent moderate-to-severe disease), or the definition of short- and long-term response. There is also a lack of consensus regarding the induction and maintenance dose as well as in the concomitant use of immunomodulators. Hyams et al (301) prospectively evaluated the efficacy and safety of Infliximab for inducing and maintaining benefit in paediatric patients with moderately to severely active UC. In this study paediatric patients (ages 6 to 17 years) who had active UC and had not responded to or tolerated conventional treatment were given 5 mg/kg Infliximab at weeks 0, 2, and 6. The primary end point was the response at 8 weeks. Patients, who responded, were then randomized to 2 groups and received Infliximab every 8 or 12 weeks and followed through 54 weeks. At week 8, Infliximab induced a response in 73.3% of patients. Among responders, twice as many were in remission at week 54 after 8weeks (8 of 21, 38.1%) than 12 weeks (4 of 22, 18.2%; p=0.146) therapy. The overall remission rate at week 54 for all enrolled patients was 28.6% (301). Eidelwein et al (297), reported the outcome of 12 paediatric patients with UC who received Infliximab for the treatment of fulminant colitis, acute exacerbation of colitis, steroid-dependent colitis and steroid refractory colitis. Nine patients had a complete short-term response, and 3 had partial improvement. With a median follow-up time of 10.4 months in this study, three patients underwent colectomy (297).

Another study by Mamula et al (298) have reviewed charts of 17 children with UC that were unsuccessfully treated with a traditional pharmacologic approach and underwent Infliximab therapy clinical improvement, i.e. avoidance of restorative proctocolectomy or lack of need for rescue medications, was observed in 75% of the patients. A response to the initial dose of Infliximab seen in 14 of 17 (82%), while 10 of 16 (63%) had a sustained response. Concomitant corticosteroid could be discontinued in six patients; two patients underwent colectomy after two years follow-up (298;302). Russell and Katz (299), described 14 children with UC treated with Infliximab: a clinical response occurred in 9 (299). Serrano et al (300) reported Infliximab to be associated with remarkable clinical improvement. McGinnis et al (303) conducted a largest paediatric study to date evaluating the effectiveness of Infliximab in UC and reported 75% response rate. Although the few published reports about the use of Infliximab in paediatric UC patients are encouraging, but still larger studies with more extensive follow-up are needed to adequately position this drug in paediatric UC also. Adalimumab has not been proven to be effective for paediatric ulcerative colitis.

Table 1.8.2: Published paediatric Adalimumab studies to date (adapted from Russell et al (304))

Study	N	Prior Infliximab	Initial dosing used in mg (no of patients)	Clinical Response	Comments
US 2012 (296)	192	46.3%	160/80(93),80/40(95)	63%	No safety signals detected, Included >18years
UK and ROI 2011(304)	72	94%	160/80(3), 80/40(41), 24 mg/m² (16) other (10)	71%	2 deaths
USA 2008(294)	10	100%	80/40(4), 40(5), 80(1)	80%	Mean PCDAI 12 at start
USA 2008(292)	15	100%	80/40(11), 40(1), 80(1), 40/20(1), 160/80(51)	64%	Included >18years
USA(RESEAT) 2009 (295)	115	95%	160/80(22), 80/40(51), 40/40 (17) other/unknown (9)	65%	Short follow-up period
Israel 2010(293)	14	71%	Dosing schedule not available	85%	Included >18years
Italy 2009(291)	23	61%	160/80(13), 120/80(2)	91%	Prospective;80mg maintenance in most

In addition to the case series listed there have been at least four published paediatric case reports (287-290)

Table 1.8.3: Studies evaluating the efficacy of Infliximab in paediatric patients with UC (adapted from (305;306)

Study	N	Severity (mild/mod/severe)	Pre- Imm (N)	Repeated infusion	Short-term outcome	Duration (weeks)	Long-term outcome	Duration (months)
Hyams et al, 2012 (301)	60	0/0/60	-	Yes	Response: 44/60 (73.3%)	8	Response 8 weekly group (38.1%) Response 12 weekly group (18.2%) Overall remission : 28.6%	54
Eidelwein at al, 2005(297)	12	0/6/6	8	Yes	Response: 12/12 (100%) Remission: 9/12 (75%)	2	Response: 8/12 (67%) Remission: 9/12 (75%)	10.4
Mamula et al, 2002,2004(298;302)	17	1/10/6	12	Yes	Response: 14/17 (82%) Remission: 6/9 (66%)	0.3	Response: 1016(63%)	9.5
Russell and Katz 2004(299)	14	0/5/9	7	Yes	Response: 8/14 (57%)	2.6	Remission: 8/14 (57%)	12
McGinnis et al, 2004(307)	29	0/0/29	-	-	Response: 18/29 (70%)	4	Response: 5/29 (17%)	12
Serrano et al, 2001(300)	3	-	3	-	Response: 3/3(100%)	-	Response: 1/3 (33%)	1.5
Olivia-Hemker et al, 2002(308)	5	-	3	Yes	Response: 5/5 (100%) Remission: 2/5 (40%)	2	-	-

Except one all studies are retrospective and non-controlled series

1.9 Effect of biologic therapy on growth

1.9.1 Effect of biologic therapy on growth in paediatric CD

Studies that report the effect of biologics on growth in children with CD are summarized in (Table 1.9.1). Although two studies have shown no improvement in growth after Infliximab therapy (247;309) a number of studies have reported beneficial effects of Infliximab on growth in children with CD, as early as 6 months after start of therapy (156;207;279;310;311). Cezard et al prospectively followed 21 children with severe CD and reported an improvement in a subset of 10 children who had growth measurements (279). Borrelli et al reported an improvement in SDS for height 6 months after initiating a maintenance course of Infliximab in eight patient ages 6-18 years. Another important finding in this study was that the weight and height gain were significantly higher in patients on maintenance therapy than in those treated with only three infusions of Infliximab (156). Walters et al not only reported an improvement in height velocity but also suggested that growth was more likely to improve in those who were in the earlier stages of puberty and those who showed clinical response (311).

The multicentre REACH study evaluated the role of Infliximab as a maintenance medication in 103 children responsive to Infliximab over 54 weeks. In this study, HtSDS improved significantly from baseline -0.76±1.24 to 54-week -0.49 ± -0.50 (207). In a recent review of growth and its relationship to treatment modalities in 176 children with CD, improvement in height was least likely in those receiving GC therapy, reported improvement in median height z-scores from baseline -0.5(-1.2-0.6) to -0.5(-2.2-0.1) and -0.8(-2.8-1.4) at 6 and 12 months follow-up(312). Crombé et al, demonstrated that the height z-scores of children responding to Infliximab improved from -0.57 ± 1.18 to -0.25 ± 0.99 over the period of follow-up. In contrast, non-responders to Infliximab had no catch-up growth (313). The recently published multicentre IMAgINE 1, randomized, open-label study of Adalimumab for paediatric patients with moderate to severe CD also reported significant improvement in HV z-score from BL to 26weeks and 52weeks (296).

1.9.2 Effect of biologics on growth in paediatric JIA

Biological agents, particularly Etanercept and Infliximab, have also been found to be effective in restoring normal growth in children with JIA (Table 1.9.2). Schmeling et al reported an increase in growth velocity from 3.7 ± 1.2 cm before the beginning of Etanercept therapy to 7.6 ± 1.2 cm in the first year of treatment. The average Ht SDS increased from -2.4 to -1.9 after one year and to -1.1 ± 0.9 after two years indicating catch-up growth. However, 7 out of 18 patients with refractory JIA treated with Etanercept demonstrated growth retardation leading to short stature (314). Tynjala et al observed a significant increase in growth velocity

over a two-year follow up period before and after the initiation of anti-TNF treatment. This mainly reflected the increase in the growth velocity of the 53 patients with previously delayed growth. Mean HtSDS increased by +0.45 during the two years on anti-TNF treatment. There were no differences between the patients receiving Etanercept or Infliximab treatment in the change of growth velocity, height adjusted relative weight, or BMI (315). Vojvodich et al studied the longitudinal growth in response to Etanercept treatment in pre-pubertal and pubertal patients with JIA. This is the only study which reported the effect of TNF-α blocker therapy on longitudinal growth in relation to puberty and separately analyzed the growth response to Etanercept treatment in pre-pubertal and pubertal children (316).

Two other studies have shown that the beneficial effect of biologic therapy on growth may be more likely when the patient is receiving background immunnosuppression therapy using methotraxate (317;318). Giannini et al (318) conducted a 3-year, open label nonrandomised study to evaluate the effects of longterm anti-TNF-α Etanercept treatment with or without methrotrexate on growth in children with selected categories of JIA. This study reported statistically significant increase in mean height percentiles from baseline for Etanercept monotherapy at year 3 only and for etanercept in combination with the methrotrexate group at 1,2 and 3 years (318). In a similar study Billiau et al (317) also reported a significant improvement in growth velocity allowing catch-up growth in in a comibined etanercept and Methotrexate group only (317).

Table 1.9.1: Biologics in chronic inflammatory conditions: growth outcome studies in Crohn's disease

Study	N	Study (months) duration	Study Design	Results
Hyams et al 2012 (296)	192	12	Prospective-randomized to 2 doses	Mean HV z-score increased from -0.98 at baseline to 1.66 at 26 weeks (p=<.001) and 1.80 at 52weeks (p=<.001) in low dose Adalimumab group Mean HV z-score increased from -0.37 at baseline to 1.38 at 26 weeks (p=0.008) and 2.07 at 52weeks (p=0.001) in high dose Adalimumab group
Crombé et al 2011(313)	102	32	Retrospective	Mean Ht z-scores increased from (-0.57± 1.18) to (-0.25 ± 0.99) p=0.04 in IFX responders Mean Ht z-scores decreased from (-0.54± 1.44) to (-0.52 ± 1.27) p=0.52 in IFX non-responders
Sinitsky et al 2010(312)	16	12	Retrospective	Median Ht-z-score baseline -0.5(-1.2-0.6) Median Ht-z-score 6 months follow-up -0.5(-2.2-0.1) Median Ht-z-score 1 year follow-up -0.8(-2.8-1.4)
Pfefferkorn et al 2009(310)	176	12-24	Prospective	Mean HtSDS at diagnosis: 0.49±1.2 (-0.66 to -0.31) Mean HtSDS 1 year follow-up: 0.50±1.2 (-0.67 to -0.32) Mean HtSDS 2 year follow-up: 0.46± 1.1 (0.96 to -0.23)
Diamanti et al 2009(247)	28	10	Retrospective matched control	Case group (14) Mean HtSDS baseline (146.1±0.2) to final (147.8±0.3) Control group (14) Mean HtSDS baseline (147.4±0.2) to final (148.5±0.1)
Thayu et al 2008(207)	103	12	Prospective-randomized to 2 doses	Mean HtSDS from baseline (-0.76± 1.24) to 54-week (-0.49 ± -0.50)
Walters et al 2007(311)	24	12	Retrospective	Mean HtSDS at baseline (-1.15 ±1.2) Tanner I-III: Mean ΔHVSDS +3.94 , mean ΔHtSDS +0.50, P < 0.001 Tanner IV-V: Mean Δ HV SDS +0.22, mean ΔHtSDS 0.02, P = NS
Wever et al 2006(309)	24	3	Retrospective	Mean HV cm/month before 0.32 & after 0.42 P =0.36
Borrelli et al 2004(156)	18	6	Prospective non-randomized	Overall group HtSDS baseline (-0.99 ± 0.62) to 6 months after (-0.74 ± 0.71) p<0.01 Non-retreated group HtSDS baseline (-0.86 ± 0.42) to 6 months after (0.83 ± 0.40) p=NS Retreated group HtSDS baseline (-1.15 ± 0.81)) to 6 months after (-0.62 ± 0.99) p<0.01
Cezard et al 2003(279)	10	12	Prospective non-randomized	Mean HtSDS before -0.5 (-1-1.3) & after +0.5 (0 -1.3) p=0.004

Table 1.9.2: Biologics in chronic inflammatory conditions: growth outcome studies in juvenile idiopathic arthritis

Study	N	Study duration	Study Design	Results
Giannini et al 2010 (318)	594	3 years	Open-label nonrandomized	Mean height percentiles increased from baseline in Etanercept treated patients at year 3 (4.8 percentile points) Mean height percentiles increased from baseline in Etanercept + Methotrexate treated patients at 1,2, and 3 years (2.4, 3.3, and 5.6percentile points)
Billiau et al 2010(317)	24	18 months	Prospective open-label	Median growth velocity increased in Etanercept + Methotrexate Treated group at 12 months compared with baseline and this increased persisted till 18 months
Schmelling et al 2007(314)	7	12 months	Prospective	HV cm/year before (3.7 ± 1.2) to (7.6 +/- 1.2) in the first year of treatment (p= 0.001). Average SDS increased from (-2.4 ± 1.0) to (-1.9 ± 0.9) after I & 2 years (-1.1 ± 0.9) p = 0.05
Tynjala et al 2006(315)	71	24 months	Retrospective	Patients with delayed growth Mean ΔHSDS +0.45 (0.33 to 0.56) p=0.001 Patients with normal growth Mean ΔHSDS +0.05 (0.07 to 0.16) p = 0.39
Vojvodich et al 2003(316)	52	12 months	Retrospective	Mean ΔHSDS in pre-pubertal group (+0.2± 0.1) p= 0.001 Mean ΔHSDS in pubertal group (+0.2± 0.1) p= 0.071

1.10 Effect of biologic therapy on bone health

1.10.1 Effect of biologic therapy on bone in children with IBD

To date, only one study has evaluated the impact of Infliximab therapy on bone biomarkers in children with CD (Table 1.10.1). Thayu et al (207), in REACH study of 103 children with CD observed the changes in the bone biomarkers, which had Infliximab induction therapy of 5mg/kg at 0, 2 and 6 weeks. Changes in the bone biomarkers were observed at week 10 after Infliximab induction therapy. This study also examined the association of these changes in the bone biomarkers with subsequent changes in disease activity and linear growth during the 54-week interval after initiation of Infliximab therapy. This study reported the improvement in markers of bone formation, bone specific alkaline phosphatase (BSAP) and pro-collagen type 1 N propeptide (P1NP) during the 10-week interval; the median increases were 87% and 103% respectively (both $p<.001$). Moreover, increase in BSAP at week-10 was significantly associated with the reductions in PCDAI at week 10 but no association was observed at between changes in PINP and PCDAI. The bone resorption markers CTX-1 and DPD also increased significantly during the induction period (both $p<.001$); however, the magnitude of the changes (18% and 23%) was markedly less pronounced than the changes in markers of bone formation (207).

1.10.2 Effect of biologic therapy on bone in adult IBD

Data regarding the effect of anti-TNF-α on bone mineral density (BMD) and bone metabolism are limited. Studies that report the effect of biologics on bones in patients with IBD are summarized in (Table 1.10.1). In adults, an increase in BALP by 15-18% has been observed after a single Infliximab infusion (206;319-321). Other bone formation markers, such as BALP and osteocalcin, have also been reported to increase after induction therapy. Franchimont et al (319) studied 71 patients treated with Infliximab (5 mg/kg) for refractory CD who were naïve to Infliximab and compared their results with 68 matched healthy controls. The study included 21 fistulizing refractory CD patients and 50 luminal refractory CD patients. Patients were treated with a single infusion of 5mg/kg Infliximab at baseline for luminal refractory disease and with three infusions of 5mg/kg Infliximab at baseline, week 2 and week 6 for the fistulizing refractory disease. Serum bone formation markers such as BALP, OC and pro-P1NP, and the serum caboxyterminal telopeptide (sCTX); a marker of bone resorption were measured at baseline and 8 weeks after completion of Infliximab treatment (8 week for luminal refractory CD and 14 week for fistulizing refractory CD. In this study Crohn's disease activity index score (CDAI) was considered as a confounding factor. In this study, the serum

concentration of the markers of bone formation (BSAP, OC and P1NP) were found to be lower in CD patients before Infliximab therapy as compared to the controls, and were back to normal levels at 8 weeks after Infliximab therapy. Relative improvement in bone formation (defined as increase of at least 30% in the bone formation marker (BALP, OC, P1NP) was found after Infliximab therapy in 30%, 61% and 47% of patients, respectively. Serum concentration of CTX was significantly increased in CD patients at baseline, but was no longer different from controls at 8 weeks after Infliximab therapy. Relative improvement in marker of bone resorption (decrease of at least 30% in sCTX serum levels) was observed in 38% patients after Infliximab therapy Franchimont et al (319). Ryan et al (321), in a prospective trial of 24 patients with active CD who were treated with Infliximab (5mg/kg) for the first time, reported a significant increase in serum BALP and OC in patients with active CD. No significant improvement in marker of bone resorption (serum N-telopeptide cross-linked type I) was found. In this study five time points were assayed, i.e. baseline, 1-2, 4-6, 8-10 and 12-18 weeks after Infliximab infusion. This study, reported a significant increase in markers of bone formation BALP and OC. Levels of both bone formation markers remained significantly increased even at 4 months after Infliximab therapy. No significant change was observed in the serum N-telopeptide cross linked type I levels at 4 months and was found to be lower than at baseline. This study also demonstrated that the effect of Infliximab on bone metabolism was independent of the clinical response in terms of effect on disease activity. Moreover, the trend of increase in bone formation markers and decrease in bone resorption markers was greater in those who were responders as compared to non-responders (321). Abreu et al (206) reported a significant increase in BALP at 4 week compared to the base line after a single Infliximab infusion in a prospective study of 38 patients who had refractory CD. Findings in this study were not compared to age-matched control group and only two confounding factors, i.e. the effect of glucocorticoid use and the clinical response based on CDAI on changes were analyzed in detail. In this study sera were also analyzed for the proinflammatory cytokines (IL-1α, IL-6, and TNF-α), calcium and immunoreactive parathyroid hormone (iPTH) at baseline and at 4weeks after Infliximab therapy. At 4 weeks PTH levels were reduced and serum calcium levels were increased significantly. Serum cytokine levels remained unchanged from baseline to week 4. This study suggested that the effect of Infliximab on bone formation was independent of glucocorticoid use and Infliximab response, as improvement in bone formation markers were observed in both the responders and non-responders as well as in those patients who were receiving glucocorticoid therapy and those who were not on glucocorticoids (206). Miheller et al (322), also studied the effect of Infliximab on bone formation and resorption markers in 27 patients with fistulizing CD and compared the results with 54 patients with inactive CD (controls). Clinical response to the

therapy based on defined as the decrease in the number of draining fistulas by half (responders) and no improvement in the number of draining fistulas by half (non-responders). A significant difference in β-Cross Laps (bCL) a marker for bone resorption on days 0 and 14, and days 0 and 42. OC, levels were found to be increased between day 0 and 42. This study reported the beneficial effect of Infliximab only in those who were Infliximab responders (320). There are currently no published studies looking at the effect of Adalimumab on bone metabolism in IBD patients. However, only one open label prospective study reported the effects of Adalimumab on bone mineral density (BMD) in 50 patients who had active rheumatoid arthritis. In this study the BMD of both the lumbar spine (L1 and L4) and left femoral neck was measured before treatment and one year after by DXA. In this study, BMD of lumbar spine and femoral neck remained unchanged after 1 year of Adalimumab therapy and both disease activity at baseline and disease duration were found to be inversely correlated with lumbar spine and femoral neck BMD. This study concluded that Adalimumab therapy can stop the progression for bone loss (323).

1.10.3 Effect of biologics in bone mineral density in paediatric IBD

To, date only one study has looked at the effects of Infliximab on bone mineral density in children with CD and suggested that bone density improves following treatment with Infliximab (324). This study reported significantly lower BMAD in those patients who had never received Infliximab than those undergoing biological therapy with Infliximab (324) (Table 1.10.1).

1.10.4 Effect of biologics in bone mineral density in adult IBD

To, date few published studies have looked at the effects of biologics on actual bone loss in CD patients by measuring BMD (Table 1.10.1). Pazianas et al (325), conducted a retrospective study on 61 CD patients who had low BMD. In this study a number of confounding factors which could affect study outcome were analyzed including sex, number of postmenopausal women, and number of women on hormone replacement therapy, glucocorticoid use and Infliximab infusions. In this study 23 patients were on Infliximab therapy and 36 patients were on bisphosphonates. After controlling for corticosteroid use, patients with concurrent Infliximab and bisphosphonate treatment had a greater increase in BMD as compared to those who had bisphosphonate alone; corticosteroids inhibited this effect. However, Infliximab alone had no effect on BMD (325;326). Mauro et al (326), conducted a retrospective study of 15 CD patients who had Infliximab therapy for the first time and who underwent DXA before and during Infliximab therapy. These patients were then compared with 30 CD patients who were naïve to Infliximab therapy and who had DXA done

at least one year apart. Age, gender, age at diagnosis, disease duration, final weight, change in weight, disease activity and the use of bisphosphonate and corticosteroids were used as confounding factors in this study. Patients in this study had Infliximab therapy (5mg/kg) at intervals of 4-8 weeks for a mean period of 18 months. Those patients who had Infliximab therapy had a significant increase in lumbar bone area, bone mineral content (BMC) and BMD as compared to the control group. The increase in BMC in patients who had Infliximab therapy was significant when compared with controls who had received glucocorticoid therapy or who had evidence of disease activity (326). Bernstein et al (327), also found that the maintenance treatment with Infliximab (5mg/kg) at 6-8 weeks interval for 1 year in 46 CD patients resulted in the improvement in BMD after 1 year in lumbar spin, at the femoral trochanter and at the femoral neck and this effect was independent of the glucocorticoids, calcium supplementation or changes in C-reactive protein (CRP) (327).

1.10.5 Effect of biologics on GH/IGF-1 axis

The effect of Infliximab on the GH/IGF-I axis has been assessed in a series of 14 adult IBD patients with low levels of IGF-I and IGFBP-3 treated with three induction doses (weeks 0, 2 and 6) plus two additional infusions every eight weeks (328). Peripheral resistance to GH was apparently reversed after the second infusion of Infliximab, as judged by the significant increase in the serum levels of IGF-I andIGFBP-3 as compared to baseline. However, this effect was not sustained since they return to baseline values between the first and second maintenance dose (328). TNF blockade by Infliximab could account for the initial improvement of GH sensitivity as increased TNF activity suppressed the GH/IGF-I axis in the liver. The late impairment of GH sensitivity during maintenance therapy was harder to explain, but one could speculate that during this period some degree of subclinical mucosal inflammation might persist. Another study conducted by Eivindson et al (329) assessed changes in the IGF system in patients with active CD before and during Infliximab treatment and studied 13 patients with therapy refractory CD, treated with Infliximab (5 mg/kg body-weight) at baseline and after 2 weeks. The IGF system and markers of inflammation were examined at baseline, on days 2-5 and after 1, 4, and 8 weeks. Ten healthy age- and gender-matched persons served as controls. This study has shown treatment with Infliximab normalized circulating levels of total IGF-I and IGFBP-3, and partially normalized IGFBP-2, whereas free IGF-I remained suppressed and suggest that the changes in the IGF system may be part of the catabolic state in active CD and may have an association with metabolic bone disease and muscle wasting (329).

Table 1.10.1:Effect of biologics on bone metabolism and BMD in Crohn's disease

Study	N	Study duration	Study Design	Results
		ADULT STUDIES		
Pazianas et al 2006(325)	61 CD	12 months	Retrospective	Patients with concurrent Infliximab and bisphosphonate treatment exhibited a greater increase in BMD compared to those on bisphosphonates alone (+6.7%/year vs. +4.46%/year, P = 0.045). Infliximab alone had no effects on BMD
Mauro et al 2007(326)	15 CD, 30 controls	12 months	Retrospective	Infliximab group had a significant increase in the lumbar bone are (4.15%±6.6%), BMC (12.8%±13.6), and BMD (8.13%±7.7%) (p<0.01), than the control group
Miheller et al 2007(320)	29 CD	6 weeks	Retrospective	OC concentrations increased from 28.93 ± 14.95 ng/mL to 36.33 ± 20.05 ng/mL (p<0.005) at days 1 and 42 sRANKL concentrations increased from 0.0112 ± 0.028 ng/mL to 0.0411 ± 0.123 ng/mL (NS) at days 1 and 42 bCL concentrations decreased from 0.636 ± 0.594 ng/mL to 0.519 ± 0.235 ng/mL (NS) at days 1 and 42 OPG concentrations decreased from 3.739 ± 1.485 ng/mL to 3.419 ± 1.618 ng/mL (p<0.05) at days 1 and 42
Miheller et al 2006(322)	27 CD, 54 controls	6 weeks	Prospective	Significant differences in bCL concentrations on days 0 and 14 p<0.01 and days 0 and 42 p<0.05. OC levels increased significantly between day 0 and 42 p<0.05
Abreu et al 2005 (206)	38 CD	4 weeks	Prospective	Median BAP increased from 6.6(2.5,27.0) to 7.2 (2.1,26.0) p=0.010 and Median NTX remained unchanged from 15.5(7.9,36.0) to 15.5(11.0,36.0) p= 0.801 at 4 weeks following Infliximab therapy
Ryan et al 2004(321)	24 CD	18 weeks	Prospective	Increase in bALP p=0.022 and OC p=0.008 and remained significantly higher at 4 months post Infliximab therapy. No significant change in sNTx p=0.5 at 4 months post Infliximab therapy

Franchimont et al 2004(319)	71CD, 68 controls	8 weeks	Open-label nonrandomized	Median BALP increased from 7.3(5.8, 9.1) to 15(6.2, 12) p=0.0008, OCS 15(8.7, 18.6) to 17.2(12.8, 28.3) p=0.001, P1NP 30.3(20.2, 40.6) p= 0.003, at 8 week following Infliximab therapy. Median CTx decreased from 256.6(157,427.2) to 224.3(103.1, 374.1) p=0.04 at 8 week following Infliximab
Bernstein et al 2005(327)	46 CD	12 months	Prospective	Mean BMD increased at Lumbar spine from 1.117 ± 0.022 to 1.145 ± 0.023 (p<0.001), femoral neck 0.76 ± 0.018 to 0.915 ± 0.017 (p=0.002), femoral trochanter 0.021 ± 0.022 to 0.767 ± 0.020 (p=0.02) from baseline to 1 year
			PAEDIATRIC STUDIES	
Thayu et al 2008(207)	103CD	12 months	Randomised open-label	Median BSAP increased from 44.2(14.1-161.7) to 86.2(12.2, 285.6) p<.001 from baseline to week 10 Median P1NP increased from 253.8(29.2,1026.5) to 615.1(25.3,1706.2) p<.001 from baseline to week 10 Median CTX-1 increased from 768.0(229.0.2203.9) to 925.2(134.9,345.6) p<.001 from baseline to week 10 Median DPD increased from 12.8(1.8,38.2) to 13.7(2) p<.001 from baseline to week 10
Paganelli et al 2007(324)	35 CD, 25 controls	6 month	prospective	Infliximab treated group had increase in mean BMAD z-score (-1±0.8), than those never treated with Infliximab (-1.8 ±0.8) P<0.05

1.11 Overall conclusions and proposed areas of research

The extensive literature review presented above has reviewed the effects of biologic therapy on growth and skeletal development in chronic inflammatory conditions particularly IBD. The main aim of this review is to summarize and evaluate effects of inflammation and biologic therapy on growth and skeletal development in children with chronic inflammatory conditions and to explore the areas of interest for further research. The role of conventional therapies on growth is not clear; the existing evidence from paediatric IBD studies is limited. It is unclear whether recent therapeutic advances have had a beneficial impact on the growth of children with CD. Despite advances in therapy, short stature and slow growth continue to be encountered in children with CD. There is a need for simple definitions of growth that can identify poor growth in children with chronic disease and this is explored in Chapter 2.

As was described in Section 1.9, infliximab therapy has a positive impact on growth in paediatric CD. However, factors that may improve growth e.g. changes in dose of corticosteroid, background immuno-suppressants, and surgery have not been accounted for. Not all studies have reported change in growth rates in relation to changes in puberty. This is investigated in Chapter 3.

The published clinical studies to date of Adalimumab use in children are relatively limited with no studies yet conducted to examine the effects of Adalimumab on growth in children with CD. This is explored in Chapter 4.

As was described in Section 1.10, biologic therapy has a positive impact on bone health in paediatric CD. To date, few published studies have examined the effect of anti-TNF-α therapy on bone density and bone metabolism and most of the data originate from studies performed in adult patients with CD. To date only one study has reported the effect of biologic therapy on bone formation, body composition and muscle mass in children with CD. The effect of biologic therapy on insulin-like growth factor (IGF) system in IBD has rarely been investigated. There are no published data regarding effect of biologic therapy on IGF-1 system in paediatric patients with CD. The impact of biologic therapy on volumetric bone mineral density (vBMD), bone structure, and muscle mass measured by using pQCT is not reported so far. This is investigated in chapter 5.

The hypothesis of this study was that the biologic therapy improves linear growth, puberty, bone health, body composition and muscle function in children with CD and this is associated with changes in the IGF-1 axis and markers of bone metabolism.

CHAPTER 2

Growth in children receiving contemporary disease specific therapy in children with Crohn's disease

2.1 Summary

Introduction: It is unclear whether recent therapeutic advances have had a beneficial impact on the growth of children with Crohn's Disease (CD).

Aim: To assess the frequency of short stature and poor growth and their relationship to disease course and therapy in children with CD.

Subjects & methods: The anthropometric and treatment details of 116 children (68 male) with a mean (range) age at diagnosis of 10.8yrs(4.9, 15.5) and a mean age at maximum follow-up(MF) of 15.4yr(9.4,19.3) were studied retrospectively at diagnosis(T0), 1-yr(T1), 2-yr(T2) and 3-yr(T3) after diagnosis and at MF.

Results: At T0, mean height standard deviation score (HtSDS) was -0.5(-3.3, 2.6) compared to a mid-parental HtSDS of 0.2 (-2.0, 01.4) (p=0.002). At T1, T2, T3 and MF, mean Ht SDS was -0.6(-4.8,7.8), -0.6(-2.9,2.2), -0.7(-3.6, 2.5) and -0.5(-3.5, 2.9) respectively. Mean Ht Velocity (HV) SDS at T1, T2, T3 and MF was -1.4(-7.4, 7.4),-0.6(-7.5, 6.1),-0.1(-6.6, 7.6), and 0.6(-4.8,7.8) respectively,($p<0.05$). In final models, HtSDS was associated negatively with the use of prednisolone (p=0.0001), Azathioprine (p=0.0001), Methotrexate (p=0.0001) and WtSDS (p=0.0001). HVSDS was associated positively with age (p=0.0001) and WtSDS (p=0.01). ΔHtSDS was associated negatively with the use of prednisolone ($p<0.02$).

Conclusion: Although contemporary therapy of CD is associated with an improvement in rate of growth over the first few years of therapy, a substantial proportion of children continue to remain short. This study highlights the need for consistency in describing growth in children with chronic diseases.

2.2 Introduction

Poor growth and short stature are common complications of CD and may not only be present at diagnosis but also during treatment (330). Short stature has also been described in those with CD who have reached final height (234;331). The prevalence of short stature and poor growth at diagnosis may be related to the duration and severity of symptoms before diagnosis as well as whether the child has pubertal delay and was reported to be as high as 88% in the 1980's (332). Studies that have reported growth problems during therapy are those where the use of glucocorticoids (GC) has generally been more prevalent (235;333;334). Although the use of GC therapy has reduced and more attention has been paid to the nutrition of affected children, there is a persisting concern that poor growth may still exist (310;335). Whilst GC therapy plays a direct role in affecting longitudinal growth and skeletal development, there is ample accumulating evidence that proinflammatory cytokines by themselves exert an important detrimental effect on growth. This effect may be mediated directly at the level of the growth plate or through the systemic GH/IGF-1 axis (219) and in vivo and in vitro studies suggest that these effects may be abrogated by administration of GH or IGF-1 (336;337) . Promoting growth through manipulation of this axis may be a therapeutic option which requires further exploration. However, designing such studies requires a clear assessment of the prevalence of growth problems in children receiving contemporary therapy. The aim of the present study was to assess the frequency of short stature and poor growth and their relationship to disease course and therapy in children with CD.

2.3 Methods & subjects

Clinical records of all children with a confirmed diagnosis of CD, who were between 2yrs and 18yrs at the Royal Hospital for Sick Children, Glasgow were examined retrospectively. Data were collected at diagnosis (T0), 1-yr (T1), 2-yr (T2) and 3-yr (T3) after diagnosis and at (maximum follow-up) MF. Of the 165 children with CD, case notes were available in 142 and of these children, 26 were excluded as 5 had growth data missing at diagnosis, 2 were less than 2yrs old at diagnosis and 19 were recently diagnosed and had less than one year growth data (Figure 2.3.1). Mean (range) age at diagnosis in the 116 children who were studied was 10.8yrs (4.9,15.5) and mean age at maximum follow-up (MF) was 15.4yr (9.4,19.3). Crohn's disease location and behaviour at diagnosis were classified according to the Montreal classification (5). Data on pubertal status at all time points were available in 33 children. On the basis of pubertal stage, the children were categorized as, pre-pubertal (n=14) and pubertal (n=19).

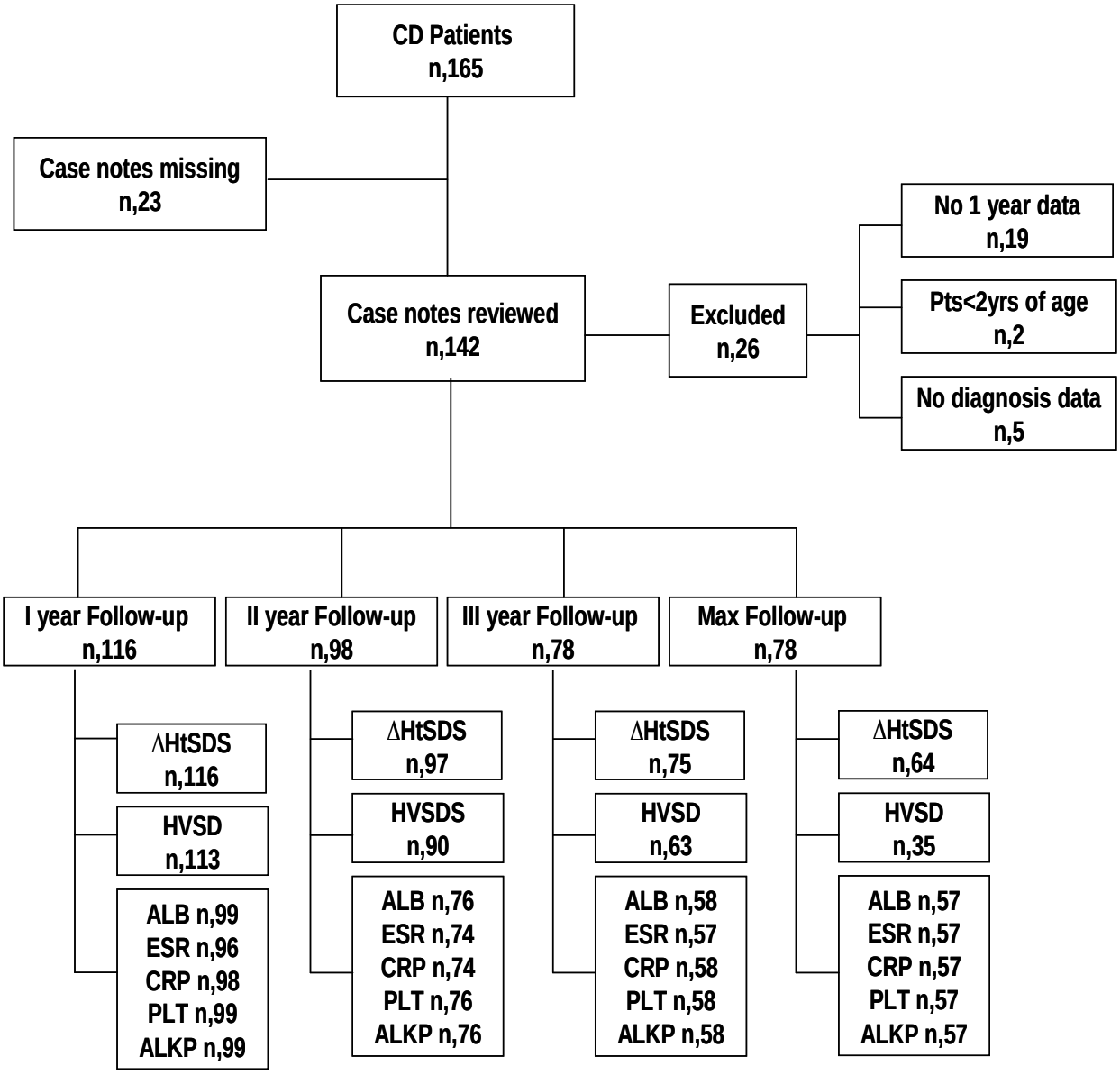


Figure 2.3.1: Flow diagram of study recruitment

Cumulative data on erythrocyte sedimentation rate (ESR), platelets, C-reactive protein (CRP), serum albumin (Alb), and total Alkaline Phosphatase (ALP), were collected in the year prior to each time point. Details of therapeutic interventions including enteral therapy, medications and resectional surgery were collated from the case records within prior each year of treatment. Those who had growth-promoting endocrine therapy (T2-4, T3-5, MF-5) were excluded from further study in the year after starting this therapy. Growth was assessed in relation to systemic markers and therapeutic interventions. Height was measured with a Harpenden stadiometer and used to calculate annual height velocity (HV) and body mass index (BMI). Tanner stage was estimated by either clinical examination or self-estimation (338;339). Height (Ht), Weight (Wt) and body mass index (BMI) were converted into SDS for chronological age using 1990 UK standards (340;341). Height velocity (HV cms/yr) was calculated using a two-point technique from the difference between the two height measurements divided by the decimalized time interval. For boys under 16.6 yrs and girls under 15 years, HV was converted to height velocity standard deviation scores (HVSDS) using the 1966 standards (342;343).

2.4 Statistical analysis

All data were analysed using Minitab software version 16.1 and are described as mean, and range. Univariable analysis was performed using general linear models (GLM) to account for repeated measures for HtSDS, WtSDS, BMISDS and HVSDS. Random effects were used to incorporate the repeated measures structure of the data into GLMs. Factors significant at the $p<0.2$ level in the univariable analysis were included in the initial multivariable models. Multivariable linear regression models were tested to evaluate the influence of factors on growth. Final multivariable models were derived using stepwise forward elimination procedure. At each step of the forward stepwise regression analysis, the eligible independent variable with the highest statistical value ($p<0.05$) were included in the model. Clinical/therapeutic/laboratory data for the groups with the worst and best growth outcomes were compared using Fishers' exact test.

2.5 Results

2.5.1 General characteristics

Clinical and therapeutic details at all time-points are outlined in (Table 2.3.1). Disease location was most commonly panenteric (Ileo-colonic and upper GI tract, L3 + L4) in 49(42%) and behaviour type B1 98(85%) with 23 (20%) having perianal disease and 4(3%) having genital involvement. The use of GC remained constant and was used in 28(24%) of cases at

T0 and then in 31(27%), 20(21%), 13(17%) and 22(21%) of children at T1, T2, T3 and MF respectively. Exclusive enteral nutrition at T0 was recorded in 54(63%) of cases; in subsequent years of study it ranged between 11% to 16% in any one year. Throughout the study period, a total of 23 children had resectional surgery.

2.5.2 Growth

Mean HtSDS at diagnosis -0.5(-3.3, 2.6) was lower than the mean mid-parental height SDS of 0.2(-2.0, 1.4) (p=0.002). After a small but significant fall in Ht SDS between T0 and T1 there was no change in Ht SDS subsequently (Figure 2.3.2a).The percentage of children with height SDS (HtSDS) <-2 was 9.4%, 10.3%, 12.2% and 11.5% at T0, T1, T2 and T3, respectively, and was 8.9% at MF. The mean ∆HtSDS did not change from -0.0(-0.9,2.0) to -0.0(-0.8,1.0), (p=0.45) between T1 and T2, -0.0(-0.8,1.0) to 0.0(-0.9,0.7), (p=0.07) between T2 and T3 and from 0.0(-0.9,0.7) to 0.0(-0.5,0.7), (p=0.72) between T3 and MF (Figure 2.3.2b). However, the mean HVSDS increased from -1.4(-7.4,7.4) to -0.6(-7.5,6.1), (p=0.005) between T1 and T2, and from -0.6(-7.5,6.1) to -0.1(-6.6,7.6), (p=0.01) between T2 and T3 and from -0.1(-6.6,7.6), to 0.6(-4.8, 7.8), (p=0.03) between T3 and MF (Figure 2.3.2c).The percentage of children with HVSDS between -1 and -2 changed from 49% at T1 to 9%, 22%, and 20% at T2, T3 and MF. Children with a HVSDS <-2 changed from 17% at T1 to 39%, 24% and 20% at T2,T3 and MF, respectively. Weight and BMI SDS showed an improvement over the first year of therapy and then remained within the normal range (Table 2.3.1).

2.5.3 Puberty and growth

In 33 children, data on pubertal status were available from diagnosis to 3-year follow-up and of these children, 14 remained pre-pubertal and 19 were pubertal. Mean age of children in the pre-pubertal group and pubertal group at T1 was 8.6yrs (5.9, 11.4) and 12.2yrs (9.4, 14.7) respectively. Those who remained pre-pubertal had a mean HVSDS of -1.1(-6.8,1.8), -1.6(-4.4,2.7) and -1.8(-6.5,5.0) at T1, T2 and T3, respectively, and did not improve between T1 and T3 (p=0.61). Mean ΔHtSDS of this group was 0.2 (-0.2,0.7), -0.1(-0.7,0.5) and -0.0(-0.8,0.3) at T1, T2 and T3 respectively. Mean ΔHtSDS of this group reduced between T1 and T2 (p=0.04). In the pubertal group, mean HVSDS was -1.8(-4.0, 3.3), -1.0(-6.5,5.0) and 1.2(-6.7,6.9) at T1, T2 and T3, respectively. In this group HVSDS increased between T1 and T3 (p=0.007). Mean ΔHtSDS of this group was -0.2(-0.6,0.5), -0.1(-0.6,0.6) and 0.2(-0.3,0.7) at T1, T2 and T3 respectively. Mean ΔHtSDS increased between T1 and T3 (p=0.001) and T1 and T2 (p=0.01) (Figure 2.3.3 a,b,c,d).

Table 2.3.1: Demographic and clinical details of children with CD at1-yr (T1), 2-yr (T2) and 3-yr (T3) after diagnosis

	T0	T1	T2	T3	MF
Gender (M/F)	68/48				
Age (mean) years	10.8(4.9,15.5)	11.9(5.9,16.5)	12.9(6.8,17.7)	13.5(7.9,18.7)	16.1(9.4,19.3)
Follow-up duration (mean) years					4.6(1.4,8.6)
HtSDS	-0.5(-3.3,2.6.)	-0.6(-4.8,7.8)	-0.6(-2.9,2.2)	-0.7(-3.6,2.5)	-0.5(-3.5,2.9)
BMISDS	-0.6(-3.8,3.7)	-0.1(-2.2,2.7)	0.1(-2.8,2.6)	-0.0(-2.1,4.5)	0.0(-2.5,2.6)
WtSDS	-0.7(-3.4,2.6)	-0.4(-3.6,2.5)	-0.3(-2.2,2.5)	-0.5(-3.1,3.3)	-0.3(-2.5,3.2)
ΔHtSDS		-0.0(-0.9,2.0)	0.0(-0.8,1.0)	0.0(-0.9,0.7)	0.0(-0.5,0.7)
HVSDS		-1.4(-7.4,7.4)	-0.6(-7.5,6.1)	0.1(-6.6,7.6)	0.6(-4.8,7.8)
Concomitant Medication (n)%					
-Aminosalicylic acid	33(28)	52(45)	46(47)	42(54)	33(42)
-Exclusive enteral nutrition	63(54)	15(13)	16(16)	11(14)	9(12)
-Azathioprine	14(12)	50(43)	36(37)	30(28)	23(29)
-Prednisolone	28(24)	31(27)	20(21)	13(17)	22(21)
-Methotrexate	2(2)	22(19)	39(40)	32(41)	43(55)
-Biologic	1(1)	16(14)	11(11)	16(21)	16(21)
-Resection surgery	4(3)	3(2)	4(5.1)	3(4)	9(10)
Biomarkers mean(range)					
Serum albumin (g/l)		36.1(18-55)	37.8(26-45)	36.9(20-49)	35.3(12-44)
≤30 (g/l) %		33.3	27.6	32.7	31.5
ESR (mm/hr)		23.5(1.0,68)	20.1(3.0,56)	24.8(2.0,59)	18.5(1.0,52)
>20 mm/hr %		48.9	40.5	54.3	38.5
CRP (mg/l)		15.8(7,93)	15.2(7,82)	18.1(7,154)	13.9(7-84)
>7 (mg/l)		46.9	37.8	50.0	42.1
Platelets (10^9/L)		411.8(207,877)	384.5(193,717)	383(180,797)	325.4(310.657)

Figure 2.3.2: Growth in children with CD at diagnosis (T0), year 1(T1), year 2(T2), year 3(T3) and at Maximum follow-up (MF), expressed as mean (range)

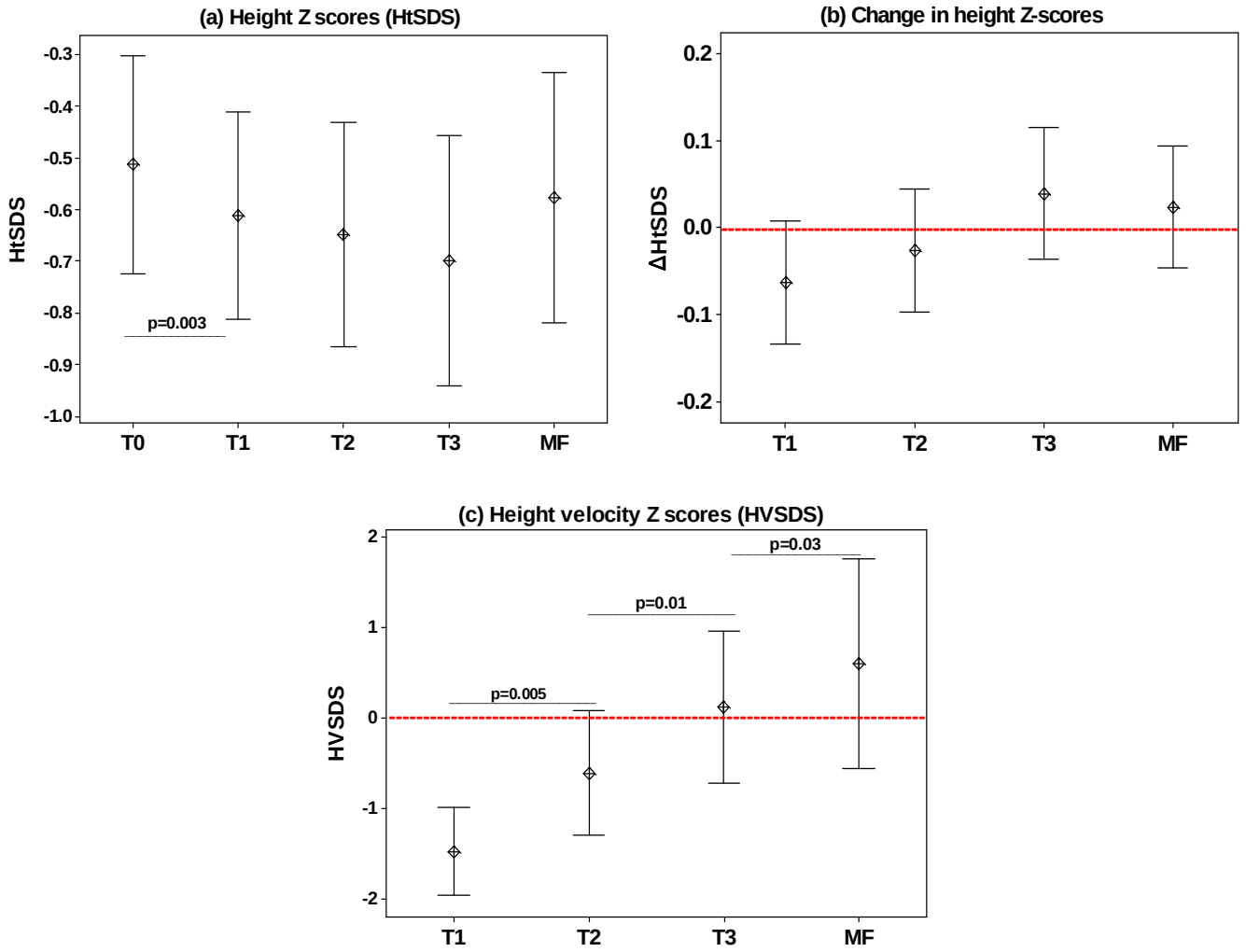
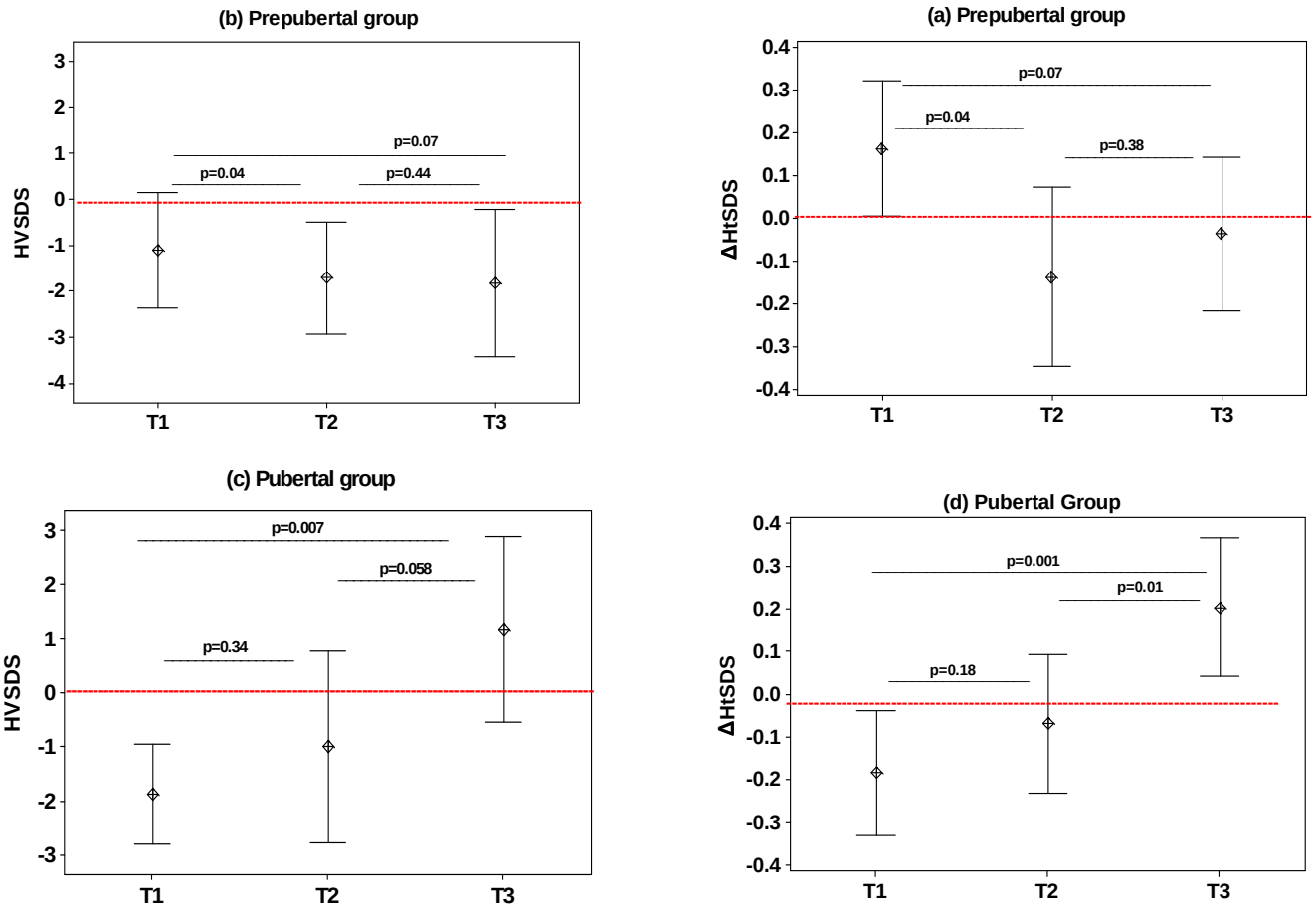


Figure 2.3.3: Growth in children with CD at year 1(T1), year 2(T2), and year 3(T3) after diagnosis expressed as mean (range)



2.5.4 Surgery and growth

Throughout the study period, 23(M,9) children had resectional surgery. The most common surgical procedure was hemicolectomy in 17(73.9%); 3 children had colectomy, 3 children had stricturoplasty and 1 child had ileocolonic resection. The impact of surgery on growth was assessed in children with CD 12 months(T-12) prior to surgery, at the time of surgery(T+0) and 12 months(T+12) post surgery (Table 2.3.2). Of 23, 14(M,6) had sufficient growth data available at all time points. The mean age at diagnosis and at the time of surgery was 10.6yrs (5.0,13) and 14.6yrs(12.0,17.6) respectively. The mean change in height SDS (ΔHtSDS) in the whole group increased from -0.1 (-0.6,0.8) at T0 to 0.2(-0.4,0.7) at T+12, (p=0.01) (Figure 2.3.4 a & b).

2.5.5 Association of growth to therapy and disease

Clinical/therapeutic/laboratory data for the groups with the worst and best growth outcomes were compared in terms of ΔHtSDS at T1 and T3 (Table 2.3.3). At T1 the use of Methotrexate was significantly associated with better growth, (p=0.01), and at T3 the use of prednisolone, (p=0.01) and raised ESR, (p=0.02) were significantly associated with worse growth.

Multivariable regression models were used to determine the association of disease and therapy on growth (HtSDS, WtSDS, BMISDS, HVSDS and ΔHtSDS) at T1, T2, T3 and MF by fitting linear mixed effect models. In final models, HtSDS was associated negatively with the use of prednisolone (p=0.0001), Azathioprine (p=0.0001), Methotrexate (p=0.0001) and WtSDS (p=0.0001). HVSDS was associated positively with age (p=0.0001) and WtSDS (p=0.01). ΔHtSDS was associated negatively with the use of prednisolone (p<0.02). BMISDS was associated positively with prednisolone (p=0.0007), and serum albumin (p=0.0001) (Table 2.3.4).

Table 2.3.2: Demographic details of children with CD who had surgery

	T-12 N,14	T+0 N,14	T+12 N,14	p-value T-12 VS T+0	p-value T+0 VS T+12	p-value T-12 VS T+12
Age mean(range)	12.5 (9.9-15.7)	13.5 (11.1-16.7)	14.6 (12.1-17.6)			
Concomitant Medication						
-Enteral nutrition		9	4			
-Aminosalicylic acid		8	5			
-Azathioprine		4	6			
-Methotrexate		7	5			
-Prednisolone		3	3			
-Biologics		2	-			
Growth failure and Pubertal delay		5				
Anthropometry Mean(range)						
Height SDS	-0.8 (-3.4, 1.4)	-0.9 (-2.8,-0.5)	-0.6 (-2.6,0.1)	0.43	**0.004**	0.37
BMI SDS	-0.1 (-1.8, 1.9)	0.1 (-2.4,2.2)	0.4 (-1.7,2.3)			
Height Velocity (cm/yr)		4.2 (0.4,9.1)	5.8 (0.4,11.7)		0.13	
∆HtSDS		-0.1 (-0.6,0.8)	0.2 (-0.5,0.7)		**0.01**	
Pubertal status (n,11)						
-I	8	5	2			
-II	1	4	5			
-III	-	-	2			
-IV	-	-	-			
-V	1	1	1			

Figure 2.3.4: Growth in children with CD during the 12 months prior to surgery (T0) and in the 12 months following surgery (T+12)

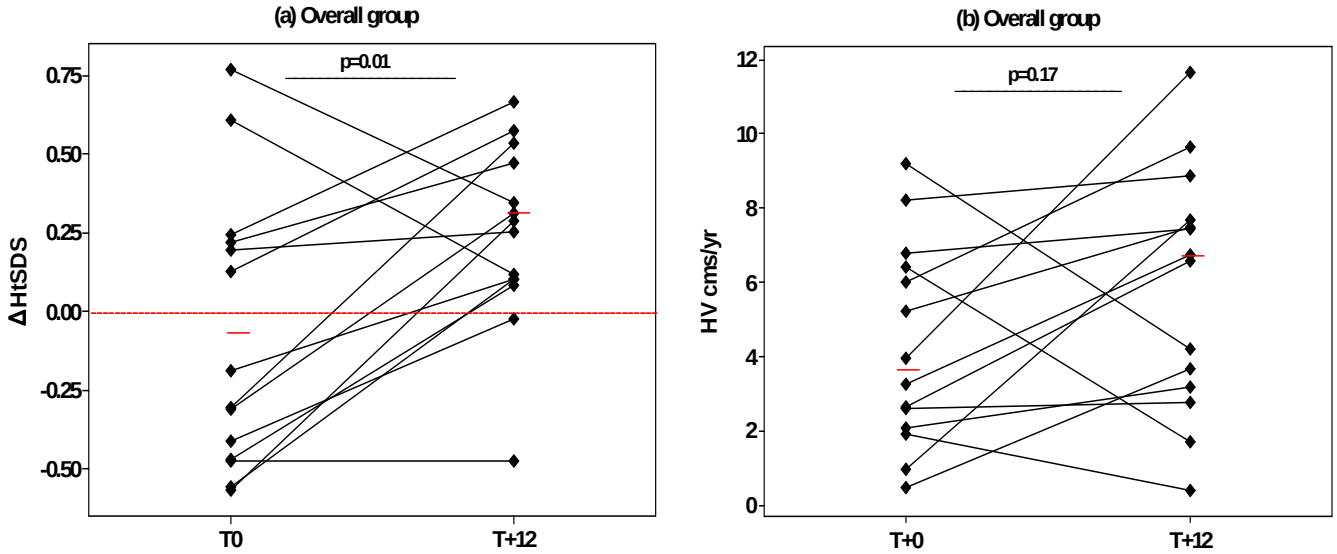


Table 2.3.3: Clinical/therapeutic/laboratory data for the groups with ΔHtSDS ≥0.50 (good growth) and ≤-0.50 (worst growth) at T1(Year-1), T3(year-3)

	T1			T3		
Growth	**ΔHtSDS ≥0.50**	**ΔHtSDS ≤-0.50**	**p-value**	**ΔHtSDS ≥0.50**	**ΔHtSDS ≤-0.50**	**p-value**
N,	8	12		6	5	
Sex M/F	4/4	7/5		5/1	2/3	
Age (median)	11.7	13.9		14.7	13.5	
ΔHtSDS (median(range)	0.6(0.5,2.0)	-0.6(-0.8,-0.4)		0.7(0.5,0.7)	-0.6(-0.8,-0.5)	
-Aminosalicylic acid						
Yes	6	6	0.20	2	2	0.45
No	2	6		4	3	
-Exclusive EN						
Yes	1	4	0.25	2	1	0.45
No	7	8		4	4	
-Azathioprine						
Yes	0	5	**0.05**	3	3	0.43
No	8	7		3	2	
-Prednisolone						
Yes	0	4	0.10	0	4	**0.01**
No	8	8		6	1	
-Methotrexate						
Yes	6	2	**0.01**	2	2	0.45
No	2	10		4	3	
-Biologic						
Yes	1	1	0.50	1	2	0.36
No	7	11		5	3	
Serum albumin (g/l)						
≥35 (g/l)	6	7	0.28	6	2	0.06
≤35 (g/l)	2	5		0	3	
ESR (mm/hr)						
<20 mm/hr	4	6	0.35	6	1	**0.02**
>20 mm/hr	4	6		0	4	
CRP (mg/l)						
<7 (mg/l)	3	6	0.30	5	1	0.06
>7 (mg/l)	5	6		1	4	

Table 2.3.4:Multivariable analysis (General Linear Model), SDS (standard deviation scores), HV (height velocity), ∆Ht (change in height)

Parameter	t	HtSDS p-value	t	WtSDS p-value	t	BMISDS p-value	t	HVSDS p-value	t	∆HtSDS p-value
Age	-	-	-	-	-	-	6.25	**0.0001**	-	-
WtSDS	7.8	**0.0001**	-	-	-	-	2.39	**0.01**	-	-
Azathioprine	-3.62	**0.0001**	-	-	-	-	-	-	-	-
Methotrexate	-3.66	**0.0001**	-	-	-	-	-	-	-	-
Prednisolone	-3.78	**0.0001**	-	-	2.69	**0.0007**	-	-	-2.34	**0.02**
Serum albumin (g/l)	-	-	4.8	**0.0001**	3.79	**0.0001**	-	-	-	-

2.6 Discussion

Although the majority of children with CD were not particularly short and there was an improvement in growth over the first year after diagnosis, this study provides clear evidence that despite advances in therapy, short stature and slow growth continue to be encountered in a sub-group of children with CD. The mean HtSDS of our population as well as the percentage of children with a HtSDS of less than -2 were similar to that reported in other contemporary studies (310;335). Our observation that HtSDS did not show an improvement despite a significant improvement in HVSDS is also similar to the study by Pfefferkorn et al (310) and may suggest that the reduction in growth deceleration, as reflected by an improving HV is not sufficient to improve overall height but simply prevents any further deterioration in height. We observed significant improvement in HVSDS of children who were pubertal as compared to those who were pre-pubertal, suggesting that the improvement in growth may in part be due to progression through puberty (310). However, the reliability of calculating HVSDS for chronological age in older children with delayed puberty is somewhat questionable as they may have a higher calculated HVSDS based on age-matched normal data.

Several inter-related factors may influence growth in children with CD, including poor nutrition, long-term use of GC and uncontrolled disease. In our cohort, early use of enteral nutrition was greater and the use of GC in our population was lower than that reported recently by the North American group (310). However, despite these differences, the prevalence of growth problems was similar. As described previously, we observed an improvement in ΔHtSDS in the children who had surgery during the study period (344). Treatment of CD continues to change over time and it is possible that the children at T3 and maximum follow-up represent those who had greater exposure to drugs such as glucocorticoids. This may introduce a degree of selection bias in the study. Pubertal status was assessed in a small subgroup of patients and given that it was not performed routinely in the clinic, it is possible that it may have been performed in those where there was a concern about growth and puberty, thus introducing further selection bias. A routine surveillance programme of growth and puberty in IBD clinics would eliminate such selection bias.

In our study, HVSDS was particularly poor in the first year after diagnosis when children were more likely to be exposed to GC therapy. We examined the relationship of a number of factors including therapeutic modalities to two commonly used anthropometric markers of growth - HVSDS and ΔHtSDS. We did not find a consistent association between individual markers of disease activity and growth and given that an association between individual markers of disease and disease activity may not exist (345), our finding is not unexpected. By examining temporal associations, we could also compare the association of growth with

markers of disease activity and therapeutic modalities that would play a dominant role in induction of remission of disease and those which play a role in maintenance of remission. The negative association between the use of prednisolone and markers of growth was only evident on multivariable analysis with the ΔHtSDS and not HVSDS. The negative association between other steroid sparing drugs and growth may be explained by the fact that these children would have been the ones who had more severe disease. However, interestingly, during the first year of therapy, the use of Methotrexate was associated with better growth. Our temporal observations suggest that the relationship between these factors and growth needs to be carefully interpreted. It also suggests that there may be specific windows which could be targeted for growth promoting endocrine-therapy but this need to be explored through prospective studies.

There are a number of methods of defining change in growth and in the context of growth promoting therapy, there is little consensus. However, change in HtSDS (346;347) and HVSDS (348) remain the most commonly used parameters. These parameters of growth have been studied most widely for assessing response to growth hormone therapy in children with GH deficiency where an adequate first year response in gain in Ht SDS in pre-pubertal children may range between 0.4 to 0.9 SDS (349).The marked age dependency of HVSDS particularly around the peripubertal years makes HVSDS a less reliable marker of growth especially when assessing growth in children with pubertal delay. Furthermore, the normative data for calculating HVSDS are based on older reference data than HtSDS data. We would, therefore, recommend that change in HtSDS may be a more valid method of assessing and reporting longitudinal growth in children with chronic disease, particularly when there is a high prevalence of children of a peripubertal age. This will also allow comparison with studies that report results of endocrine growth promoting therapy.

In summary, in our population of children with CD, contemporary therapy was associated with a reduction in growth deceleration but this was not associated with sufficient catch up growth for an improvement in overall height SDS. A substantial proportion of children continues to show poor growth irrespective of the therapeutic regimen for the disease and may benefit from novel therapeutic strategies aimed at improving growth.

CHAPTER 3

Improvement in growth of children with Crohn's disease following anti-TNF-α therapy with Infliximab

3.1 Summary

Introduction: Treatment with anti-TNF-α antagonists therapy such as Infliximab may improve growth in children with Crohn's disease (CD) but the extent of improvement in growth and its relationship to pubertal progress and glucocorticoid therapy are unclear.

Aim: A retrospective study of growth, puberty and disease activity over the 6 months prior (T-6) to starting Infliximab, at baseline (T0) and for the following 6 months (T+6) and 12 months (T+12) in children with CD.

Subjects & methods: The growth and treatment details of 28 children (M: 17) who were started on Infliximab at a median (10th, 90th) age of 13.1yr (10.0, 15.7) were reviewed. Data on disease markers (CRP, ESR, and Albumin), total alkaline phosphatase (ALP) and a physician global assessment were also collected. Results are expressed as median (10th, 90th).

Results: Out of 28 cases, 21 (75%) demonstrated a clinical response to Infliximab treatment. Overall, height velocity (HV) increased from 3.6cm/yr (0.4, 7.8) at T0 to 5.5cm/yr (2.1, 9.2) at T+6 ($p=0.003$). In Infliximab responders, HV increased from 2cm/yr (0.3, 7.1) to 6.4cm/yr (2.3, 9.1) ($p=0.004$) and in the non-responders, HV remained static at 4.3cm/yr (2.5, 8.6) at T0 and 3.0cm/yr (2.0, 11.3) ($p=0.701$) at T+6. HV also increased in the subgroup of 13 children who had remained pre-pubertal from 4.5cm/yr (0.4, 8) to 5.5cm/yr (3.3, 8.4) ($p=0.05$). In the subgroup of 11 children who had a reduction (n,2) or cessation in GC (n,9), HV increased from 1.8cm/yr (0.3,8.3) at T0 to 5.6cm/yr (2.2,9.2) at T+6 ($p=0.14$), whereas those children who did not receive GC over the 12 months had an increase from 3.7cm/yr (0.6,6.5) to 6.4cm/yr (2.9,9.0) ($p<0.05$).HV at T0 and T+6 showed a significant association with the average ALP over the prior 6 months(r,0.39, $p<0.05$). HV did not show any association with individual markers of disease activity.

Conclusion: Clinical response to Infliximab therapy is associated with an improvement in linear growth over the short term in children with CD. This increase in height may not be simply due to progress in pubertal status or reduction in glucocorticoid dose.

3.2 Introduction

Abnormalities of growth, puberty and skeletal health are commonly encountered in children with IBD, especially, CD (330). Poor growth may persist in a substantial proportion of children despite optimal management of disease by contemporary regimens. Poor nutrition, abnormalities of sex steroid and the growth hormone (GH)/insulin like growth factor-1 (IGF-1) axis, and chronic use of glucocorticoid (GC) are important contributory factors; interdependent with these factors are the modulatory role of disease severity mediated via pro-inflammatory cytokines such as tumour necrosis factor (TNF α), interleukin (IL) 1β and IL6 (219). Whilst GC therapy plays a direct role in affecting longitudinal growth and skeletal development, there is ample accumulating evidence that proinflammatory cytokines by themselves exert an important effect on growth. This effect may be mediated directly at the level of the growth plate or through the systemic GH/IGF-1 axis (219) . Studies by our group, as well as others, have shown that pro-inflammatory cytokines play a vital role in inhibiting growth plate chondrogenesis and promoting bone resorption (187;233).

Biological therapy such as Infliximab, a chimeric monoclonal antibody against TNF-α has been shown to be effective in childhood onset CD for both induction and maintenance of disease remission (273;279). Although preliminary studies suggest that children starting Infliximab show an improvement in growth (156;311) over a third may continue to demonstrate poor growth (247;309;350) and the factors that are associated with this growth response are unclear. It is also unclear whether any demonstrable improvement is related to a reduction in GC dose or due to a progression through puberty. In children, pubertal progress and growth are intricately linked and abnormalities of growth in childhood CD may be manifested as poor growth before puberty, during puberty and, sometimes as a reduction in final adult height (234;330). Current reports of Infliximab therapy suggest that growth is most likely to improve in those who are in early stages of puberty and this observation may be a function of pubertal progress akin to similar results from studies of resectional surgery (351). A study of children who remain pre-pubertal throughout the treatment period or who did not have GC therapy would improve our understanding of the effects of Infliximab on growth.

The aim of this study was to assess growth, puberty, markers of disease and concomitant therapy over the six months prior to starting Infliximab and for the six months and 12 months following treatment. By paying particular consideration to puberty and GC usage, we attempted to tease out the respective importance of these factors, as well as determine the extent of the growth promoting effect of Infliximab that was independent of these factors.

3.2.1 Subjects and methods

Clinical records of all children with IBD who were started on Infliximab therapy between 2003 and 2008 at the Royal Hospital for Sick Children were examined retrospectively. Out of a total of 42 children, there were 38 children (male, 24) who were diagnosed with CD as defined by standard criteria (352;353). Sufficient data on growth were available for the 6 months before starting Infliximab (T-6), at starting Infliximab (T0) and 6 months after starting Infliximab (T+6) in 34 children. Out of these 34 children, 6 had completed their growth and reached final height during the period of study so these children were excluded from further analysis (Figure 3.1.1). The median age of the remaining 28 (M, 17) children at diagnosis and start of Infliximab was 10.8yr (6.6, 13.4) and 13.1yr (9.9, 15.7) respectively. Disease location was small and large bowel in 13 cases, large bowel only in 11 cases, small bowel only in 3 cases and perianal only in 1 case. In 20 of the 28 children, data on pubertal status were available at pre and post 6 months. Pubertal status was measured by a validated self-assessment form. Thirteen children remained pre-pubertal throughout the study period, whereas those who progressed in puberty were considered as pubertal. In 25 children growth data were available over the 12 month period following Infliximab therapy. Data on erythrocyte sedimentation rate (ESR), platelets, C-reactive protein (CRP), serum albumin (Alb), total alkaline phosphatase (ALP), alanine transaminase (ALT) were assessed as systemic markers of disease and growth.

Height was measured with a Harpenden stadiometer and used to calculate height velocity (HV) and body mass index (BMI). Tanner pubertal stage was estimated by either clinical examination or self-estimation (338;339). Height (Ht) and body mass index (BMI) were converted into SDS using 1990 British childhood standards (340;341). T-6,T0,T+6 and T+12 HV(cm/yr) were calculated for the prior 6 months of these time points with T0 representing the point of starting Infliximab therapy. To account for delayed puberty, HV at T-6,T0,T+6 and T+12 was converted into SDS adjusted for breast stage for girls >11 yrs and genital stage for boys >12 years by using age-related references for attainment of pubertal stages (354). A Physician's Global assessment was used to classify patient's response to therapy based on remission or improvement in symptoms as well as general patient well-being. Using this assessment, children were categorized as a "Responder" or a "Non-responder" to Infliximab; the former category included those who reached clinical remission. Details of concomitant medications were obtained from the case notes and are presented in (Table 3.3.1). Only 3 children at T0 and 1 child at T+6 were on supplemental feeds. None of the children were on any form of direct growth or puberty promoting drugs. Eight children received scheduled

maintenance with Infliximab for 12 months after starting Infliximab therapy; none of these had resectional surgery.

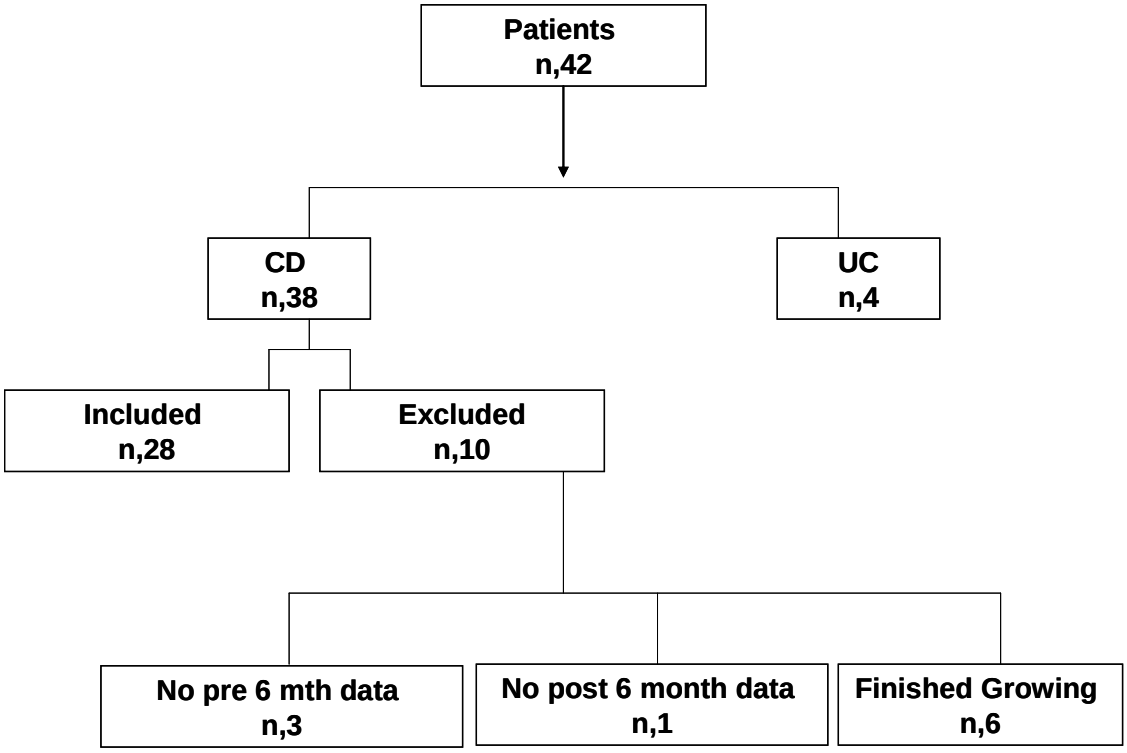


Figure 3.1.1: Flow diagram of study recruitment

3.2.2 Statistical analysis

All data are described as medians and 10th and 90th centiles and were analysed with 1-sample Wilcoxon non-parametric test by using Minitab software version 15. Univariate linear regression analysis was used for univariate analysis of association. Statistical significance was set at $p < 0.05$. Assessment of a relationship of HV at T0 and T+6 with systemic biochemical and haematological markers was performed by comparing the HV at T0 to the average value of the marker at T-6 and T0 and by comparing HV at T+6 to the average value of the marker at T0 and T+6. Logistic regression analysis was used to assess independent factors associated with change in absolute HV at T+6. The dependent reference group included those with a greater than two-fold rise in absolute HV at T+6. Independent factors assessed were prednisolone use at T0 and T+6, and Methotrexate use at T0 and T+6.

3.3 Results

3.3.1 General Characteristics

Over six months from T0 to T+6, each patient received a median of 4 Infliximab infusions (2, 6). 27 children started an induction regime of 5mg/kg at 0, 2wk and 6wk, but one of 27 children did not complete induction and in one child the corresponding dose was 7mg/kg.12 children progressed to maintenance therapy. Table 3.3.1 shows the clinical details of these children 6 months prior (T-6) to starting Infliximab, at baseline (T0) and for the following 6 months (T+6). Out of the 28 children, 21(75%) demonstrated a clinical response to Infliximab therapy out of whom 10(36%) achieved clinical remission. 4 of 28 cases, had an immediate adverse reaction which could be attributed to the Infliximab. These included headache, urticaria, anaphylactic reaction, nausea and vomiting. The patient with anaphylactic reaction completed the induction regime. During the 12 month period of study none of these children had resectional surgery.

3.3.2 Linear growth & disease status

The median HV in the overall group increased from 3.6cm/yr(0.4,7.9) at T0 to 5.5cm/y(2.1,9.2) at T+6, (p=0.003).The median change in height SDS (∆HtSDS) of this group also increased from -0.1 (-0.31, 0.16) at T0 to 0.05 (-0.14, 0.45) at T+6, (p=0.006) (Figure 3.3.1 a & b). This was reflected in the HtSDS which increased from a median of -1.01 (-2.35,-0,03) at T0 to -0.93(-2.13,-0-04) at T+6,(p=0.005) (Table 3.3.1).

Out of the 28 children, 17 had a reduction in HtSDS between T-6 and T0 and 11 out of these 17 (65%) children showed an increase in HtSDS between T0 and T+6, 2(12%) showed no

change in HtSDS and the remaining 4(23%) continued to show a deterioration in HtSDS after starting Infliximab therapy. In the 10 children who had an increase in HtSDS before Infliximab therapy, 7(70%) showed a continuing improvement in HtSDS after starting therapy and 3(30%) showed a deterioration after starting therapy (Figure 3.3.2). HV at T0 and T+6 in the 7 non-responders was 4.3cm/yr (2.5,8.6) and 3.0cm/yr (2.0, 11.3), respectively (p=0.71) (Figure 3.3.3a). The median ΔHtSDS of this group also remained unchanged from -0.0 (-0.1, 0.4) at T0 to -0.1 (-0.2, 0.6) at T+6, (p=0.60) (Figure 3.3.3b), whereas, in those who showed a clinical response, HV increased from 2cm/yr (0.3, 7.0) to 6.4cm/yr (2.3, 9.1) (p=0.004) (Figure 3.3.3c). The median ΔHtSDS of this group also increased from -0.1 (0.00, 0.10) at T0 to 0.05 (0.00, 0.4) at T+6, (p=0.001) (Figure 3.3.3d).

HV at T0 and T+6 showed a positive association to the average serum ALP, a recognized marker of growth and skeletal development, during the 6 months prior to starting Infliximab (r,0.39, p=0.046) and the 6 months after starting Infliximab (r,0.53, p=0.003), respectively (Figure 3.3.4). HV T+0 and T+6 did not show an association with any of the systemic disease markers that were studied.

In 25 children growth data were available over the 12 month period following Infliximab therapy. The median HV in the overall group changed from 3.6cm/yr(0.4,7.9) at T0 to 5.2cm/y (0.4,9.0) at T+12, (p=0.4). The ΔHtSDS of this group also increased from -0.1 (-0.31, 0.2) at T0 to 0.12 (-0.4, 0.61) at T+12, (p=0.050) (Figure 3.3.5 a & b).

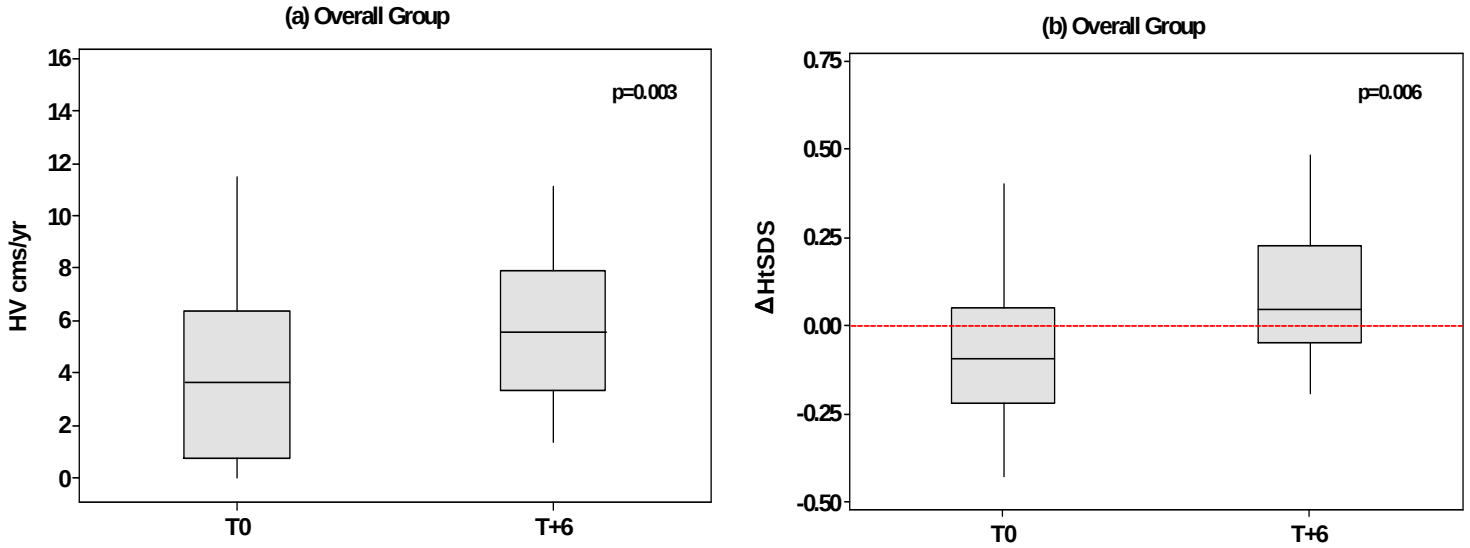
3.3.3 Pubertal status & growth

Of the 20 children with complete pubertal data, 13, with a median age of 10.3 years (8.09-12.91) at T-6, remained pre-pubertal 6 months prior (T-6) to starting Infliximab, at baseline (T0) and for the following 6 months (T+6). In the 20 children who had puberty assessed and in whom HV could be adjusted for pubertal age, HV SDS was -2.2 (-8.3, 5.3) and 1.0 (-2.9, 4.0) at T0 and T+6, respectively (p=0.03). Analysis in the sub-group of pre-pubertal children demonstrated an increase in HV from 4.5cm/yr (0.4, 8) at T0 to 5.5cm/yr(3.3, 8.4) at T+6 (p=0.05) (Figure 3.3.6).

Table 3.3.1: Clinical details of children at 6 months before starting Infliximab (T-6), at starting Infliximab (T0), at 6 months (T+6) and 12 months (T+12) after starting Infliximab

					p-Value				
	T-6 N,28	**T+0 N,28**	**T+6 N,28**	**T+12 N,25**	**T-6 VS T+0**	**T+0 VS T+6**	**T-6 VS T+6**	**T+6 VS T+12**	**T+0 VS T+12**	**T-6 VS T+12**
Age	12.6 (9.2-15.4)	13.1 (10.0-15.7)	13.6 (10.4-16.4)	14.3 (10.8-17)						
Concomitant Medication										
-Aminosalicylic acid		17	16	14						
-Azathioprine		13	9	7						
-Methotrexate		13	21	19						
-Prednisolone		12	6	5						
Anthropometry										
Height SDS	-0.83 (-2.62,0.16)	-1.01 (-2.35,-0.03)	-0.93 (-2.13,-0.04)	0.12 (-0.43,0.61)		**0.006**		0.9	0.050	**0.0049**
BMI SDS	-0.29 (-1.94,0.93)	-0.56 (-2.00,1.47)	-0.38 (-1.52,1.31)	-0.3 (-1.5,1.24)						
Height Velocity (cm/yr)	2.5 (0.4,6.9)	3.64 (0.4,7.8)	5.55 (2.1,9.2)	5.2 (0.4,9.0)		**0.003**		0.22	0.4	**0.05**
Pubertal status (n,20)										
-I	17	15	13	6						
-II	2	3	5	6						
-III	1	2	1	7						
-IV	-	-	1	-						
Biomarkers				1						
Serum albumin (g/l)	35 (27,42)	35 (24,39)	38 (33,43)	37 (28,42)	0.86	**0.007**	**0.006**	0.31	**0.01**	0.5
ALP(U/l)	119 (83,186)	92 (60,146)	157 (108,208)	166 (86,234)	0.013	**<0.0001**	**0.01**	0.8	**0.0001**	0.15
ALT(U/l)	13 (8,26)	8 (7,20)	16 (11,26)	16 (10,34)	0.12	**0.0007**	**0.04**	1.0	**0.007**	0.08
ESR (mm/hr)	28 (10,46)	40 (13,56)	16 (11,26)	22 (6,42)	0.23	**0.002**	**0.002**	0.5	**0.02**	0.13
CRP (mg/l)	7 (7,31)	7 (7,36)	7 (7,11)	7 (7,30)	0.19	**0.04**	0.4	0.8	0.2	0.8
Platelets (109/L)	435 (262,563)	403 (289,670)	342 (213,516)	321 (250,298)	0.97	**0.04**	**0.03**	0.4	**0.01**	**0.02**

Figure 3.3.1: Growth in children with CD during the 6 months before Infliximab therapy (T0) and in the 6 months following the start of therapy (T+6). Box and whisker plots representing median 10th, 25th, 75th, and 90th centiles



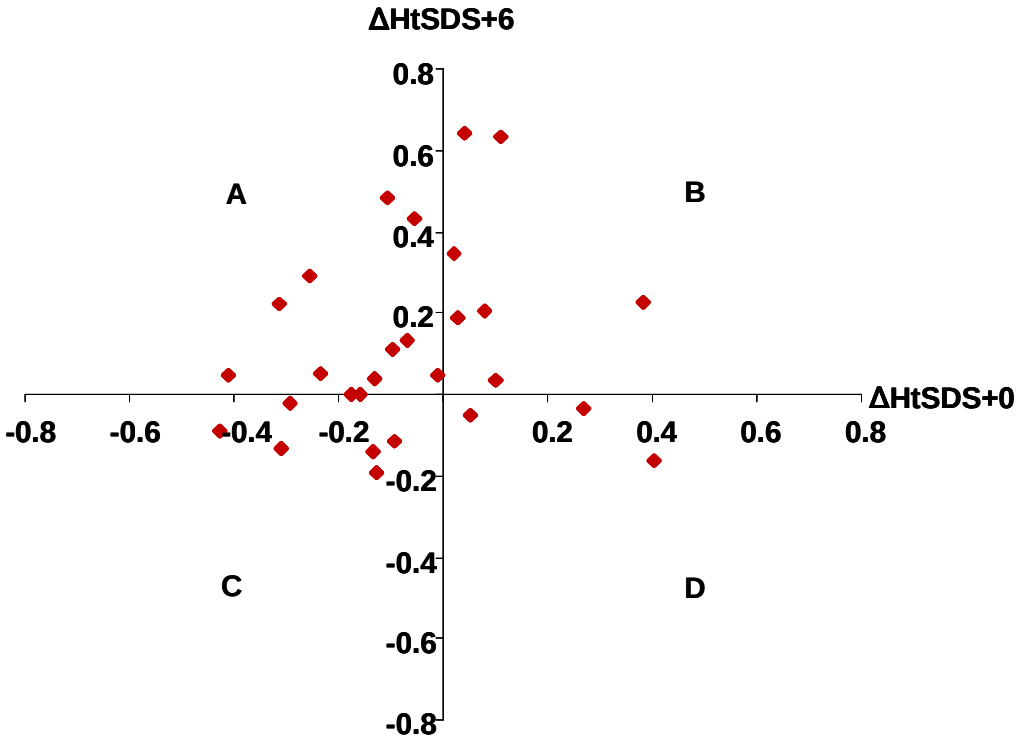


Figure 3.3.2: The relationship between change in height standard deviation scores (ΔHtSDS) during the 6 months before (ΔHtSDS+0, x-axis) and the 6 months after (ΔHtSDS+6, y-axis) starting Infliximab therapy in children with CD. The children in quadrant (A) had slow growth before starting Infliximab therapy but had improved growth after treatment. The children in quadrant (B) had normal growth both before and after Infliximab therapy. The children in quadrant (C) had normal growth before but had slow growth after starting Infliximab therapy. The children in quadrant (D) had slow growth before and after starting Infliximab therapy

Figure 3.3.3: Growth in children with CD during the 6 months prior to Infliximab therapy (T0) and in the 6 months following start of therapy (T+6); Box and whisker plots representing median, 10th, 25th, 75th, 90th centiles

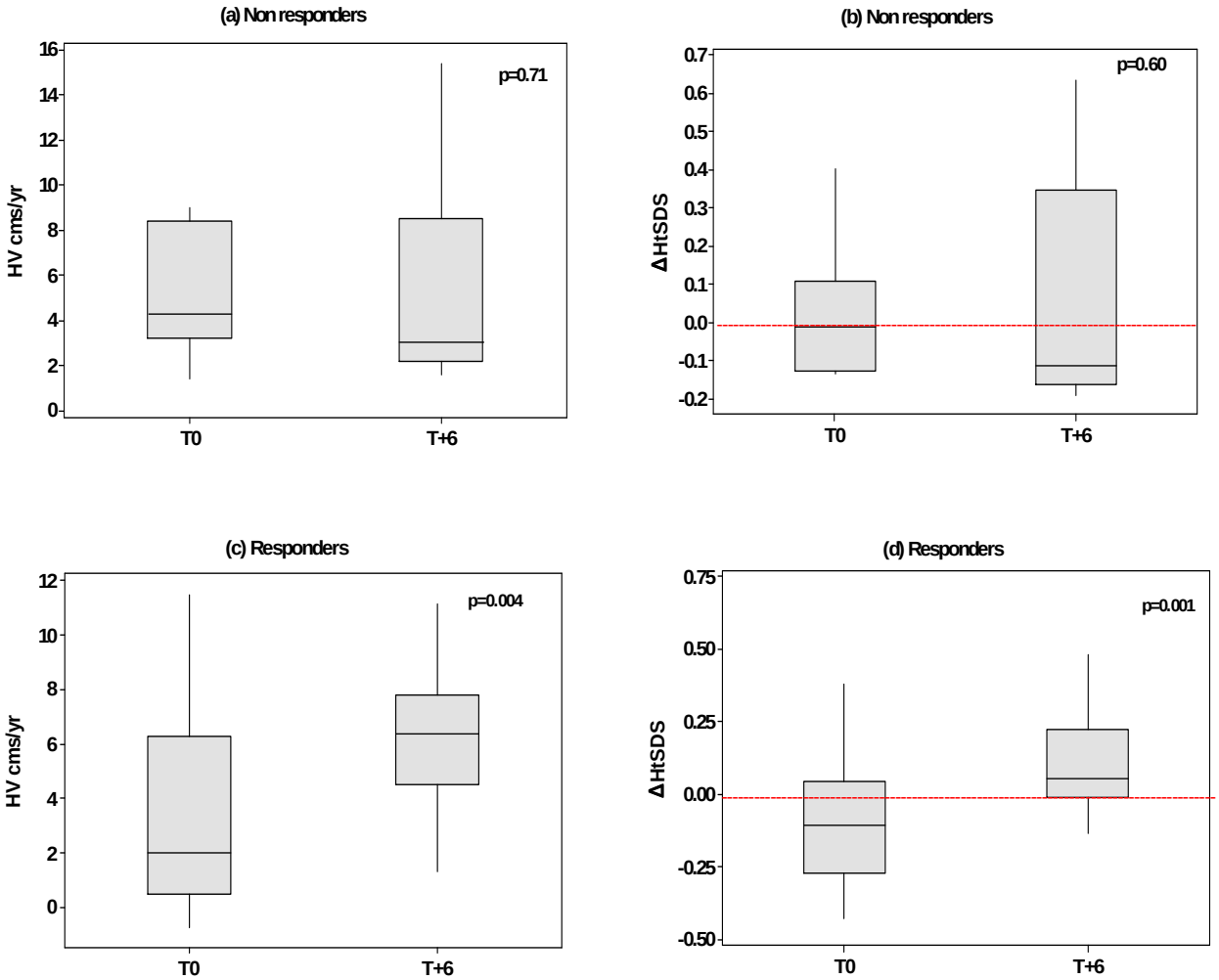


Figure 3.3.4: The association between HV (cm/y) and ALP (U/L)

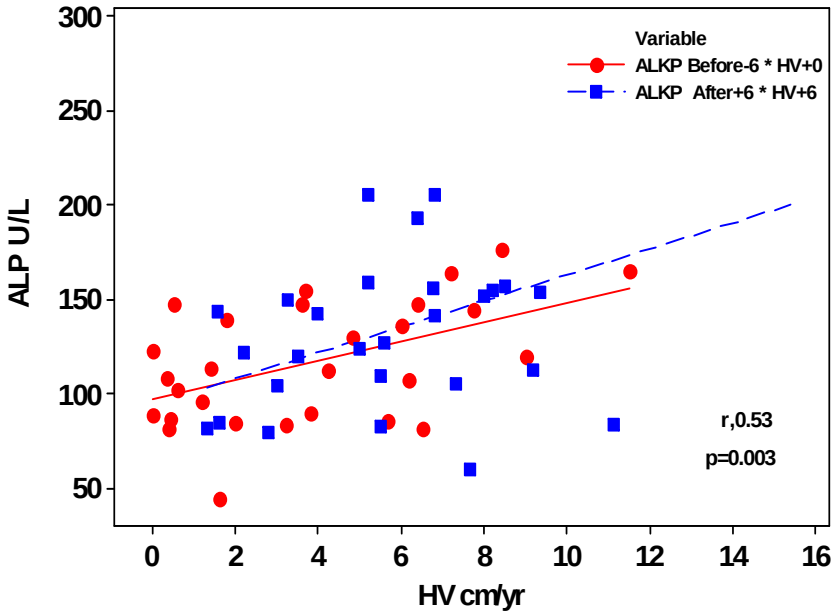


Figure 3.3.5: Growth in children with CD during the 6 months before Infliximab therapy (T0) and in the 6 months (T + 6) and 12 months (T + 12) following start of therapy. Box and whisker plots representing median 10th, 25th, 75th, and 90th centiles

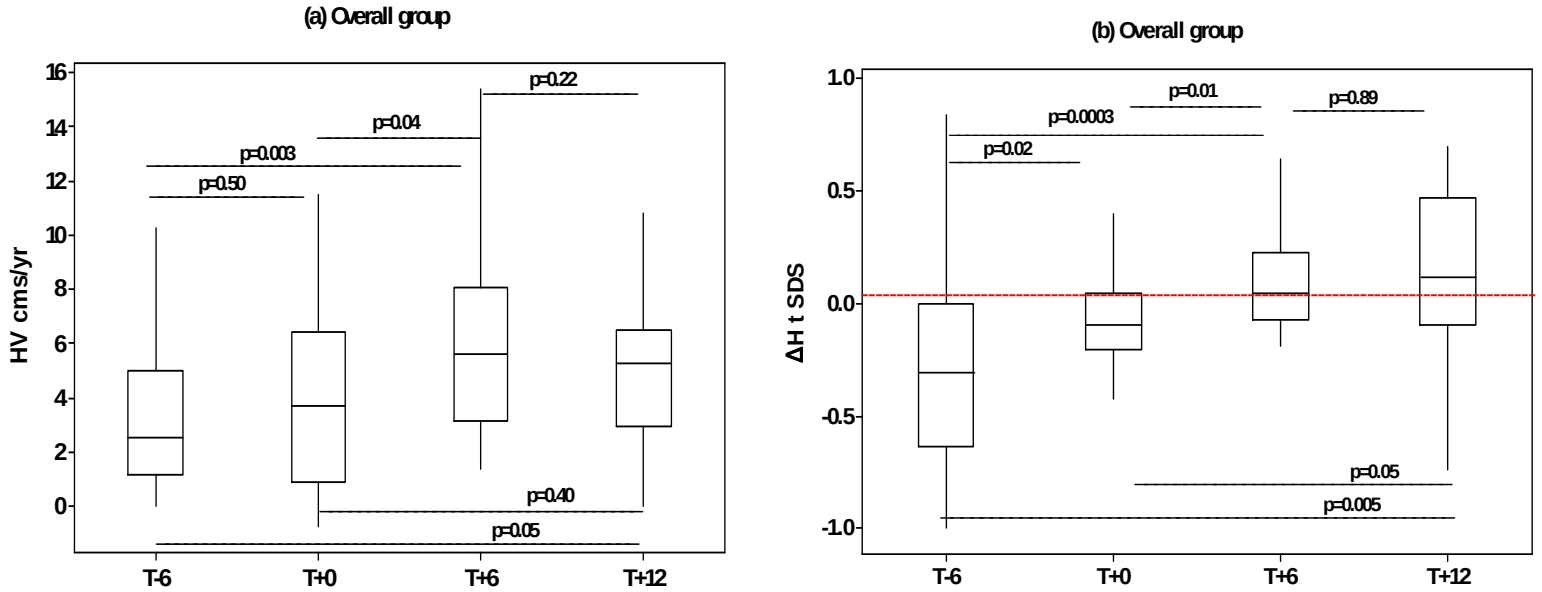
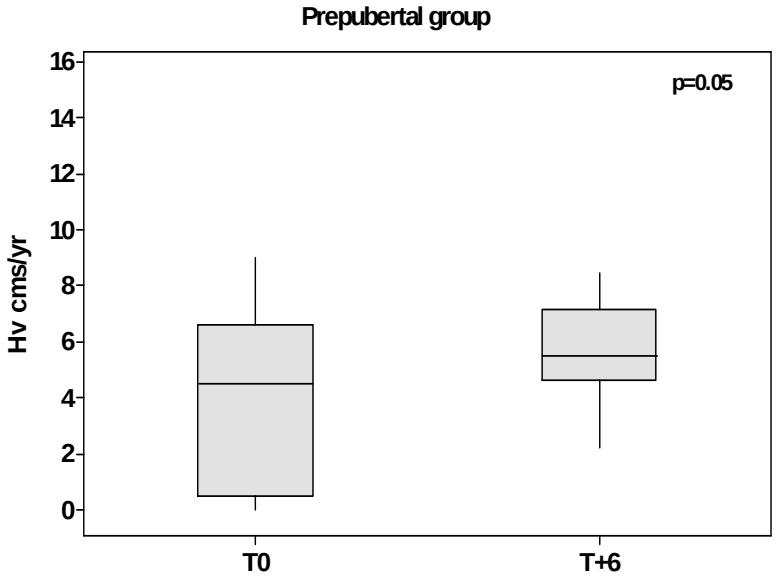


Figure 3.3.6: Height velocity (HV, cm/yr) in pre-pubertal children with CD during the 6 months prior to Infliximab therapy (T0) and in the 6 months following start of therapy (T+6). Box and whisker plots representing median 10th, 25th, 75th, and 90th centiles



3.3.4 Glucocorticoid status & growth

The cohort of children could be divided into three groups based on whether they received prednisolone in the 6 months prior to starting Infliximab or in the 6 months after starting Infliximab. Out of the 28 children, 13 (45%) did not receive prednisolone either before or after starting Infliximab; 9(32%) had a complete cessation of prednisolone therapy and 2(7%) had a reduction; and the third group consisting of 4 children had an increase (n,1) or initiation (n,3) of prednisolone therapy in the 6 months after starting Infliximab. In the first group of children, HV increased from 3.7cm/yr(0.6,6.5) at T0 to 6.4cm/yr(2.2,9.3) at T+6, (p=0.04); in the second group, HV also increased from 1.8cm/yr(0.3,8.3) at T0 to 5.6cm/yr(2.9,9.0) at T+6 but the difference did not reach statistical significance (p=0.11). In the third group HV at T0 and T+6 was similar at 3.9cm/yr and 4.1cm/yr, respectively (p=0.14) (Figure 3.3.7 a, b & c).

3.3.5 Methotrexate therapy

In 12 cases, children were on Methotrexate therapy before and after starting Infliximab. Median HV before and after starting Infliximab in this group of children was 2.7(0.0, 8.2) and 7.1(1.4, 14.1), respectively (p= 0.001). In these 12 children, median cumulative prednisolone dose for the prior 6 month period at T0 and T+6 was 0.02mg/kg/day (0.00,0.04) and 0mg/kg/day (0.00,0.03) (NS). In 9 cases, children were not on Methotrexate before starting Infliximab but were commenced on Methotrexate in the 6 months after starting Infliximab. Median HV at T0 and T+6 was 5.2cm/yr (2.2,9.2) and 5.7cm/yr(0.4,11.5), respectively (0.26). For these 9 children, median cumulative prednisolone dose for the prior 6 month period at T0 and T+6 was 0mg/kg/day (0.00, 0.05) and 0mg/kg/day (0.00, 0.03), respectively (NS). In 7 cases, Methotrexate therapy was not administered before or after starting Infliximab and their HV at T0 and T+6 was 3.3cm/yr (1.6, 7.7) and 3.7cm/yr (0.4, 7.2), respectively (0.37). Median cumulative prednisolone dose at these two time points was 0mg/kg/day (0.00, 0.06) and 0mg/kg/day (0.00, 0.05) (NS), respectively (Figure 3.3.8 a, b & c).

3.3.5.1 Multivariate analysis

Multivariate analysis using logistic regression with change in actual HV at T+6 (greater than 2 fold increase in HV as reference category) and prednisolone category and Methotrexate category as independent variables (Nagerlkerke R square 0.40) revealed no significant independent factor.

Figure 3.3.7: Height Velocity (HV cm/yr) according to prednisolone therapy status in the 6 months before (T0) and the 6 months (T+6) after starting Infliximab therapy

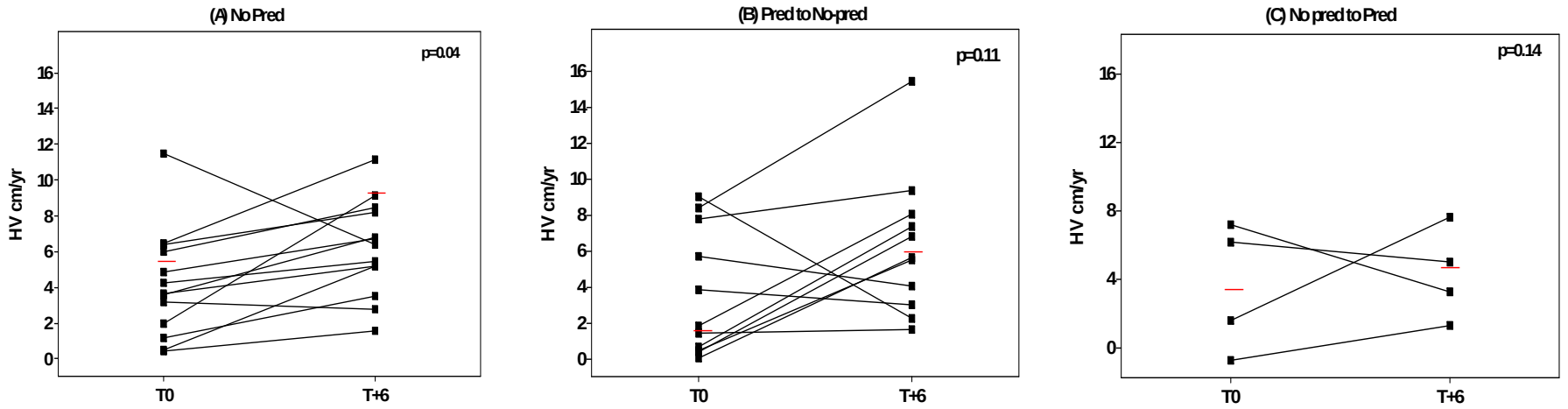
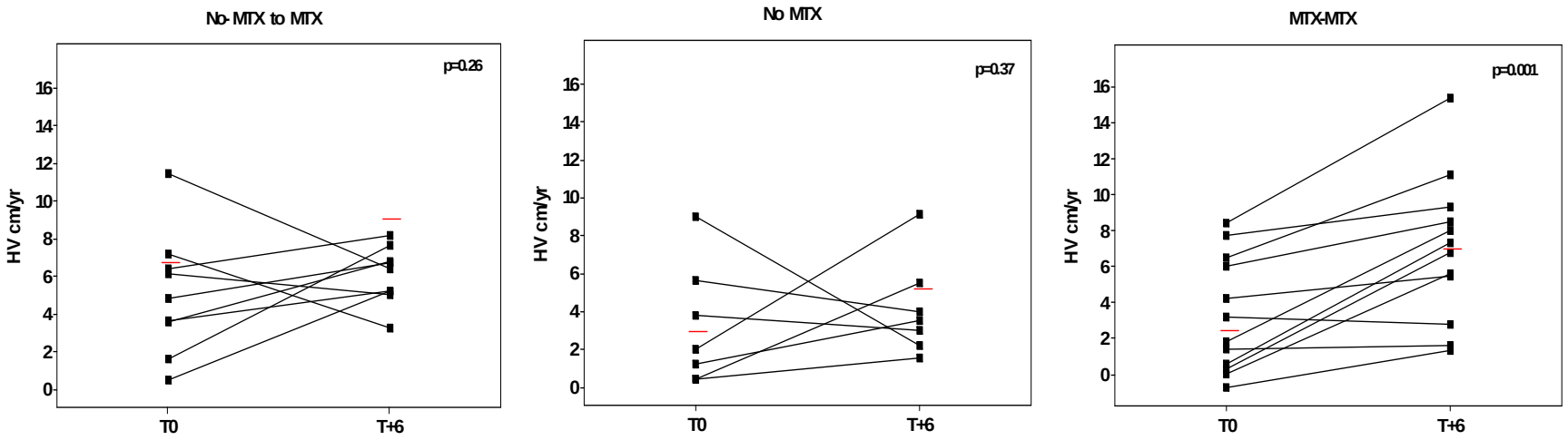


Figure 3.3.8: Height Velocity HV (cm/yr) according to Methotrexate therapy status in the 6 months before (T0) and the 6 months (T+6) after starting Infliximab therapy



3.3.6 Discussion

Infliximab therapy in children with CD has been reported to be associated with an improvement in linear growth and our study supports these findings (233;273;311). Overall, there was an average improvement of approximately 50% in height velocity in the 6 months after the initiation of therapy and improvement was further sustained for a further 6 months. Whilst this improvement in growth is noteworthy, the median age of this group at the time of starting therapy was 13yrs and a height velocity of 5.5cm/yr at this age is still relatively low. In general, boys are close to approaching their peak height velocity at this age and their expected median height velocity would be approximately 10cm/yr (355); in girls, median height velocity is approximately 5cm/yr at this age (355). However, these data assume a normal timing of puberty; accordingly, over 50% of healthy girls over the age of 13 years would be expected to reach menarche. The cohort in our study excluded all girls that had reached menarche and in the 20 cases where puberty data were available, none were more advanced than Tanner Stage 3 at the start of therapy. The relatively low height velocity for age is reflected in the height SDS and how it changed following Infliximab therapy. In those children whose height SDS was falling before start of Infliximab therapy, an improvement in height SDS was seen in approximately 60% and these findings are similar to previous reports (247;309;350).

In other groups of children, such as those with juvenile idiopathic arthritis, treatment with TNF-α antagonist receptor therapy has also been associated with an improvement in growth but this improvement has been partly attributed to a reduction in the use of GC therapy (315). In CD, a recent review of growth and its relationship to treatment modalities in 176 children suggested that poor growth was more likely in those who were receiving GC therapy and the authors called for eliminating the use of GC (310). Our observation that initiation of Infliximab was associated with an improvement in growth in those children who had not been exposed to exogenous GC at all, is noteworthy, and suggests that the growth-promoting effects of Infliximab are not solely due to its ‘GC-sparing effect’. The finding that growth was more likely to improve in those children who were judged to be ‘responders’, also suggests that growth improves as a result of improved disease control. Our observation that the increase in height velocity was more marked in the children who had never been on GC compared to those who had been on GC, suggests a possible adverse priming effect of GC on growth. It is equally plausible GC administration may also be a proxy marker of disease severity. Given that GC and proinflammatory cytokines may exert adverse effects on the growth plate through common as well as distinct mechanisms, the possibility that prior therapy with GC hinders the growth-promoting effect of Infliximab does require further study.

Proinflammatory cytokines and chronic inflammation maybe associated with altered gonadal function and reduced sex steroid synthesis and affected children and adults may exhibit a combined picture of central and peripheral hypo-gonadism (356). In adolescents, this may present as delayed onset of puberty, slow progression through puberty and/or associated growth retardation. Improvement of the disease status would be expected to be associated to pubertal progression and improved growth. Previous report of growth following Infliximab therapy have suggested that improvement is more likely in those, which are in early puberty (310;311), and this may be due to the possibility of pubertal progress following initiating Infliximab therapy. Our observation that growth improved in children who remained pre-pubertal for the 6 months before and after starting Infliximab suggests that the improvement in growth may not simply be due to pubertal onset or progression through puberty. Previously published studies have suggested that growth is more likely to improve in those who were in early puberty at introduction of Infliximab therapy (310;311). Whilst this implies that growth improvement may be secondary to progression in puberty, our study shows that growth improvement is not simply due to pubertal progress.

While our review of this cohort clearly shows the relationship between growth and clinical response to Infliximab therapy, there was no clear relationship between individual markers of disease activity and growth. A lack of an association between individual markers of disease and disease activity has been reported previously (345). Our data also suggest that the beneficial effect of Infliximab on growth may be more likely when a child is receiving a background of Methotrexate. A beneficial synergistic effect of Methotrexate and Infliximab on disease activity has been previously reported in children with rheumatoid arthritis (357). In vivo studies in children and in vitro studies at the level of bone and growth plate, suggest that Methotrexate promotes bone resorption and suppresses chondrogenesis (358;359). However, it is possible that this adverse effect of Methotrexate on the skeleton may only be observed at higher doses and the synergistic effect may only occur at tissue exposure at a lower level (357). This may further depend on the dose and route of administration of Methotrexate and these issues will require further studies in a larger cohort of cases. Nevertheless, our observations would support the early use of Methotrexate in children before Infliximab is used as the subsequent component of step-up therapy (100).The association between ALP and height velocity that was observed in our study suggests that an improvement in growth is associated with an improvement in bone formation. Although, the total ALP assay reflects intestinal, placental, liver, bone and kidney isoforms of ALP, in children without liver disease, the correlation between bone and total ALP is greater than 0.9 (360). In addition to being present in mature osteoblast and the matrix vesicles that are associated with bone mineralization; the bone isoform of ALP is also present in the

hypertrophic chondrocytes of the epiphyseal growth plate, and in the matrix vesicles associated with bone mineralization**;** it is also the predominant isoform in the plasma of growing children (185). In cross-sectional studies, both total and bone ALP parallel the childhood height velocity curve (361) and longitudinal studies of short-term and medium-term growth in healthy children have reported a high level of correlation between growth and total or bone ALP (359;362;363). Proinflammatory cytokines and GC can affect osteoblast activity independently**,** as well as through common mechanisms such as the 11β-hydroxysteroid dehydrogenase shuttle which regulates tissue glucocorticoid exposure in response to cytokines (364). Our preliminary data suggest that, in addition to improving growth, it is possible Infliximab use is associated with an improvement in bone health and this observation was recently confirmed in a report of an improvement in biomarkers of bone formation in another similar cohort of children (207). Our study suggests that this improvement may be independent of a reduction in GC and changes in pubertal stage. A recent report suggests that Infliximab therapy may also be associated with improvements in lean mass corrected for height (365). There is, therefore, a need to further understand the wider benefits of anti-TNF therapy.

In summary, linear growth in children with CD improves following Infliximab therapy in those who show a clinical response. This increase is clearly multifactorial but of note also seen in pre-pubertal and GC naïve children and cannot solely be attributed to a change in these factors alone.

CHAPTER 4

The effects of anti-TNF-α treatment with Adalimumab on growth in children with Crohn's disease

4.1 Summary

Introduction: Adalimumab is used to treat children with Crohn's disease (CD), but the effects of Adalimumab on growth in CD have not been studied.

Aim: To study growth and disease activity over 12 months (6 months prior to (T-6), baseline (T0) and for 6 months following (T+6) Adalimumab.

Subjects & methods: The growth and treatment details of 36 children (M: 22) who started Adalimumab at a median (10^{th}, 90th) age of 14.7years (11.3, 16.8) were reviewed.

Results: Of 36 cases, 28 (78%) went into remission. Overall 42% of children showed catch up growth, which was more likely in: (i) those who achieved remission (median change in height SDS (ΔHtSDS) increased from -0.2 (-0.9, 1.0) at T0 to 0.2 (-0.6, 1.6) at T+6, (p=0.007), (ii) in those who were on immunosuppression ΔHtSDS increased from -0.2(-0.9, 1.0) at T0 to 0.1 (-0.8, 1.3) at T+6, (p=0.03) and (iii) in those whose indication for using Adalimumab therapy was an allergic reaction to Infliximab median ΔHtSDS increased significantly from -0.3(-0.9, 1.0) at T0 to 0.3(-0.5, 1.6) at T+6, (p=0.02). Median change in height SDS (ΔHtSDS) also increased from -0.4 (-0.8, 0.7) at T0 to 0.0(-0.6, 1.6) at T+6, (p=0.04) in 15 children who were on prednisolone therapy when starting Adalimumab.

Conclusion: Clinical response to Adalimumab therapy is associated with an improvement in linear growth in a proportion of children with CD. Improved growth is more likely in patients entering remission and on immunosuppression but is not solely due to a steroid sparing effect.

4.2 Introduction

Crohn's disease in children often results in malnutrition, growth retardation, pubertal delay and poor bone health (330). Several interrelated factors contribute to linear growth retardation in children with CD, including poor nutrition, chronic use of GC and lack of IGF-1 interdependent with these factors are the modulatory role of disease activity mediated via pro-inflammatory cytokines such as TNF α, IL 1β and IL6 (219). Conventional treatment of CD consists of enteral nutrition, corticosteroids, anti inflammatory agents and immunomodulators. However, these treatments have been largely unsuccessful in altering the natural course of the disease (245). Growth is a vital outcome in intervention studies in paediatric IBD, yet is rarely reported as a secondary outcome let alone as the primary outcome (366). Although the initial therapeutic strategy for CD has not been changed for a long time, the emergence of biological therapy, with its record of rapid efficacy and use as maintenance therapy has changed the management of severe paediatric CD dramatically (273). Adalimumab (Humira®, Abbott UK) is a humanized anti-TNF therapy that has been shown to be efficacious for induction and maintenance of remission for adults with CD (265;267). Adalimumab is generally used in those patients who have an attenuated response or an adverse reaction to Infliximab (284-286). Infliximab treatment has been shown to allow catch up growth and reduce steroid use in paediatric patients with CD in several studies (156;207;247;279;309-312;367). Adalimumab, however, is not currently licensed for use in paediatric IBD patients in the UK at least. The published clinical studies to date of Adalimumab use in children are relatively limited (276;288;289;291-295) with no studies yet conducted to examine the effects of Adalimumab on growth in children with CD. The aim of the present study was to assess the effect of Adalimumab therapy on growth, puberty and disease activity over the 6 months prior to and 6 months after starting Adalimumab treatment in children with CD.

4.3 Subjects and methods

All members of the British Society of Paediatric Gastroenterology Hepatology and Nutrition (BSPGHAN) were approached by e-mail and invited to take part in the study. The full study methodology has been published previously (304). Data collected included; patient demographics, anthropometry, drug treatments (including previous Infliximab use) and details of any prior surgery (304). No growth data were included in the initial publication. Data for this study were collected and provided by Dr David Wilson and Michelle Wilson, School of clinical sciences, the University of Edinburgh. Of the 70 cases in the original study cohort with CD, those included in this study were children who had sufficient growth data available at all 3

time points. Sufficient data on growth were available for the 6 months before starting Adalimumab (T-6), at starting Adalimumab (T0) and 6 months after starting Adalimumab (T+6) in 36 children who were then studied further. Thirty-four of 36 had previously received Infliximab (Figure 4.5.1). The median (10^{th}, 90^{th}) ages at diagnosis and start of Adalimumab were 10.3 years (6.5, 13.1) and 14.7 years (11.3, 16.8) respectively. CD was diagnosed using standard criteria (6;353). In 28 of the 36 children, data on pubertal status was available at start of Adalimumab therapy (T0). Additionally, in 11 children growth data were also collected at 12 months following Adalimumab therapy.

Height was measured with a Harpenden stadiometer and Tanner pubertal stage was assigned using either clinical examination or self-estimation (338;339). Height (Ht) data were converted into SDS (standard deviation scores) using 1990 British childhood standards (340;341). T-6,T0,T+6 and T+12 HV(cm/year) were calculated from the prior 6 months of growth before time points with T0 representing the point of starting Adalimumab therapy. Disease phenotype was assigned using the Montreal classification (5). Adalimumab effect was assessed using the Paediatric Crohn's Disease Activity Index (PCDAI), where available, or Physicians Global Assessment (PGA) when PCDAI was not available (7;368). Using this assessment, children were then categorized as either "Remission" or "No-remission". Standard definitions were used for PCDAI disease status and remission (remission, PCDAI ≤10)(7). Details of concomitant medications were recorded and are presented in (Table 4.5.1). None of the children were on growth or puberty promoting treatments during the period of study.

4.4 Statistical analysis

All data are described as medians and 10th and 90th centiles and were analyzed with 1-sample Wilcoxon non-parametric test by using Minitab software version 16. Statistical significance was set at $p < 0.05$. To evaluate how growth was affected by Adalimumab, change in height SDS (ΔHtSDS) was calculated as well as height velocity.

4.5 Results

4.5.1 General characteristics

Thirty-four (94%) had prior Infliximab usage of whom 7 were primary non-responders, 16 had loss of clinical response and 11 had had an allergic reaction necessitating discontinuation. The induction dose of Adalimumab most commonly used was 80mg followed by 40mg 2 weeks later in 18 patients (50%), 24mg/m2 in 9 patients (25%), 160mg then 80mg 2 weeks

later in 2 patients (6%) and the 7 patients remaining received a combination of other dosing regimens(19%).

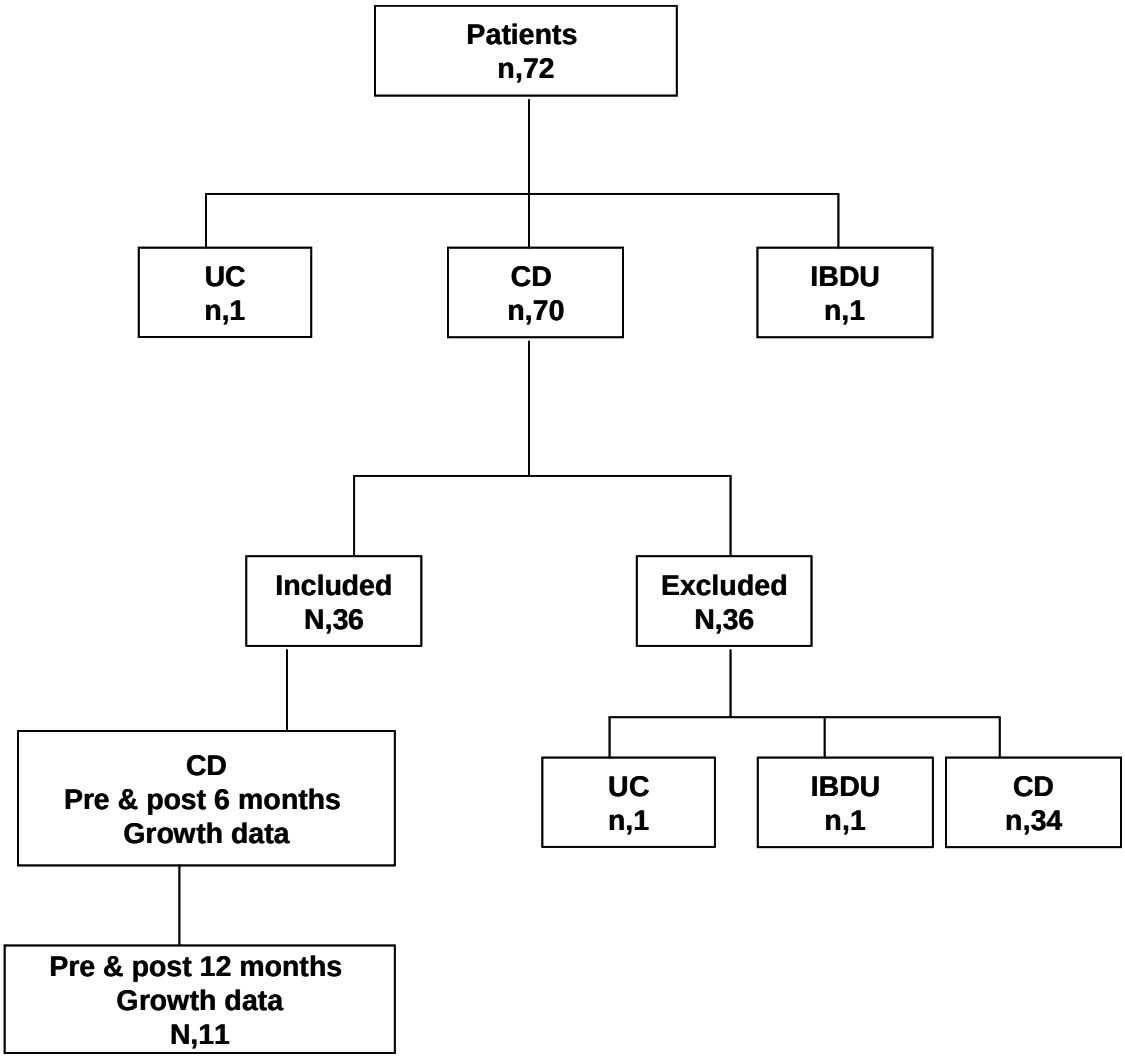


Figure 4.5.1: Flow diagram of study recruitment

Table 4.5.1: Demographic details of children at starting Adalimumab (T0) & at 6 months (T+6) after starting Adalimumab. Continuous variables are presented as medians and 10th and 90th centile

	T+0 N,36	T+6 N,36	T+0 VS T+6 p-value
Age (yrs)	14.7(11.3,16.8	15.2(11.9,17.3)	
Concomitant Medication			
Background immunosuppression (Azathorprine or Methotrexate	23		
-Prednisolone	15		
-Infliximab	36		
Anthropometry			
ΔHtSDS	-0.2 (-0.7, 0.2)	0.0(-0.5, 0.8)	**0.005**
BMI SDS	-0.8(-2.2,1.3)	-0.4(-1.3,1.2)	
Height Velocity (cm/yr)	2.0 (0.0, 5.8)	4.0 (0.0, 11.2)	**0.02**
Pubertal status (n,29)			
-I	6		
-II	7		
-III	4		
-IV	3		
-V	9		
PCDAI (n,19)	25(7.5,65)	10(0,55)	**0.0001**

All 36 patients commenced maintenance Adalimumab therapy at 40mg (n=33, 92%) or 24mg/m2 (n=3, 8%). One patient suffered an adverse reaction leading to withdrawal after Adalimumab therapy. Of 36 cases, 4 had resectional surgery during the study period. Other previous medications included, 30(83%) who had enteral nutrition, 32(89%) who had glucocorticoids, 34(94%) had prior Azathioprine/6-mercaptopurine and 20(56%) had previous Methotrexate.

4.5.2 Disease characteristics

Disease location was most commonly panenteric (Ileo-colonic and upper GI tract, L3 + L4) in 15(42%); 15 (42%) children had perianal disease. Of the 36 children, 28(78%) achieved remission on Adalimumab therapy in this cohort. A PCDAI score was available in 19 children at baseline (T0) and at 6 months (T+6) after Adalimumab therapy. Median PCDAI score decreased significantly from 25(7.5, 65) at T0 to 10 (0, 55) at T+6, (p=0.0001). Of 19 patients with PCDAI data, 14 achieved clinical remission as assessed by the PCDAI. Twelve of the 19 children had a PCDAI score available at 6 months and 12 months after starting Adalimumab therapy. In this group median PCDAI Score also was reduced significantly from 27.5(7.5, 65) at T0 to 6.25(0, 55) at T+6, (p=0.001) and 5(0, 37.5) at T+12, (p=0.0005) (Figure 4.5.2a & b).

4.5.3 Linear growth in the whole cohort

The median change in height SDS (ΔHtSDS) in the whole group increased from -0.2 (-0.7, 0.2) at T0 to 0.0(-0.5, 0.8) at T+6, (p=0.005) (Figure 4.5.3 a). Categorizing the subjects by gender showed improvement of linear growth in both genders (data not presented). The median HV of the whole group also increased significantly from 2.0cm/year(0.0,5.8) at T0 to 4.0cm/year(0.0,11.2) at T+6, (p=0.02) (Figure 4.5.3 b). Of the 36 children, 15 (42%) demonstrated catch-up growth with median ΔHtSDS of this group increasing from -0.3 at T0 to 0.3 at T+6, in 6 (14%) children median ΔHtSDS was 0 (4 both before and after the initiation of Adalimumab therapy), 1 (2.7%) had a reduction in ΔHtSDS from 0, 1 to 0.0 and 14 (39%) had ongoing growth deterioration with median ΔHtSDS of this group decreasing from -0.4 to -0.3 after starting Adalimumab therapy (Figure 4.5.4).

In 11 children growth data were available over the 12 month period following Adalimumab therapy. The median ΔHtSDS in the overall group changed from -0.2 (-0.9, 0.7) at T0 to 0.0 (-0.6, 0.7) at T+12, (p=0.10).The median HV of this group changed from 2.6cm/year (0.0, 8.9) at T0 to 4.2cm/year (0.0, 10.3) at T+12, (p=0.30). Median ΔHtSDS increased from -0.2 (-0.7, 0.2) at T0 to 0.03 (-0.5, 0.8) at T+6, (p=0.007) in children who achieved remission.

Figure 4.5.2: PCDAI values in children with CD at baseline (T0) and in the 6 months (T+6) and 12 months (T+12) following start of Adalimumab therapy

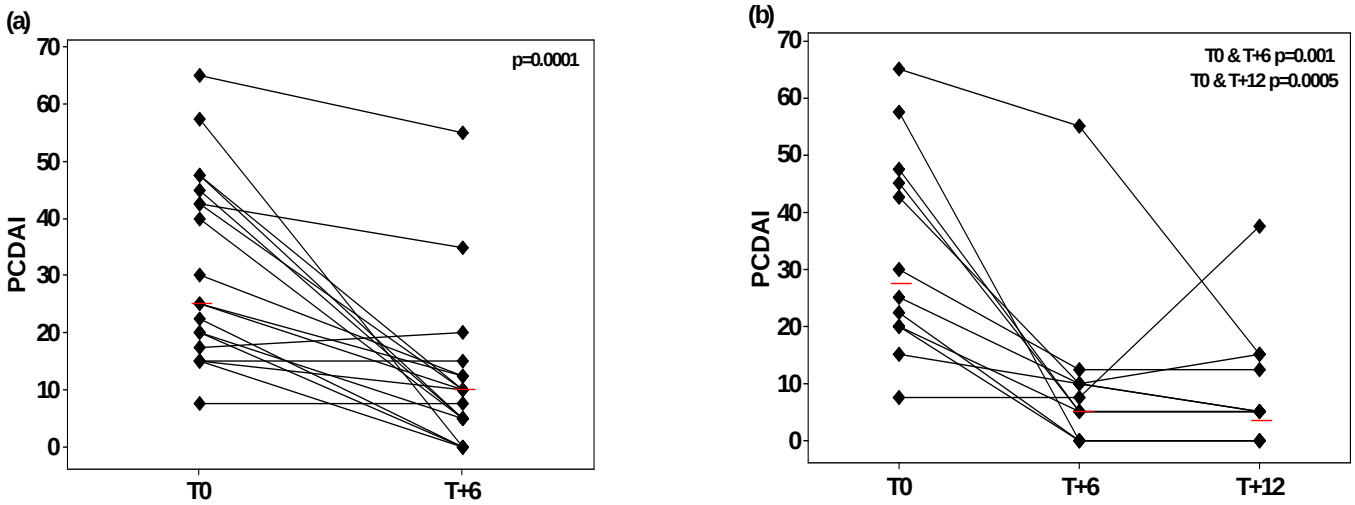
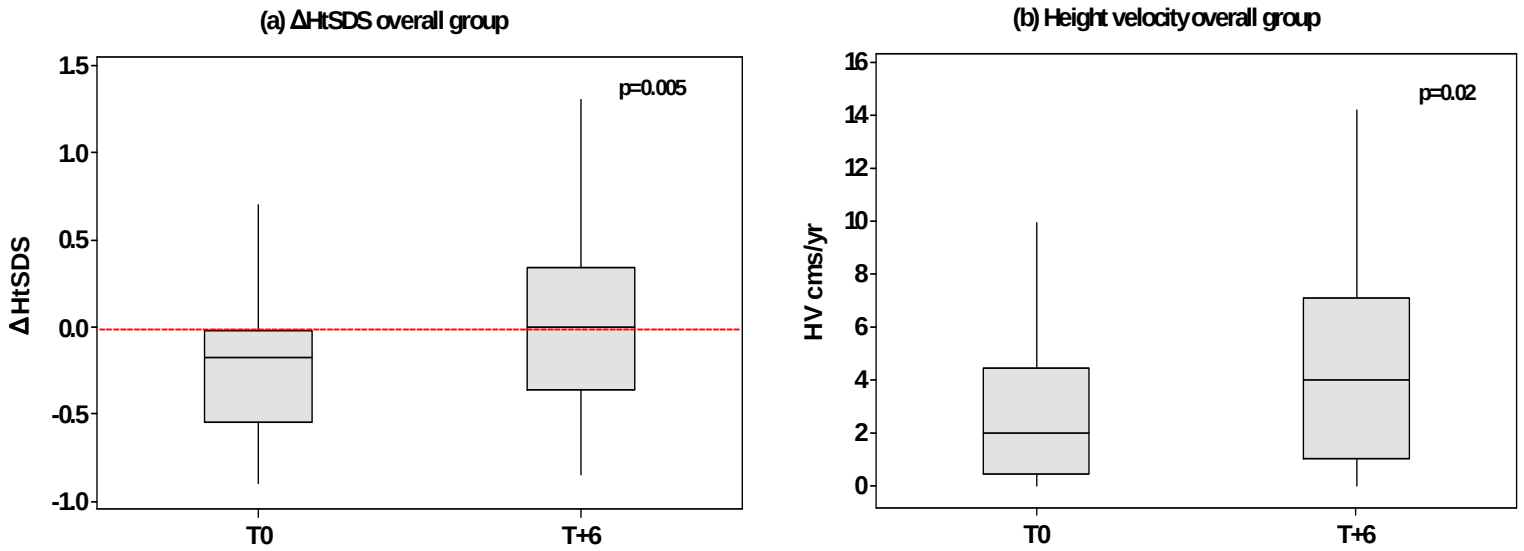


Figure 4.5.3: Growth in children with CD during the 6 months prior to Adalimumab therapy (T0) and in the 6 months following start of therapy (T+6)



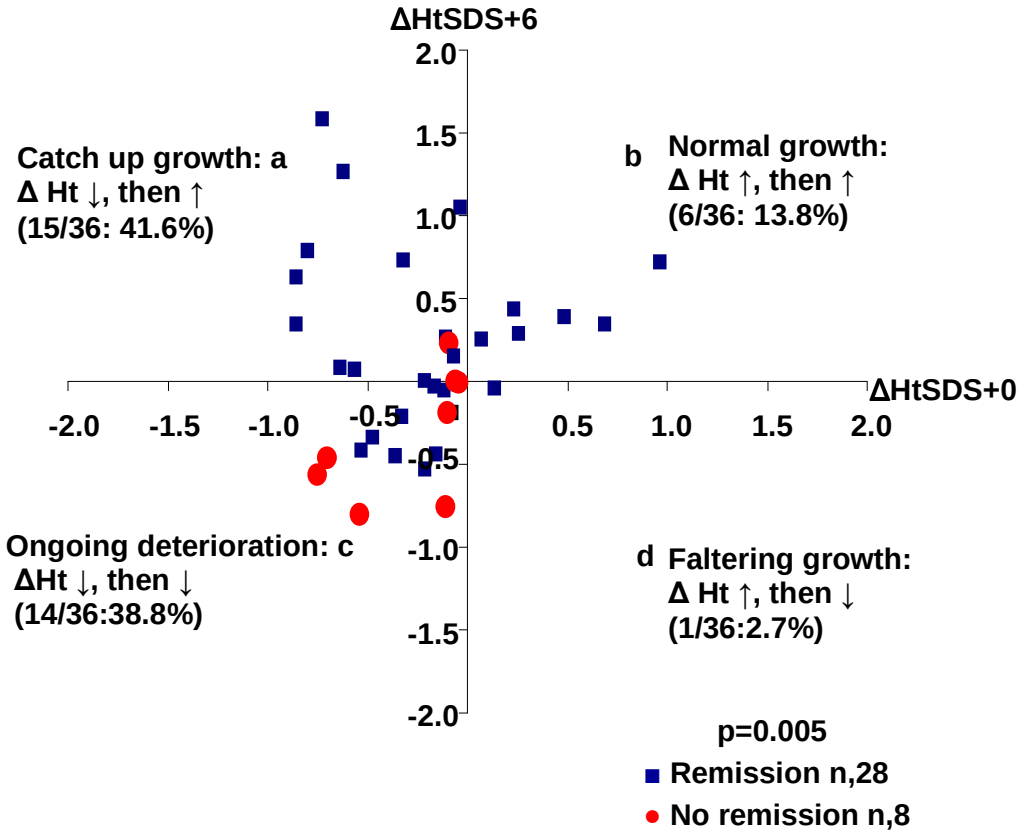


Figure 4.5.4: The relationship between change in height standard deviation scores over the 6 months before (ΔHtSDS0, x-axis) and the 6 months after (ΔHtSDS+6, y-axis) starting Adalimumab therapy in children with CD. The children in quadrant (a) had slow growth before starting Adalimumab therapy but had improved growth after treatment. The children in quadrant (b) had normal growth both before and after Adalimumab therapy. The children in quadrant (c) had normal growth before but had slow growth after starting Adalimumab therapy. The children in quadrant (d) had slow growth before and after starting Adalimumab therapy Growth in those achieving remission.

Median HV of this group increased significantly from 2.0cm/year (0.0, 9.9) to 5.1cm/year (0.0, 14.1) at T+6, (p=0.03) (Figure 4.5.5 a & b). In those who had no remission, median ΔHtSDS did not show any significant change; -0.1 (-0.7, 0.1) at T0 and -0.3 (-0.8, 0.2) at T+6, (p=0.87) and likewise median HV also did not show any improvement being 2.6cm/year (0.3, 6.1) at T0 and 1.5cm/year (0.0, 4.5) at T+6, (p=0.36) (Figure 4.5.5 c & d).

Growth response was also examined based upon the indication for using Adalimumab therapy as (i) allergic reaction to Infliximab (ii), loss-of-response to Infliximab and (iii) primary non responder to Infliximab (Figure 4.5.6 a, b & c). Of thirty four patients 11 had an allergic reaction, 16 had a loss of response and 7 were primary non responders. Median ΔHtSDS increased from -0.3(-0.9, 1.0) at T0 to 0.3(-0.5, 1.6) at T+6, (p=0.02) in children who had an allergic reaction; median HV changed from 0.2 cm/year (0.0, 8.6) to 5.3cm/year (0.0, 14.1) at T+6, (p=0.058). Median ΔHtSDS did not show any significant change from -0.1(-0.7,1.0) at T0 to -0.1(-0.8,0.7) at T+6, (p=0.73) in children who had loss of response nor in children who were primary non-responders -0.1(-0.5, 0.1) at T0 to 0.0(-0.8, 0.2) at T+6, (p=0.93).

4.5.4 Pubertal status & growth

In 28 of the 36 children, data on pubertal status were available at start of Adalimumab therapy (T0). On the basis of pubertal stage patients were categorized into two groups according to the method previously published by Walters et al (311) i.e. Tanner stages I-III and Tanner stages IV-V patient groups. Of 28 patients 17 were at Tanner stages I-III with a median age 13.5years (6.8, 6.5) and 11 were at Tanner IV-V with a median age 16.6years (14.2, 17.3). In Tanner stages I-III patient group, median ΔHtSDS increased significantly from -0.4 (-0.8, 0.6) at T0 to 0.2 (-0.8, 1.3) at T+6, (p=0.02). Median HV of this group was also increased from 2.0cm/year (0.2, 8.8) at T0 and 5.3cm/year (0.1, 14.1) at T+6, (p=0.03). In Tanner stages IV-V patient group, median ΔHtSDS did not change significantly from -0.0 (-0.8, 0.2) at T0 to -0.0 (-0.2, 1.5) at T+6, (p=0.10). Median HV of this group also showed an increase from 0.0 cm/year (0.0,8.6) at T0 to 2.0cm/year (0.0,13.6) at T+6, (p=0.34) but the difference was not significant (Figure 4.5.7 a, b c & d).

4.5.5 Glucocorticoids

The cohort of children was divided into two groups based on whether they were on glucocorticoids (prednisolone) or not when Adalimumab was commenced. Of the 36 children, 15 (42%) were receiving prednisolone therapy when starting Adalimumab and 21 (58%) were not.

Figure 4.5.5: Growth in children with CD during the 6 months prior to Adalimumab therapy (T0) and in the 6 months following start of therapy (T+6); Box and whisker plots representing median, 10th, 25th, 75th, 90th centiles.

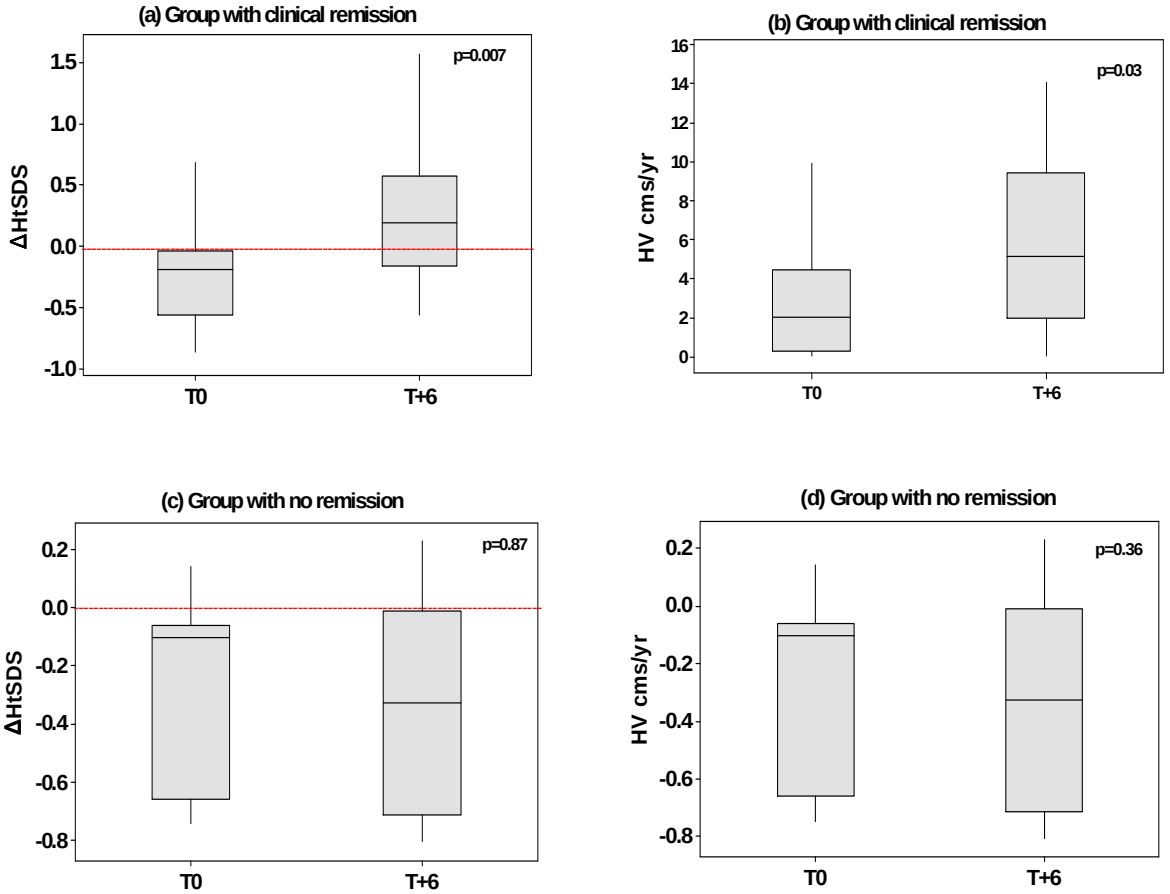


Figure 4.5.6: Growth response based upon the indication for using Adalimumab therapy

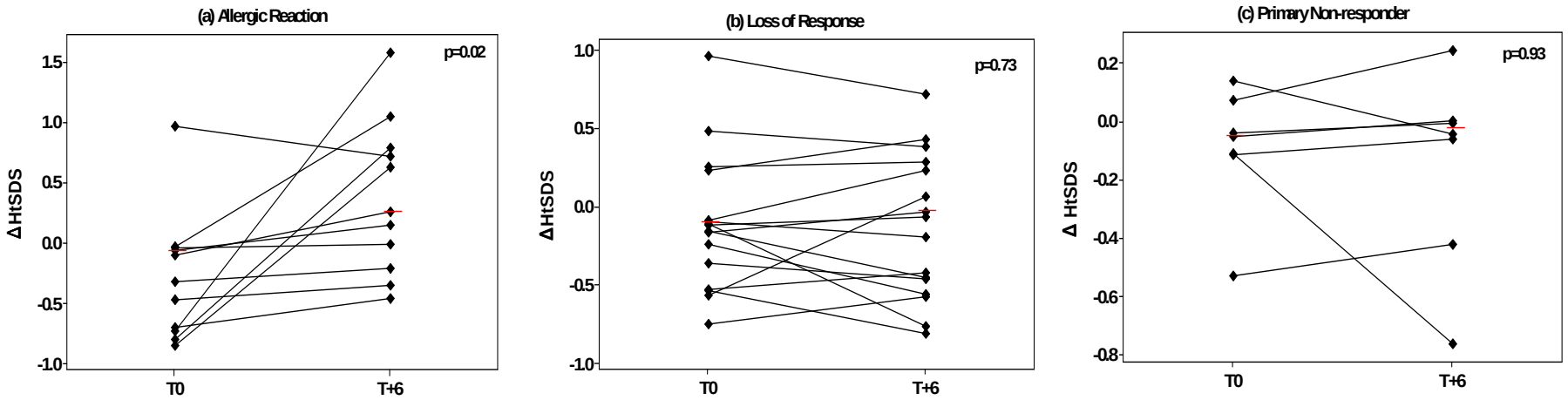
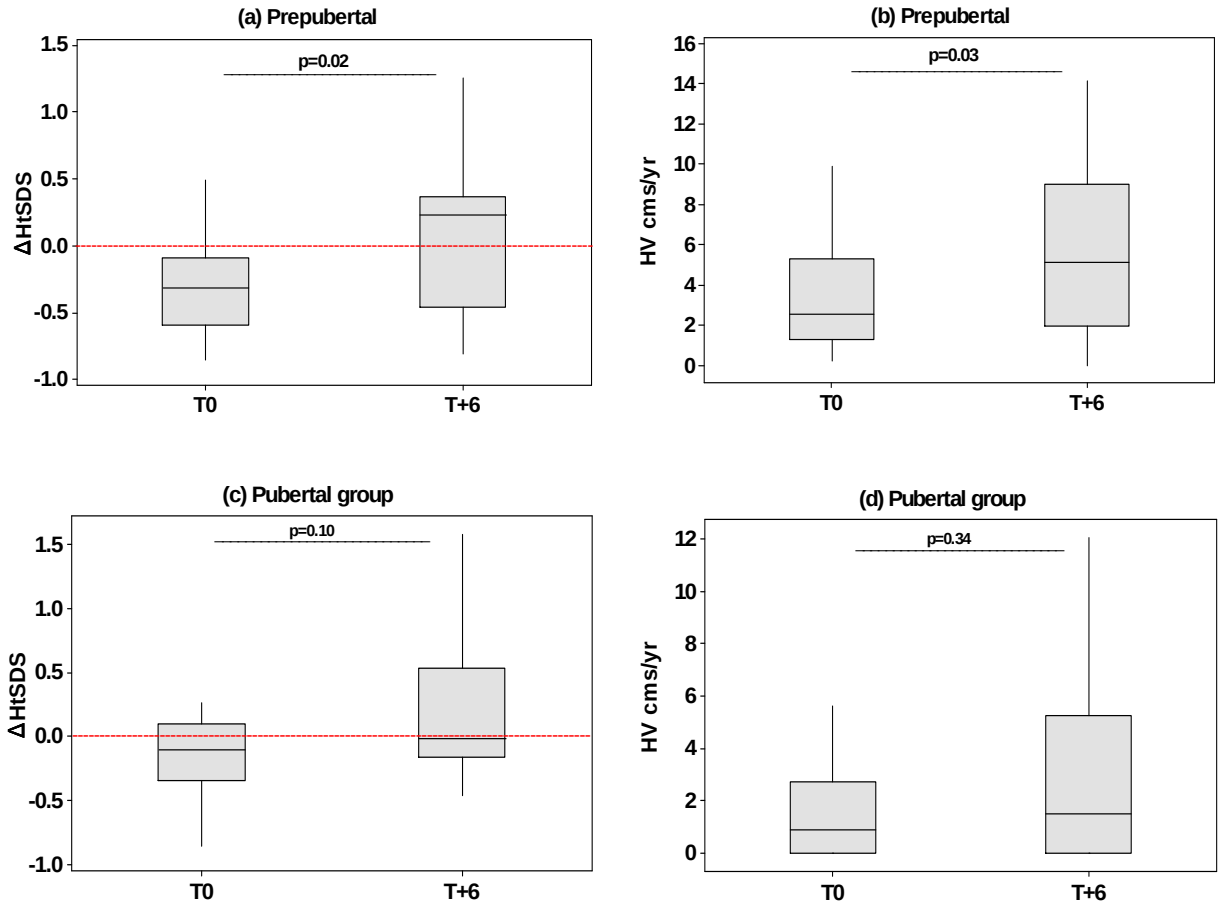


Figure 4.5.7: Growth in relation to pubertal status in children with CD during the 6 months prior to Adalimumab therapy (T0) and in the 6 months following start of therapy, Box and whisker plots representing median, 10th, 25th, 75th, 90th centiles



In the prednisolone group, median ΔHtSDS increased from -0.4 (-0.8, 0.7) at T0 to -0.0(-0.6, 1.6) at T+6, (p=0.04). Median HV of this group also changed, but not significantly, from 1.4cm/year (0.0, 9.9) at T0 to 4.0cm/year (0.0, 14.1) at T+6, (p=0.22); in the prednisolone-free group, median ΔHtSDS increased from -0.1(-0.9, 1.0) at T0 to 0.1 (-0.8, 1.0) at T+6, (p=0.02). Median HV of this group also increased from 2.7cm/year(0.0,8.6) at T0 to 4.0cm/year(0.0,12.0) at T+6 but the difference did not reach statistical significance (p=0.28) (Figure 4.5.8 a, b, c & d).

4.5.6 Immunosuppression

The cohort of children was divided into two groups based on whether or not they were on immunosuppression therapy (either thiopurine or Methotrexate) at commencement of Adalimumab. In the 23/36 (64%) children who were on immunosuppression therapy, median ΔHtSDS increased from -0.2(-0.9, 1.0) at T0 to 0.1 (-0.8, 1.3) at T+6, (p=0.03). Median HV of this group changed from 2.0cm/year (0.0,9.9) at T0 to 5.1cm/year(0.0,12.1) at T+6 (p=0.23); in the other 13 children median ΔHtSDS did not change significantly at -0.1 (-0.8,0.1) at T0 to -0.0 (-0.6,1.6) at T+6, (p=0.12). Median HV of this group also remained similar at 2.0cm/year (0.0, 5.6) at T0 to 3.0cm/year (0.0, 14.1) at T+6, (p=0.32) (Figure 4.5.9 a, b, c & d).

4.5.7 Discussion

At present there are no published studies that adequately assess the effect of Adalimumab on linear growth in children with CD. This study provides evidence that Adalimumab is associated with improvement in short term linear growth in children with CD who enter remission but not in those who do not. It is also more likely to happen in children who are on immunosuppression and those in early puberty but seems to be relatively independent of steroid use. These findings suggest that growth improves as a result of several interrelated factors, including improved disease control.

Use of co-immunosuppression therapy with biologics remains controversial (369). Our study has shown significant improvement in growth in children who were on immunosuppression therapy (either thiopurine or Methotrexate) at Adalimumab commencement compared to those children who were not. This complements the findings in the larger cohort of children treated with Adalimumab where higher response rates were seen in those who were on immunosuppression therapy at starting Adalimumab (304). There are no other published data on growth in children with CD receiving Adalimumab, but there are in other groups of

Figure 4.5.8: Growth in children with CD who were on prednisolone therapy at starting Adalimumab-Box and whisker plots representing median, 10th, 25th, 75th, 90th centiles

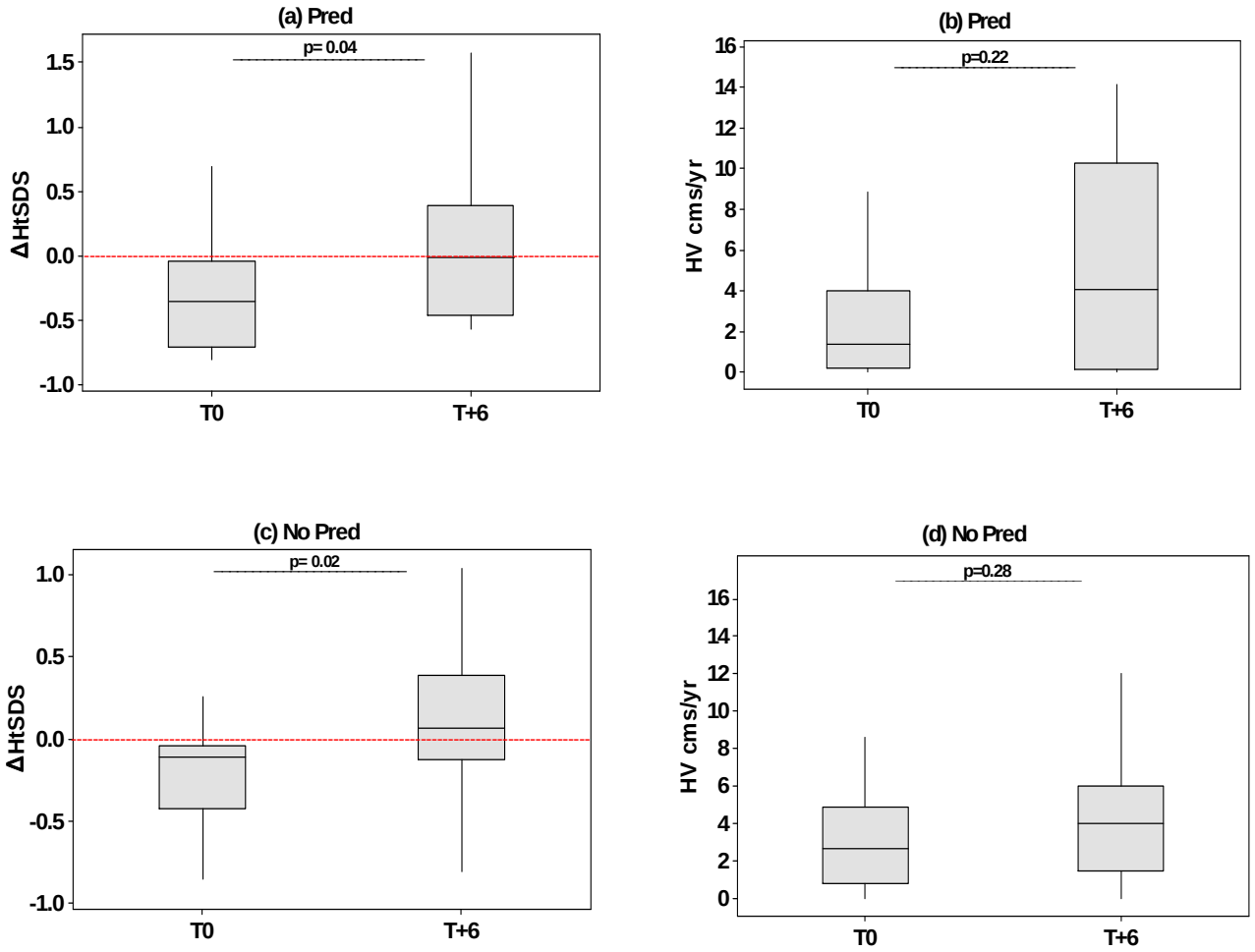
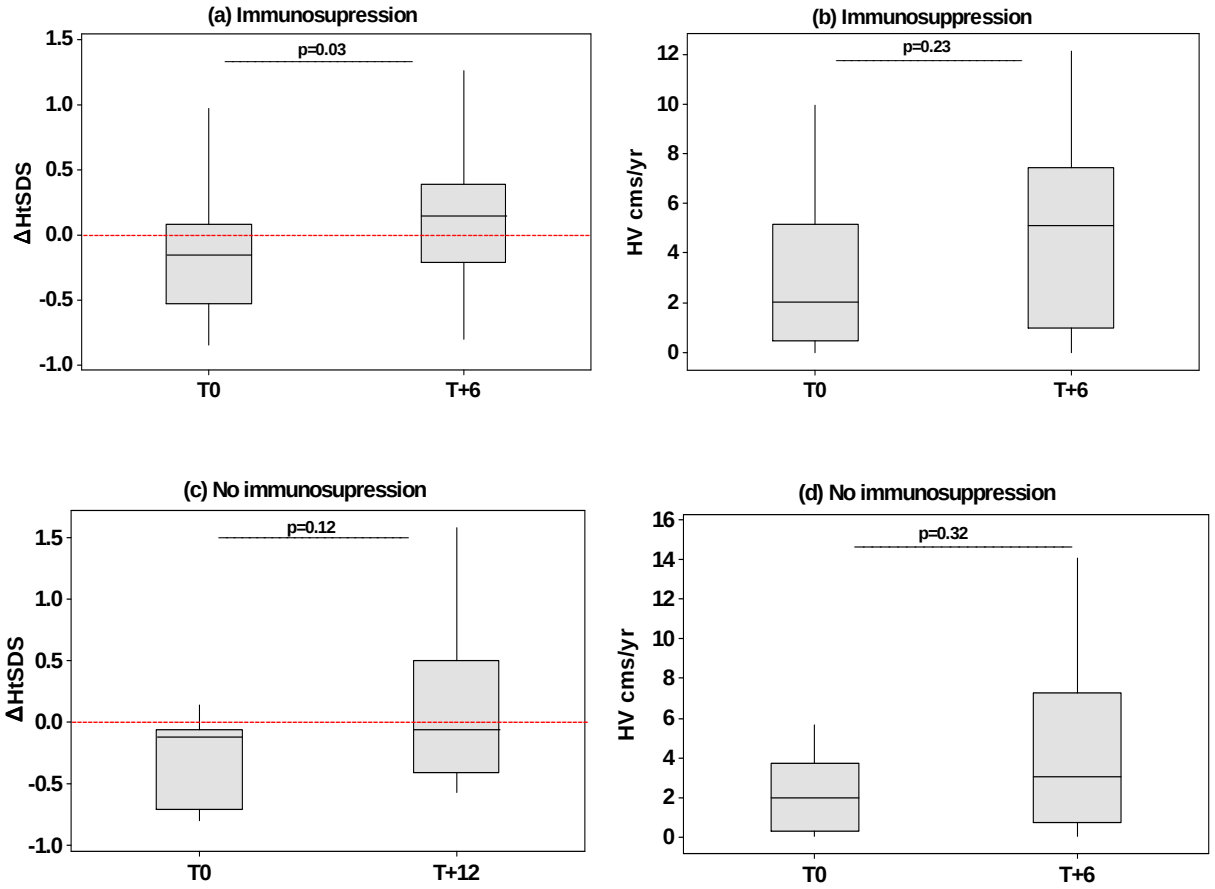


Figure 4.5.9: Growth in children with CD who were on immunosuppression therapy at starting Adalimumab. Box and whisker plots representing median, 10th, 25th, 75th, 90th centiles



children, such as those with juvenile idiopathic arthritis, which have shown that the beneficial effect of biologic therapy on growth may be more likely when the patient is receiving background immunosuppression therapy using Methotrexate (317;318). Giannini et al (318) conducted a 3-year, open label nonrandomised study to evaluate the effects of longterm anti - TNF-α etanercept treatment with or without Methotrexate on growth in children with selected categories of juvenile idiopathic arthritis. This study reported statistically significant increase in mean height percentiles from baseline for Etanercept monotherapy at year 3 only and for Etanercept in combination with the Methotrexate group at 1,2 and 3 years (318). In a similar study Billiau et al (317) also reported a significant improvement in growth velocity allowing catch-up growth in a comibined Etanercept and Methotrexate group only (317).

In our cohort there was a significant improvement in the ΔHtSDS of both children who received steroids and those who did not. There was no difference in both groups. Our data suggests that the growth-promoting effects of Adalimumab therefore is not solely due to it's ''GC-sparing effect''. This complements Infliximab data where the improvement in growth was similarly not only due to its steroid sparing effect (367).

Improvement of the disease status would be expected to be associated with pubertal progression and improved growth. We observed significant improvement in ΔHtSDS of children who were at Tanner stages I-III as compared to those who were at Tanner stages IV-V at time of starting Adalimumab. Suggesting that the improvement in growth may in part be due to progression through puberty replicating similar observations made in children treated with Infliximab (311). Pfefferkorn et al have suggested that growth is more likely to improve in those who were in early puberty at the introduction of Infliximab therapy (310), whilst this study (albeit smaller) implies that growth improvement may be *secondary* to progression in puberty.

Therapeutic interventions that have been reported to improve growth in children with CD include Infliximab, enteral nutrition and growth targeting growth hormone therapy. Short-term improvement in growth has been observed by the use of Infliximab in children with CD (156;207;279;310-312;367). Substantial evidence suggests that nutritional therapy, both elemental and polymeric formulation, has growth-promoting effects (370). Nutritional therapy has also been shown to improve height velocity (68;371) and accelerated linear development in growth in children with growth retardation (69;372). Studies have also been conducted to examine the effects of recombinant growth hormone on linear growth in children with CD and short stature (373;374). Most of these studies however, that have been conducted so far have reported changes in growth over the short term only. A recent North American study

observed changes in height z-scores at diagnosis, as well as 1 year and 2 years post diagnosis in children with CD and have reported that the distribution of height z-scores remained similar during the 2-year observation period despite improved disease activity plus the frequent use of immunomodulators and biologics (310). A recent study has also shown that at maximum follow-up no treatment was significantly associated with height improvement (335). Groups that have reported growth data in whole IBD patient cohorts including UK data from a Scottish cohort in the west of Scotland (375) have shown that current therapies do not improve final height outcome; although current treatments improve short term height most children do not improve their final height (310;335). Therefore although we are reporting short term improvement in growth, future studies need to look at final height as an outcome rather than the short term changes in height presented in most studies to date.

Improvement in linear growth after Adalimumab was observed in children who enter remission but not in those who do not. This effect of Adalimumab is similar to that of Infliximab where responders improved growth and non responders did not (311;367) . Our data also suggest that the beneficial effect of Adalimumab on growth is more likely when a child is receiving background immunosuppression therapy (either thiopurine or Methotrexate) at Adalimumab commencement. This effect of Adalimumab with concurrent immunosuppression is also similar to the findings in the cohort of children treated with Infliximab (367). In a similar manner the growth promoting effect of Adalimumab does also not seem solely due to the simple explanation of a steroid sparing effect (367). It is also interesting to note that the growth response to Adalimumab varied dependent on the reason for discontinuing Infliximab; those who had an allergic reaction to Infliximab fared best paralleling preliminary Adalimumab clinical trial data suggesting clinical response and remission are higher in patient who are anti-TNF naïve rather than those who have had no/lost response to Infliximab previously (376).

In summary, we have demonstrated that clinical response to Adalimumab therapy is associated with an improvement in linear growth over the short term in children with CD most of whom have been treated with previous Infliximab. Adalimumab continues to be mainly used at present as the second line biological agent in clinical paediatric IBD practice in the UK and elsewhere. Further prospective studies are required to clarify the effect of Adalimumab on growth, including long term follow-up to final adult height.

CHAPTER 5

Effect of biologic therapy on growth, puberty and bone health in children with Crohn’s disease

5.1 Summary

Background: Paediatric Crohn's disease (CD) is associated with abnormalities in bone health, body composition and bone metabolism. Biologic therapy (Infliximab/Adalimumab) may improve bone health, body composition and muscle function in children with CD but the extent of improvement and its relationship to disease activity are unclear.

Aim: Longitudinal observational prospective study of changes in physical growth, IGF-1 axis, bone health, body composition, muscle function and disease activity at baseline (BL), 2 weeks (2wk), 6 weeks (6wk), 6 and 12 months (6M & 12M) following biologic therapy in paediatric patients with CD.

Patients & methods: Eleven patients (M:9) commencing treatment with biologic therapy as a part of their standard medical management (<18 years), who failed to reach remission disease despite conventional therapy (corticosteroids, and/or background immunosuppression therapy with Azathioprine or Methotrexate) at a median (range) age of 14.7 years (11.2, 17.2) were recruited in the study. Data on growth, puberty, paediatric Crohn's disease activity index (PCDAI), disease markers (CRP, ESR, and Albumin), body composition using bio-electrical impedance analysis (BIA), muscle function using Leonardo mechanograph and grip strength, markers of growth hormone secretion; Insulin like growth factor-I (IGF-1 (ng/ml), Insulin like growth factor binding protein 2 (IGFBP-2 (ng/ml) and Insulin like growth factor binding protein 3 (IGFBP-3 (ng/ml), and markers of bone turnover bone-specific alkaline phosphatase (serum BALP(µg/L) and C-telopeptide of collagen cross-links (serum CTX-1 (ng/ml) were measured at BL, 2wk, 6wk, 6M and 12M. Bone and muscle parameters were measured at BL, 6M and 12M. Lumbar spine (LS), proximal femur (PF), femoral neck (FN) and total body bone mineral density (BMD (mg/cm^2) and body composition were assessed by Dual Energy X-ray Absorptiometry (DXA). DXA bone mineral content (BMC (g) data were corrected for bone area and presented as BMC z-score (SD). Bone and muscle parameters were also measured by using peripheral quantitative computed tomography (pQCT) at the non-dominant distal radius and tibia.

Results: At 12 months from baseline all patients who were receiving biologic therapy demonstrated a continued clinical response. Overall, height velocity (HV cms/yr) was 3.9cm/yr (0.2,7.4) at BL and 4.7cm/yr (0.0,9.2) at 12M (p=0.30). Median (range) BALP (µg/l) changed from 21.3 (9.2,70.5) at BL to 28.1 µg/l (5.5,117.5) at 6M(p=0.01) and 38.3 (10.9,110.5) at 12M(p=0.03). Median CTX-1(ng/ml) was 0.73(0.22,2.41) at BL and 1.21 (0.73,2.35) at 6M(p=0.04) and 1.08(0.29,2.01) at 12M(p=0.50). A positive association was observed between BALP and HV (cms/yr) at 6M (r,0.55;p=0.01) and 12M (r,0.35;p=0.049). A

significant inverse association was observed between BALP and PCDAI at BL (r,0.46;p=0.02). DXA and TANITA both indicated significant change in FFM and FFMI from BL to 12M. pQCT for tibia and radius both indicated significant change in Fat-CSA and Mus-CSA. Tibia median Fat-CSA changed 1809(892,2763) at BL to 1971(986,3505) at 6M(p=0.04) and 1899(1026,3475) at 12M(p=0.08). Tibia Mus-CSA increased significantly from 4665(3018,6184) at BL to 5356(3210,6959) at 6M (p=0.02) and 5392(3373,7055) at 12M(p=0.04). Radius median Fat-CSA was 695(684,1231) at BL and 871(600,1817) at 6M(p=0.26) and 758(366,2204) at 12M(p=0.68). Radius median Mus-CSA increased from 1827(1216,2848) at BL to 2219(1369,3094) at 6M (p=0.01) and 2539(1203,4238) at 12M(p=0.08). Leonardo mechanography showed a change in JHt(m), V-max(m/s), EFI (%), F-max(kN), P-max (kW), efficiency (%) of the movement at 1 year of biologic therapy. An inverse association was observed between JHt(m), V-max(m/s) and EFI% and PCDAI score at BL(r,0.51;p=0.01), (r,0.60;p=0.008),(r,0.63;p=0.006) and with efficiency(%) of the movement at BL (r,0.51;p=0.02) and 6wk(r,0.52;p=0.03) indicating the impact of disease activity on muscle function in patients with CD. However, no significant change was observed in total body, lumbar spine and proximal femur, femoral neck BMD. There were no significant changes in IGF-1, IGFBP-3, IGFBP-2 and ALS following biologic therapy.

Conclusion: In conclusion, these prospective studies of growth, body composition and muscle function suggests that biologic therapy in children with CD has a beneficial effect on muscle mass and muscle function and these can be observed over the first year of therapy but for comprehensive conclusion this data needs to be adjusted for body size. These positive changes are also associated with an increase in bone turnover where the change in bone formation is much greater than bone resorption. These favourable effects on bone health were not accompanied by marked changes in BMD as assessed by DXA but did show some beneficial effects on pQCT assessed SSI, a surrogate marker of bone strength. The results of this preliminary study need to be confirmed in a larger group of children.

5.2 Introduction

Children with CD have numerous risk factors for decreased bone mass, including poor growth, delayed puberty, malnutrition, changes in the growth hormone/ insulin-like growth factor axis (GH/IGF-1 axis), decreased physical activity, glucocorticoid therapy (GC) and increased inflammatory cytokines. Children with IBD, especially CD, frequently have short stature at diagnosis and height deficits which may become permanent (310). At diagnosis, growth retardation may be associated with reduced bone turnover. The pathogenesis of bone loss and growth retardation in IBD is a complex process. Both bone formation and bone resorption are decreased as reflected by the fact that biomarkers of bone formation and resorption are 30% to 50% of normal (377). Although anti-inflammatory treatment and improved nutrition are associated with normalization of bone biomarkers, bone mineral content (BMC) lags behind (377), and mechanical properties of bone may in fact worsen over time (378). In addition, height z-scores may not improve with conventional IBD therapy (310), and muscle mass deficits may also persist (213;365), which can affect the accrual of bone mass. Active inflammation may be the central mechanism responsible for alterations of normal bone metabolism. The effect of inflammation on the bone health of children with IBD has been examined in many cross-sectional (228;324) and a few longitudinal studies (377;378). Intestinal inflammation can affect bone cell function in multiple ways. Serum of children with active CD contains factors that inhibit bone formation in vitro (379). Activated T cells may shuttle inflammatory signals from gut to bone (380), and intestinal inflammation may induce an inflammatory response in the bone microenvironment (381). Sarcopenia, a specific lean mass deficit, has been found to be prevalent among both adults and children with IBD and may also adversely influence bone health (212;228;378;382).

Although the initial therapeutic strategy for CD has not been changed for a long time, the emergence of biological therapy, with its record of rapid efficacy and use as maintenance therapy has changed the management of severe paediatric CD dramatically (273;273). Studies have reported that biologic therapy restores bone formation and linear growth in children with IBD (207;311;367;383). Recent evidence suggests that Infliximab is also associated with increased muscle mass in children with CD, which should further stimulate bone mass accrual (207). Collectively, these data suggest that adequate control of inflammation is both anti-catabolic and anabolic to bone. In vitro studies have demonstrated that TNF-α inhibits bone formation by osteoblasts and promotes bone resorption by osteoclasts (199;384;385). Consistent with the hypothesis that TNF-α contributes to bone deficits in CD, recent studies in adults have demonstrated significant increases in bone

formation markers and reductions in bone resorption markers following anti-TNF-α therapy with Infliximab (319-322). To date, few published studies have examined the effect of anti-TNF-α therapy on bone density and bone metabolism and most of the data originate from studies performed in adult patients with CD (206;319;321;322;325-327). Data regarding the effect of biologic therapy on bone metabolism, body composition and BMD in children with CD is scarce and to date only one study has evaluated the impact of Infliximab therapy on bone biomarkers in children with CD (207;324). Thayu et al (207), in the REACH study of 103 children with CD observed the changes in the bone biomarkers at week 10 after Infliximab induction therapy. This study also examined the association of these changes in the bone biomarkers with subsequent changes in disease activity and linear growth during the 54-week interval after initiation of Infliximab therapy. Low BMD is relatively common complication in IBD. Osteopenia and osteoporosis are reported to affect up to 70% of both adult (386) and paediatric (382;387) patients with IBD. The mechanism by which inflammatory process affects BMD in IBD is unclear. Few studies of adults with IBD have described the beneficial impact of both short and long lasting administration of Infliximab using dual energy x-ray absorptiometry (DXA) (325-327) and only one study has reported effect of Infliximab on BMD in children with IBD (324). The impact of biologic therapy on volumetric bone mineral density (vBMD), bone structure, and muscle mass measured by using peripheral quantitative computed tomography (pQCT) is not reported so far.

To date longitudinal changes in the insulin-like growth factor (IGF) system in IBD has rarely been investigated. The majority of these studies have mainly investigated the alterations of the IGF system in growth-retarded IBD children (371;374;388). It has been generally thought that the serum IGF system which includes IGF-1, IGF-2 and their binding proteins (IGFBPs), IGFBP-3 being the most important, is down-regulated during active IBD through several systemic mechanisms (389). Moreover, only three studies (328;329;390) in adult patients have documented the impact of biologic therapy on the IGF-1 system. There are no published data regarding effect of biologic therapy on IGF-1 system in paediatric patients with CD. The administration of Infliximab leads to highly variable concentrations among patients. Therapeutic drug monitoring of Infliximab may be useful because there is an inter-individual variability of Infliximab pharmacokinetics associated with an increase in clinical response with Infliximab trough concentrations as shown in CD patients (391;392). Maser et al (392) observed a relationship between Infliximab concentrations and clinical outcomes when

studying a population of CD patients as a whole. Data regarding pharmacokinetics of Infliximab in the paediatric population is scarce at the moment there are no published studies which reported relationship between Infliximab pharmacokinetic levels and disease activity in paediatric patients with CD.

5.2.1 Aims

The aim of this longitudinal observational prospective study was to assess changes in physical growth, puberty, IGF-1 axis, bone health; body composition and muscle function following biologic therapy in paediatric patients with CD.

5.2.2 Objectives

The objective of the study was to collect clinical data and biochemical data at baseline (BL), 2 weeks (2wk), 6 weeks(6wk), 6 and 12 months (6M & 12M) following biologic therapy in paediatric patients with CD in line with the current clinical practice in management of children with CD at the Royal Hospital for Sick Children Yorkhill, Glasgow.

5.2.3 Hypothesis

Biologic therapy improves linear growth, bone health and body composition in children with CD and this is associated with changes in the IGF-1 axis and markers of bone formation and bone resorption.

5.2.4 Outcome measures

The primary outcome measures were (i) changes in height standard deviation scores (HtSDS) and Height velocity (HV), from baseline to 6 and 12 months after starting biologic therapy. Secondary, outcome measures were (i) change in pubertal status (ii) changes in systemic markers of growth hormone secretion i.e. insulin-like growth factor-1(IGF-1), insulin-like growth factor binding protein-2 (IGFBP-2), insulin-like growth factor binding protein-3 (IGFBP-3) and acid labile subunits (ALS) (iii) changes in markers of bone formation (BALP) and bone resorption (CTX-1) (iv) changes in skeletal health, body composition and muscle function measured by pQCT, DXA and bioelectrical impedance analysis (BIA), grip strength and mechanograph (v) changes in disease activity including and blood inflammatory markers erythrocyte sedimentation rate (ESR), platelets, C-reactive protein (CRP), serum albumin

(Alb), Alanine transaminase (ALT), platelets and paediatric Crohn's disease activity index (PCDAI) (V) changes in Infliximab pharmacokinetic levels.

5.2.5 Subjects and methods

In the period between June 2009 and March 2011, all children with CD who have not completed growth (Tanner stage 1-4) attending Royal Hospital for Sick Children Yorkhill, Glasgow commencing treatment with biologic therapy as part of their standard medical management (<18 years), who failed to achieve disease remission despite conventional therapy (EEN and/or corticosteroids, and/or background immunosuppression therapy with Azathioprine or Methotrexate) were eligible to participate in the study. These patients were either newly diagnosed or patients with long-lasting disease in clinical relapse. Patients on the same biologic over the prior six months were excluded from the study. All patients were approached prior to starting biologic therapy and were provided with information sheets. Information sheets can be seen in (Appendix B to D). Written informed consent was obtained from all parents/guardians and assent was obtained from children to enter the study protocol. Consent and assent forms can be found in (Appendix F to H).

Between June, 2009 and March, 2011 a total of thirteen patients started biologic therapy (Infliximab/Adalimumab). Eleven of 13 patients were recruited (10 Infliximab and 1 Adalimumab); two patients refused to participate (1 Infliximab and 1 Adalimumab) (Figure 5.2.1). All patients were diagnosed with CD using standard criteria (6;353). None of the patient was on growth or puberty promoting treatments during the period of study. The median (range) ages at diagnosis and start of biologic therapy were 11.5 years (6.3, 15.5) and 14.7 years (11.2, 17.2) respectively.

5.2.5.1 Ethical Approval

The study protocol was approved by the local research ethics committee (LREC) Reference Number: 09/S0703/58 and the research and development office. Informed consent was obtained from patients and the parents or guardians.

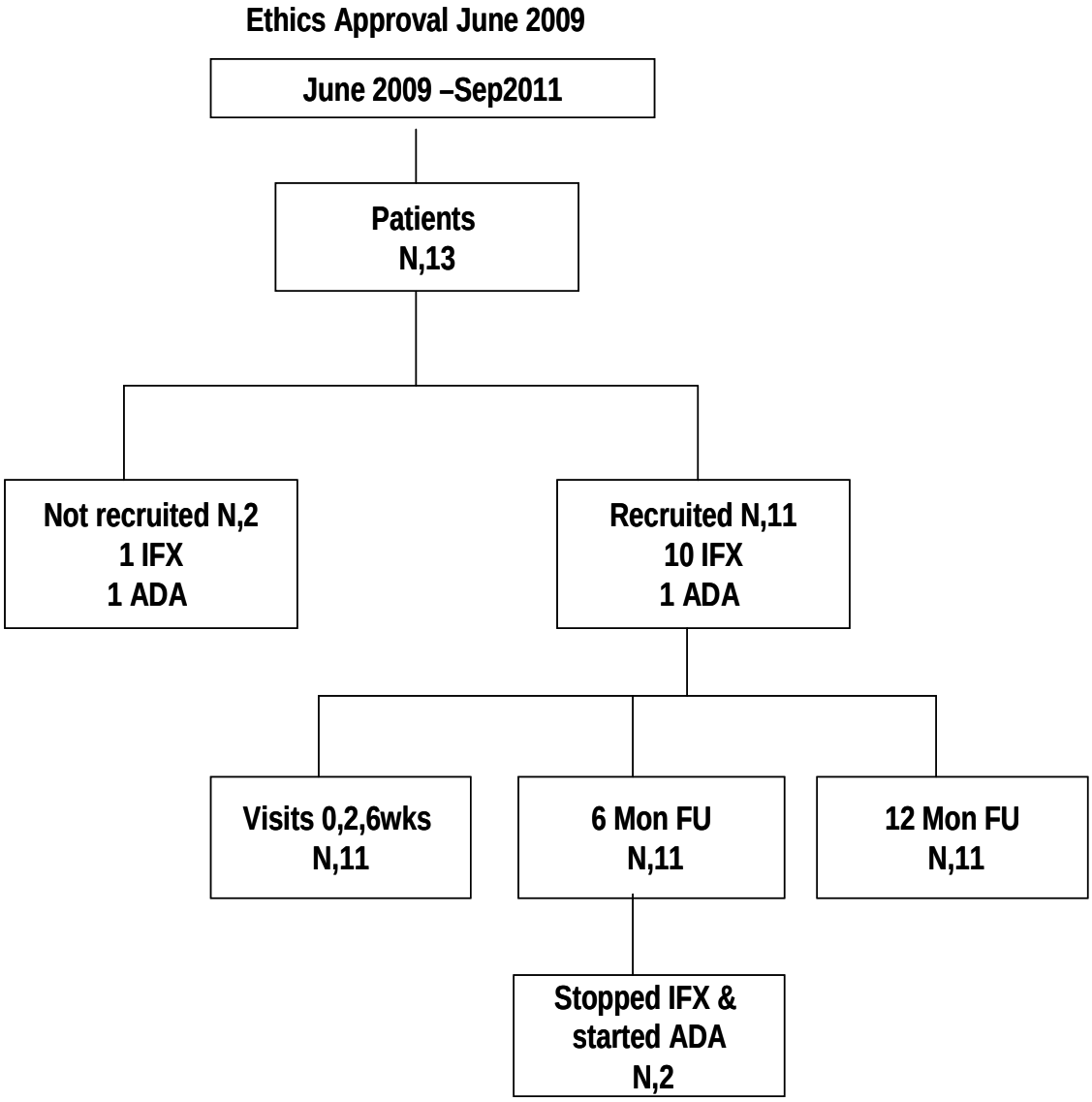


Figure 5.2.1: Flow chart of the Study Recruitment process

5.2.5.2 Anthropometric measurements

Height was measured with a Harpenden stadiometer. Height (Ht) and BMI (body mass index) data were converted into SDS (standard deviation scores) using 1990 British childhood standards (340;341), -6M, BL, 6M and 12M height velocity (HV cms/year) and change in height standard deviation scores (ΔHtSDS) were calculated from the prior 6 months of growth before time points with BL representing the point of starting biologic therapy.

5.2.5.3 Disease activity

Disease phenotype was assigned using the Montreal classification (6). Disease activity was assessed at all time points using the Paediatric Crohn's Disease Activity Index (PCDAI) based on symptoms (30%), physical examination (30%, laboratory parameters (20%, and growth data (20%, with scores ranging from 0-100) (7). An overall score was calculated taking into consideration a week history recall of symptoms, laboratory markers of disease and clinical examination. Standard definitions were used for PCDAI disease activity and remission (remission, PCDAI ≤10) (7).

5.2.5.4 Pubertal and skeletal maturation

Pubertal status was determined at BL, 6M and 12M follow-up. Puberty was self-assessed by all children, indicating their stage of development using pictures of the Tanner stages of pubertal development (338). The categories include: pre-pubertal (stage I), peri-pubertal/pubertal (stages II-III), and late pubertal/post-pubertal (stages IV-V). Bone age (BA) was assessed at BL) and 12M after the initiation of anti-TNF treatment and was assessed using the Tanner-Whitehouse (TW2) RUS method clinically by one single observer (393).

5.2.5.5 Body composition

5.2.5.5.1 Dual energy X-ray absorptiometry (DXA)

The DXA technique is based on the attenuation properties of bone, lean and fat tissues at two different x-rays energies and it measures directly the bone mineral content, lean and fat mass (FM) (394;395). Densitometric evaluation included bone area, bone mineral content (BMC), and BMD (BMD=BMC/projected area of the region scanned [g/cm^2]) for the lumbar spine

(LS), total body (TB), femoral neck (FN) and proximal femur (PF). Bone mineral apparent density (BMAD) is another frequent method for bone size adjustment used in paediatric DXA. BMAD was developed to minimize the size-related effects of DXA aBMD measurements. BMAD at lumbar spine can be calculated by estimating the vertebral depth as the square root of the area measured by DXA (Bone area). Then, the vertebral volume is calculated by simply multiplying the height x width(BA) x depth(136). Recently, Zemel et al (396). have shown that DXA BMC/BMD z-scores adjusted for height for age z-score (HAZ) provided the least biased approach for estimating the effect of short (or tall) stature on measures of BMD. Adjustments using HAZ were the least biased compared with age z-score, height age z-score, height z-score and BMAD. Therefore, this approach can be applied to assess the effect of short or tall stature on BMC/BMD z-score.

BMAD was calculated for LS and FN and was converted to z-scores (397). DXA scans were obtained at the baseline, 6M and 12M visit and were performed with a narrow fan beam lunar prodigy densitometer (GE Medical Systems, Waukesha, Wisconsin, U.S.A) and phantoms analyzed using the Encore software (Version 8.80.001). The measurements were obtained with standard positioning techniques and were analyzed to generate estimates of WB-BMC (g) and whole body lean mass (WB-LM, kg). Each scan took approximately 10-15 min to complete. All DXA scans were carried out by Dr Sheila Khanna at Yorkhill hospital for sick children, Glasgow.

5.2.5.5.2 Foot to foot impedance measurements

Bioelectrical impedance analysis (BIA) was performed u the foot to foot technique using a TANITA body fat analyzer (TBF-300, Tokyo, Japan) which provides a print out of the measured weight, impedance, and two compartment body composition analysis. Gender, age and height to the nearest centimeter were entered manually into the keypad interface. Subjects stood on the two metal sole-pad electrodes embedded on the platform scales. The electrode for each foot was subdivided into anterior and posterior electrodes. A current was applied through the anterior portion of the footpad electrodes and the voltage drop was measured in the posterior portion. The impedance measurement used a 50 kHz, 500 μA insensible current. Subjects were asked to void their bladder before the measurements. All the measurements were performed after a period of at least 10 min standing upright to

minimize potential errors from acute shifts in fluid distribution. Every measurement was performed in triplicate and averaged (398).

5.2.5.6 Bone health assessment

5.2.5.6.1 Peripheral quantitative computed tomography (pQCT)

Peripheral quantitative computed tomography is used to analyze trabecular and cortical bone characteristics, volumetric bone mineral density (vBMD; mg/cm^3), bone strength and to assess bone and muscle (CSAs) (212;399). As the radiation dose is extremely low with 0.6 µSv, pQCT is highly suitable for a longitudinal investigation of bone strength and muscle mass in children and adolescents. The most common peripheral skeletal sites selected for measurement are the radius and tibia. In this study pQCT analysis were performed using (XCT-2000; Stratech, Pforzheim, Germany). All pQCT scans were carried out by Dr Sheila Khanna at Yorkhill hospital for sick children, Glasgow. The scanner was equipped with a low-energy (38 keV) X-ray tube. The effective radiation was about 45kV at 150 µA. Calibration of the machine was performed once every three days (single slice) or once per month (multiple, slice cone phantom), respectively using phantoms provided by the manufacturer. The measuring time for a measurement run was about 4-5 minutes depending upon the cross-sectional size of the leg and the forearm. A voxel size of 0.4mm was used for both sides scan. The speed of the translational scan movement was set at 15 mm/sec. In this study pQCT measurements were performed for both radius and tibia, because the impact of impaired biomechanical stimulation and bone modeling may be more pronounced at a weight-bearing site, and cortical thickness is greater in the tibia than in the radius and is therefore less subject to partial volume effects (147). Cross-sectional slices (2mm) of the non-dominant distal radius and tibia were measured by pQCT at relative distance of 4% and 66%.The distal metaphysis (4% distal cross section) of the radius and tibia was used to determine total BMD (TotBMD) and trabecular vBMD. The diaphysis (66% distal cross section) was used to determine total, cortical cross-sectional area (CSA, mm^2); cortical vBMD. The muscle cross sectional area (CSA (mm^2), FatCSA (mm^2) and polar strength strain index (pSSI mm^3) were derived from the diaphyseal measurements and used as surrogates for muscle bone and strength. Analysis was performed with manufacturer supplied software (v.5.50; stratec). Dominance was determined by the subject report of left or right handedness.

As growth impairment is common in CD children and adolescents, all radius bone size-dependent parameters were corrected for height.

5.2.5.6.2 Distal radius

Cross-sectional slices of the non-dominant distal radius were measured by pQCT. The length of the forearm to be scanned (arm length) was measured from the ulnar styloid process to the olecranon. The subject's arm was then extended into the instrument gantry where their hand rested on a hand-grip. This allowed the subject to keep his/her arm still, thus avoiding movement artefact. The scanner was positioned on the distal forearm and a scout view was carried out. The scout view was used to position the scanner at the measurement site, and then the 4% distal cross section was identified and measured by scanner. The subject's forearm was repositioned to align the gantry positioning laser and the 66% cross section was measured (400).

5.2.5.6.3 Tibia

Tibia length was measured manually from the medial malleolus to the superior margin of the medial chondyle. The average of two measurements was used to determined tibia length. The 66% measurement site was calculated (tibia length [cm] × 0.66), measured distally from the medial malleolus and marked on the subject's calf with nonpermanent ink. The subject's leg was then extended into the instrument gantry. A scout scan was performed to visualize the distal growth plate and reference line placed at the most proximal line of the growth plate or at the end plate in case of the fussed growth plate. After establishing the distal line, the 4% distal cross section was identified and measured by scanner. The subject's leg was repositioned to align the gantry positioning laser with the reference mark on the subject's calf and the 66% cross section was measured (146).

5.2.5.7 Muscle function

5.2.5.7.1 Maximal isometric grip force

Maximal isometric grip force (MIGF) was determined for both the dominant and non-dominant hand with a standard adjustable-handled Jammer dynamometer (Preston, Jackson, MI,

U.S.A.). The test was performed sitting down with the shoulder adducted and elbow flexed at 90°. The starting position was sitting with the fore-arm held freely across the stomach. The patients were told to put maximal force on the dynamometer and to squeeze the handle as forcefully as possible for a few seconds and then released. Both hands were alternately measured in triplicate, with 60-ses rest between each test. The highest of the three measures was used for analysis. The MIGF was measured at all study time points and the result for each patient was transformed to an age- or height-dependent SDS (401).

5.2.5.7.2 Jumping mechanography

The Leonardo Mechanograph® Ground Reaction Force Platform (GRFP) Novotec Medical GmbH, Pforzheim, Germany), is a device used to assess the dynamic (kinetic) parameters deriving from motor performance (402). It is a force platform with a length of 66 cm, a width of 66 cm and a height of 7 cm. The platform is composed of two symmetrical force plates that separate the platform into a left and a right half. The resonance frequency of each plate is at 150 Hz. Each plate contains four strain gauge force sensors (the whole platform thus has eight force sensors). The force sensors measure the vertical ground reaction force exerted on the platform. The sensors are connected to a laptop computer via a USB 2.0 connection. The signal from the force sensors is sampled at a frequency of 800 Hz and is analyzed using the Leonardo mechanography GRFP software (402).

The Leonardo mechanograph software version 4.2 was used to measure lower limb muscle force, power, velocity, and jumping height (403;404). Other measurements include the Esslinger Fitness Index, which compares the maximum power relative to body weight to an age- and gender-matched reference population (405;406) and efficacy which is the maximum power relative to the peak force, i.e. maximum output for the lowest amount of force expenditure. To measure power (W/kg), velocity (m/sec), height (m), the Esslinger Fitness Index (%), and each patient performed a counter movement jump with arms moving freely, each jump was Single Two-legged Jump (S2LJ), and patients were instructed to jump as high as possible, standing upright before and after the jump. Prior to each test, patients were provided a description of the procedure and physical demonstration of the test. The force platform was adjusted to indicate a task of mass zero kg before the patient stepped onto it. Following a single-tone pitch, the patient performed the test. The termination of the test was

indicated by a double-tone pitch. Test was performed three times with a 60-sec rest between each test. The S2LJ of greatest height was selected for the analysis (402).

5.2.5.8 Biochemical markers of bone metabolism

In all children blood samples (10mls) were collected at BL, 2wk, 6wk, 6 & 12 months when possible prior to Infliximab infusion and Adalimumab injection. Samples were immediately centrifuged for 5min at 2500 rpm and then stored at -70°C until the assays were performed. Insulin like growth factor binding protein-2(IGFBP-2) and Insulin like growth factor binding protein-3(IGFBP-3) were measured by enzyme immunoassay (ELISA; Mediagnost) with intra-assay and interassay coefficients of 3.92% and 5.65 % for IGFBP-2 and 8.7% and 15.3% IGFBP-3 and no cross-reaction with other IGFBPs. Insulin-like growth factor-I was measured by an IGFBP-blocked specific EIA (IGF-R20; Mediagnost, Tuebingen, Germany) with a cross-reactivity to IGF-II of less than 0.05% and intra- and interassay coefficients of variation of 6.0% and 4.9%. Biomarkers of bone formation bone-specific alkaline phosphatase (serum BALP) and bone resorption C-telopeptide of collagen cross-links (serum CTX-1) were measured by a commercial immunoenzymetric assay kit (Immunodiagnostic Systems (IDS) Ltd), with intra-assay and interassay coefficients of variation of 7.7% and 8.8% for BALP and 3.4% and 2.7% for CTX-1. ALS was measured by enzyme immunoassay (ELISA; Mediagnost) with intra-assay and interassay coefficients of variation of 8.6% and 14.5%. IGF-1 and IGFBP-3 were converted to standard deviation scores (SDS) using computer software SDS Easy; Mediagnost GmbH, Reutlingen, Germany). In vitro determination of pharmacokinetic concentrations of Infliximab was performed by ELISA using (Immundiagnostik assay kit). The ELISAs for IGF-1, IGFBP-2, IGFBP-3, BALP, CTX-1, ALS and Infliximab pharmacokinetic concentrations) were performed by Martin McMillan in the department of child health, Yorkhill, Glasgow.

5.3 Statistical analysis

All data were analysed using by using Minitab software version 16.1 and are described as median and range and were analyzed with 1-sample Wilcoxon non-parametric test. Statistical significance was set at $p < 0.05$. Change in markers of bone metabolism, change in markers of growth hormone secretion and bone parameters measured by DXA and pQCT were expressed as percentage change between the baseline and 6 and 12 months visits. The

Pearson correlation and Spearman rank test was used to determine correlation coefficients for different variables.

5.4 Results

5.4.1 General characteristics

Infliximab induction therapy of 5 mg/kg was administered at 0, 2 and 6 weeks in all patients followed by 8 weekly infusions in those who had maintenance therapy. In one patient who had therapy with Adalimumab, induction dose of 80mg followed by 40mg 2 weeks later was used and the maintenance regimen was 2 weekly initially. One patient suffered a hypersensitivity reaction leading to withdrawal after Infliximab induction therapy and was switched to Adalimumab therapy and had an induction dose of 40mg followed by 20mg 2 weeks later was used and the maintenance regimen was 2 weekly initially. One patient lost response to Infliximab therapy after 8-months and switched to Adalimumab and had an induction dose of 80mg followed by 40mg 2 weeks later was used and the maintenance regimen was 2 weekly initially. At baseline, 6 patients were taking corticosteroids (prednisone [median 0. 07 mg/kg/day]), 9 patients had Methotrexate, 2 patients had Azathioprine, and 6 patients had enteral nutrition (n,5 exclusive and n,1 supplemental) (Table 5.4.1)

Table 5.4.1: Crohn's disease therapies at BL, 6M & 12M following biologic therapy

Treatment	BL	6M	12M
5-Aminosalicylates	6	5	4
Systemic Glucocorticoids	6	2	3
Methotrexate	9	9	9
Azathioprine	2	2	2
Enteral Nutrition			
Exclusive	5	2	2
Supplemental	1	1	-

5.4.2 Biologic therapy response

Of 11 patients, 9 demonstrated a clinical response to induction therapy, one was intolerant and developed Infliximab related peripheral neuropathy and one was primary non-responder. Two patients had an episodic therapy with Infliximab; none of these had resectional surgery. At 12 months from baseline all patients who were still receiving biologic therapy

demonstrated a continued clinical response (Table 5.4.2). Based on histopathology two patients had complete mucosal healing, seven patients had clinical remission, one was non-responder and one had no follow-up endoscopic reports available (Table 5.4.3).

5.4.3 Disease characteristics

Distribution of CD was as follows: colonic (n,6), Ileo-colonic (n,3), upper GI tract (n,1), upper and Ileo-colonic (n,1), perianal involvement(n,6). Individually, the majority of the children experienced improvement in clinical activity and improvement of the systemic inflammatory markers. All participants had active disease at BL as indicated by the PCDAI and the systemic inflammatory markers (Table 5.4.4). The median (range) value of the PCDAI decreased significantly from 35(0,55) at BL to 15(0,37.5) after 2wk, 5(0,37.5) after 6wks, 5(0,25) after 6M and 5(0,22.5) after 12M respectively, ($p<0.01$) at all time points compared with baseline (Table 5.4.5;Figure 5.4.1). PCDAI remained the same (PCDAI=0) for one patient from BL to 6M but found to be increased at 12M.

5.4.4 Change in markers of disease activity

The median(range) serum albumin (g/l) remained unchanged from 33(22,38)at BL to 35(17,39) after 2wk(p=0.62), 34(26,38) after 6wks(p=0.09), 36(26,41) after 6M(p=0.16) and 35(26,40) after 12M(p=0.09). The median ALT(U/l) remained unchanged from 13(6,64) at BL to 14(10,38) after 2wk (p=0.32), 10(6,42) after 6wks(p=0.26), 12(9,14) after 6M(p=0.31) and 13(7,36) after 12M(p=0.72).The median ESR(mm/hr) decreased significantly from 24(5,70) at BL to 12(4,98) after 2wk(p=0.01), 12(3,51) after 6wks(p=0.12), 13(4,56) after 6M(p=0.23) and 12(2,48) after 12M, (p=0.15).The median CRP(mg/l) remained unchanged from 10(3,95) at BL to 7(3,86) after 2wk(p=0.44), 7(3,56) after 6wks(p=0.28), 7(3,39) after 6M(p=0.14) and 6(3,23) after 12M(p=0.15). The median platelet count (10^9/L)(g/l) changed from 345(193,447) at BL to 329(148,489) after 2wk(p=0.87), 295(148,624) after 6wks(p=0.91), 319(174,565) after 6M(p=0.62) and 281(115,480) after 12M(p=0.06)(Table 5.4.5).

Table 5.4.2: Biologic therapy response in paediatric patients with CD

Pt.No	Drug	3M	Tot INF/INJ	6M	Tot INF/INJ	12M	Tot INF/INJ	Episodic	Maintenance	ADR
1	IFX	Responder	3	Responder	6	Responder	9	N	Y	N
2	IFX	Intolerant	3	Stopped IFX Started ADA	3	Responder	38	N	Y	Hypersensitivity reaction
3	IFX	Responder	3	Responder	5	Responder	9	N	Y	N
4	ADA	Responder	3	Responder	13	Responder	38	N	Y	N
5	IFX	Responder	3	Responder	6	Responder	9	N	Y	N
6	IFX	Responder	3	Responder	5	Responder	9	N	Y	N
7	IFX	Responder	3	Responder	5	Responder	9	N	Y	N
8	IFX	Responder	3	Responder	5	Responder	9	N	Y	N
9	IFX	Responder	3	Stopped IFX Bridging to AZA	3	Responder	6	Y	N	N
10	IFX	Primary non-responder	3	Stopped IFX Secondary loss of response	4	Started ADA Responder	6	N	Y	N
11	IFX	Responder /Intolerant	3	Stopped IFX	4	Responder	9	Y	N	Peripheral neuropathy

Table 5.4.3: Biologic therapy response in paediatric patients with CD based on histopathology

Pt.No	Drug	Baseline to follow-up
1	Infliximab	Complete mucosal healing
2	Infliximab/Adalimumab	Intolerant to Infliximab but perianal disease responded for Adalimumab
3	Infliximab	Clinical responder
4	Adalimumab	Clinical remission but no endoscopic remission
5	Infliximab	Responder to Infliximab but not in remission
6	Infliximab	Clinical responder but still have microscopic disease
7	Infliximab	Complete mucosal healing
8	Infliximab	No follow-up endoscopic reports
9	Infliximab	Clinical remission
10	Infliximab/Adalimumab	Non-responder to Infliximab
11	Infliximab	Clinical remission

5.4.5 Change in linear growth

Height standard deviation scores (HtSDS) remained below zero and did not change significantly during the study interval overall although some patients demonstrated improvement in HtSDS from BL to 12M. Median (range) HtSDS at diagnosis -1.6(-2.1, 1.5) was lower than the mean mid-parental height SDS of 0.0(-0.9,1.1) (p=0.62). The median HtSDS was -0.7(-2.6,1.6) at BL, -0.7(-2.9,1.7) after 6M and -0.4(-3.1,1.7) after 12M respectively. Median SHtSDS was -0.8(-1.7,1.5) at BL, -1.1(-2.3,1.5) at 6M(p=0.35) and -0.2(-2.2,0.9) at 12M (p=0.19). The median BMISDS at diagnosis was -1.6(-2.1,-0.0), and remained below zero during the study interval overall, although some patients demonstrated improvement in BMISDS from BL to 12M. Median BMISDS was -0.3(-2.6,0.9) at BL, -0.6(-2.2,0.1) after 6M(p=0.03) and -0.5(-1.8,1.1) after 12M(p=0.16) respectively (Table 5.4.6;Figure 5.4.2). Median ΔHtSDS did not show any significant change from -0.1(-0.5,0.3) at BL to -0.1(- 0.1,-0.5) at 6M (p=0.61) to 0.1(-0.3,0.4) at 12M (p=0.22) (Figure 5.6.3). Median HV of overall group was 3.9cm/year (0.2,7.4) at T0, 2.1cm/year (0.7,9.8) at 6M(p=0.78) and 4.7(0.0,9.2) at 12M (p=0.30) (Table 5.4.6;Figure 5.4.3).

5.4.6 Change in skeletal maturation and bone age

Median bone age changed from 14.2(9.3,16.9) at BL to 15.3(9.7,18.2) (p=0.009) and did not differ significantly when compared to chronological at BL and 12M. At BL, 7 patients were in tanner stage (TS) I-III and three were in TS-IV none of the patients were in TSV. At 6M seven patients were in TS (II-III), two were in TS-IV and two progressed to TS-IV. At 12M four patients were in TS-III, three in TS-IV and four in TS-V (Table 5.4.6).

5.4.7 Change in muscle function

5.4.7.1 Maximum isometric grip force

Individual patient measurements for MIGF for both dominant and non-dominant hand are show in (Table 5.4.7). The median measurement of the MIGF(kg) dominant hand was 19(10,25) at BL and 22(8,35) at 2wk (p=0.68), 21(8,39) at 6wk(p=0.44), 27(11,36)(p=0.02) and 22(11,36) at 12M(p=0.01) (Table 5.4.8).The median measurement of the MIGF(kg) non-

dominant hand was 18(8,27) at BL, 19(8,30) at 2wk (p=0.18), 18(6,37) at 6wk(p=0.90), 22(9,33)(p=0.01) and 20(10,34) at 12M (p=0.01) (Table 5.4.8).

5.4.7.1.1 Height adjusted MIGFSDS

The median measurement of the dominant hand height adjusted MIGFSDS changed from -1.0(-2.3,1.4) at BL to -1.1(-2.0,1.6) at 2wk(p=1.0), -0.5(-2.3,1.8) at 6wk(p=0.67), 0.3(1.9,1.7)(p=0.05) at 6M and -0.5(-1.7,1.2) at 12M(p=0.04). The median measurement of the non-dominant hand height adjusted MIGFSDS changed from -1.5(-2.7,0.5) at BL to -1.3(-2.2,1.0) at 2wk(p=0.26), -1.3(-2.9,1.9) at 6wk(p=0.81), -0.7(-2.2,1.1)(p=0.06) and -0.9(-2.1,0.5) at 12M(p=0.04)(Table 5.4.8; Figure 5.4.4)

5.4.7.1.2 Age adjusted MIGFSDS

The median measurement of the dominant hand age adjusted MIGFSDS changed from -1.9(-3.5,-0.1) at BL to -1.3(-3.8,0.6) at 2wk (p=1.0), -0.7(-4.3,0.7) at 6wk(p=1.0), -0.8(-3.2,0.9)(p=0.04) and -1.3(-3.6,0.4) at 12M (p=0.08). The median measurement of the non dominant hand age adjusted MIGFSDS changed from -2.1(-3.3,-0.5) at BL to -1.6(-4.2,0.0) at 2wk (p=0.26), -1.8(-5.5,0.8) at 6wk(p=1.0), -1.3(-4.0,0.1)(p=0.10) and -1.2(-4.0,0.3) at 12M (p=0.15) respectively (Table 5.4.8; Figure 5.4.4)

Figure 5.4.1: Individual changes in paediatric Crohn's disease activity index (PCDAI) at BL, 2wks, 6wks, 6M & 12M following biologic therapy in paediatric patients with CD

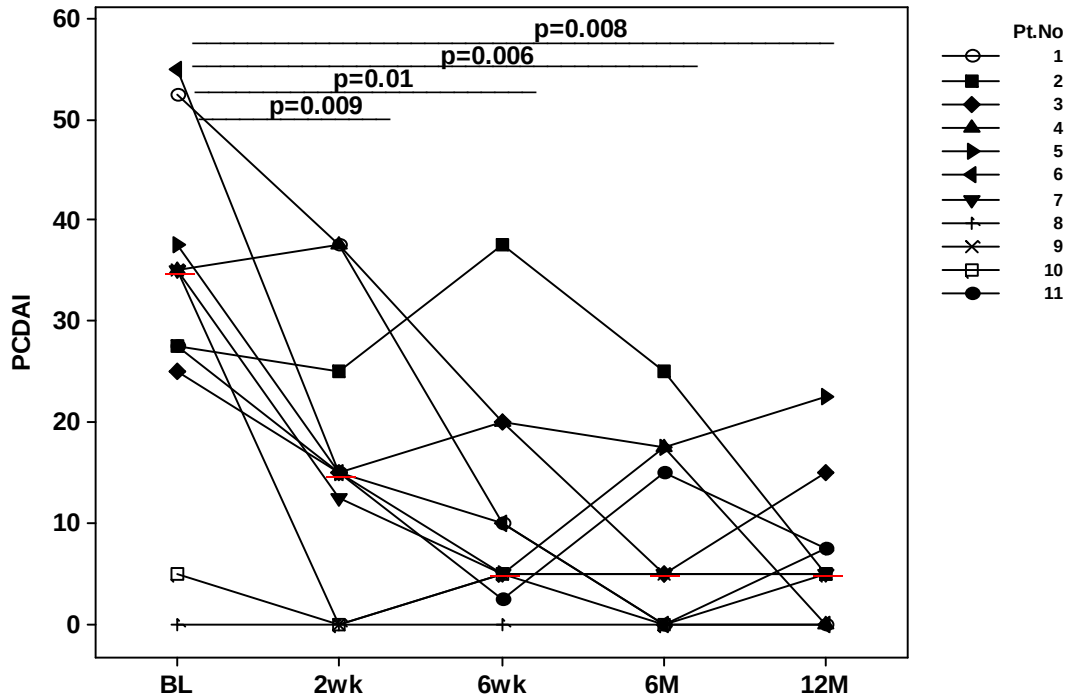


Table 5.4.4: Individual changes in PCDAI and systemic markers of disease activity at BL, 2wks, 6wks, 6M & 12M following biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
PCDAI											
BL	52.5	27.5	25	35	37.5	55	35	0	35	5	27.5
2wk	37.5	25	15	37.5	15	15	12.5	0	0	0	15
6wk	10	37.5	20	20	5	10	5	0	5	5	2.5
6M	0	25	5	17.5	17.5	0	5	0	5	0	15
12M	0	5	15	0	22.5	0	5	7.5	5	5	7.5
Serum albumin (g/l)											
BL	26	30	33	25	28	22	33	36	38	38	34
2wk	30	26	35	17	30	25	35	35	39	36	36
6wk	34	26	31	28	32	29	37	37	38	38	37
6M	36	26	32	27	28	*	40	37	41	*	37
12M	36	34	29	36	26	32	37	35	39	40	35
ALT(U/l)											
BL	28	13	11	7	54	13	6	18	16	6	64
2wk	21	10	10	11	38	14	17	16	10	10	35
6wk	25	8	10	12	10	6	19	23	10	6	42
6M	9	14	12	14	12	*	14	12	9	*	14
12M	11	36	13	18	19	11	7	8	21	11	18
ESR (mm/hr)											
BL	33	45	15	70	13	66	*	5	22	13	25
2wk	26	50	6	98	5	19	11	4	12	12	9
6wk	15	40	6	51	8	4	17	6	12	3	25
6M	8	49	6	56	25	*	4	11	13	*	16
12M	18	10	4	12	48	6	5	25	2	12	22
CRP (mg/l)											
BL	29	11	10	70	9	95	15	7	7	3	3
2wk	7	29	7	86	7	7	7	7	7	3	3
6wk	7	28	7	56	7	7	7	7	3	13	6
6M	7	9	7	27	39	*	7	3	3	*	3
12M	7	7	3.7	7	18	3	3	23	3	3	6
Platelets (10^9/L)											
BL	342	447	323	420	362	329	*	347	193	258	357
2wk	329	471	263	489	323	445	381	320	148	318	347
6wk	241	624	258	487	311	409	234	295	148	264	337
6M	237	565	303	450	316	*	319	350	174	*	351
12M	239	282	293	361	269	480	271	115	165	281	312

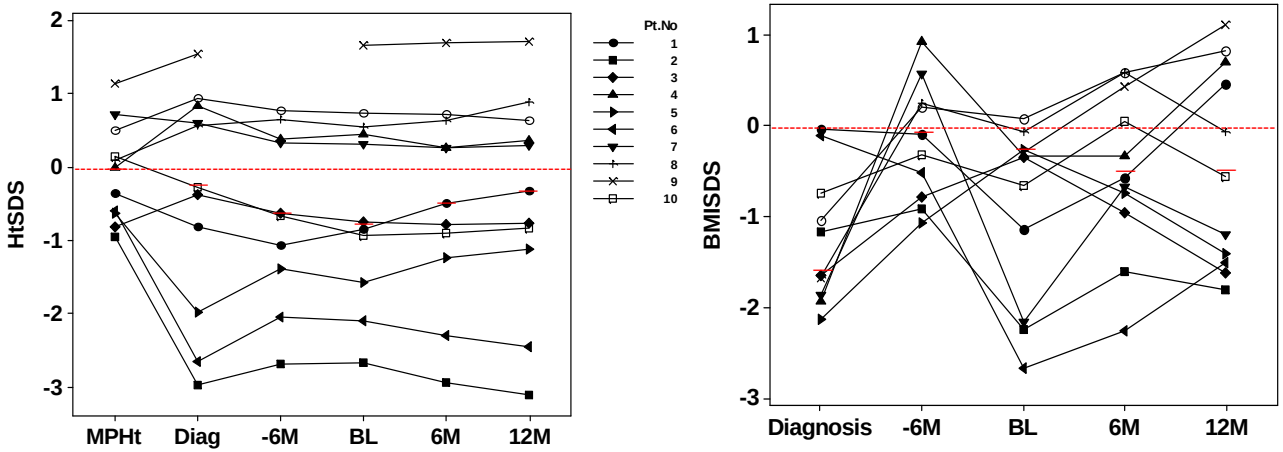
Table 5.4.5: Changes in PCDAI and systemic markers of disease activity at BL, 2wks, 6wks, 6M & 12M following biologic therapy in paediatric patients with CD Median(range)

	BL N,11	2wk N,11	6wk N,11	6M N,11	12M N,11	p-Value			
						BL -2wk	BL- 6wk	BL -6M	BL-12M
PCDAI	35 (0,55)	15 (0,37.5)	5 (0,37.5)	5 (0,25)	5 (0,22.5)	**0.009**	**0.01**	**0.006**	**0.008**
Serum albumin (g/l)	33 (22,38)	35 (17,39)	34 (26,38)	36 (26,41)	35 (26,40)	0.62	0.09	0.16	0.09
ALT(U/l)	13 (6,64)	14 (10,38)	10 (6,42)	12 (9,14)	13 (7,36)	0.32	0.26	0.31	0.72
ESR (mm/hr)	24 (5,70)	12 (4,98)	12 (3,51)	13 (4,56)	12 (2,48)	0.12	**0.01**	0.23	0.15
CRP (mg/l)	10 (3,95)	7 (3,86)	7 (3,56)	7 (3,39)	6 (3,23)	0.44	0.28	0.14	0.15
Platelets (10^9/L)	345 (193,447)	329 (148,489)	295 (148,624)	319 (174,565)	281 (115,480)	0.87	0.91	0.62	0.06

Table 5.4.6: Anthropometric details of children (n,11) at starting biologics (BL) at 6 & 12 months (6M &12M) following biologic therapy in paediatric patients with CD. Results are presented as median (range)

	BL N,11	T+6 N,11	T+12 N,11	p-Value BL - T+6	p-Value BL- T+12
Age (yrs)	14.7 (11.2,17.2)	15.3 (11.8,17.7)	15.9 (12.4,18.2)		
Bone age (yrs)	14.2 (9.3,16.9)		15.3 (9.7,18.2)		**0.009**
Anthropometry					
Height SDS	-0.7 (-2.7,1.7)	-0.5 (-2.9,1.7)	-0.4 (-3.1,1.7)	0.82	0.89
∆Height SDS	-0.1 (-0.5,0.3)	-0.1 (-0.1,-0.5)	0.1 (-0.3,0.4)	0.61	0.22
BMI SDS	-0.3 (-2.7,0.1)	-0.6 (-2.3,0.6)	-0.6 (-1.8,1.1)	**0.03**	0.16
Height Velocity (cm/yr)	3.9 (0.2,7.4)	2.1 (0.7,9.8)	4.7 (0.0,9.2)	0.78	0.30
SHt SDS	-0.8 (-1.7,1.5)	-1.1 (-2.3,1.5)	-0.2 (-2.2,0.9)	0.35	0.19
Pubertal status (n ;%)					
-I	2 (18.1)	-	-		
-II	2(18.1)	2(18.1)	-		
-III	3(27.2)	5(45.4)	4(36.3)		
-IV	4(36.3)	2(18.1)	3(27.2)		
-V	-	2(18.1)	4(36.3)		

Figure 5.4.2: Individual changes in height SDS (HtSDS) and body mass index SDS (BMISDS) and sitting height SDS (SHtSDS) following treatment with biologic therapy in paediatric patients with CD



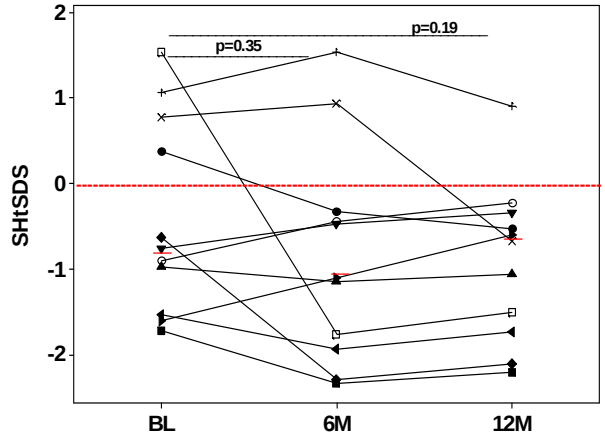


Figure 5.4.3: Individual changes in height velocity (HV) and change in height SDS (ΔHtSDS) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

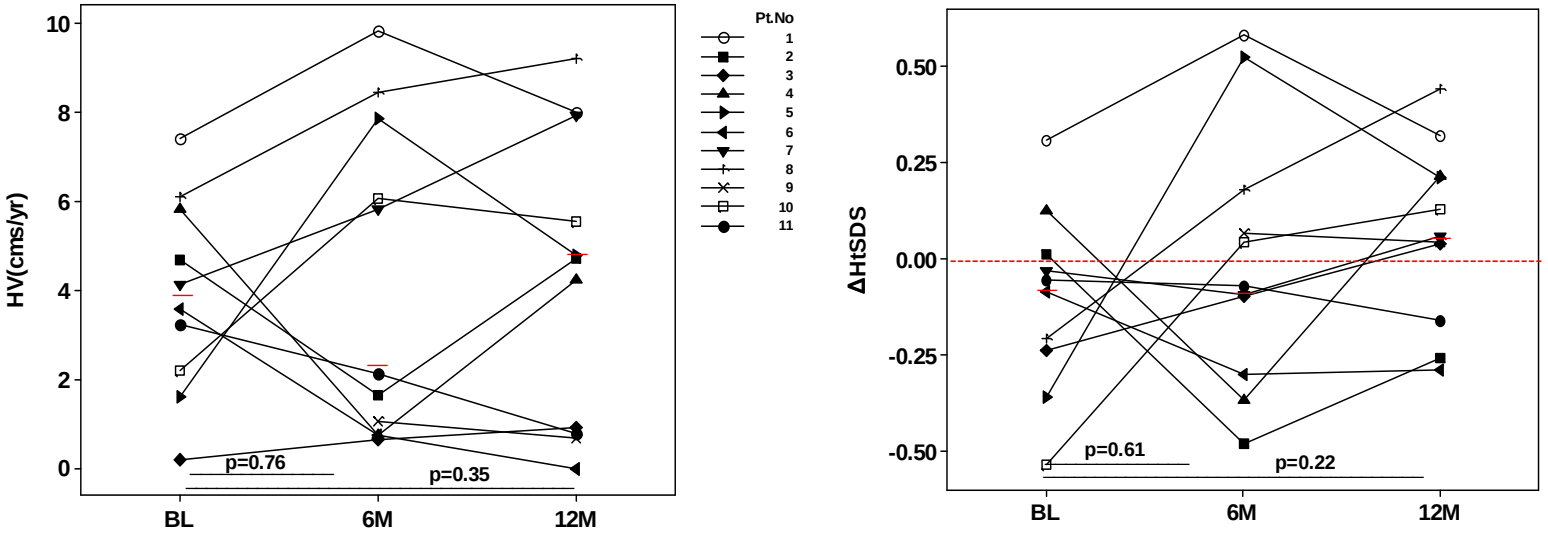


Table 5.4.7: Individual changes in maximum isometric grip force (MIGF) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients

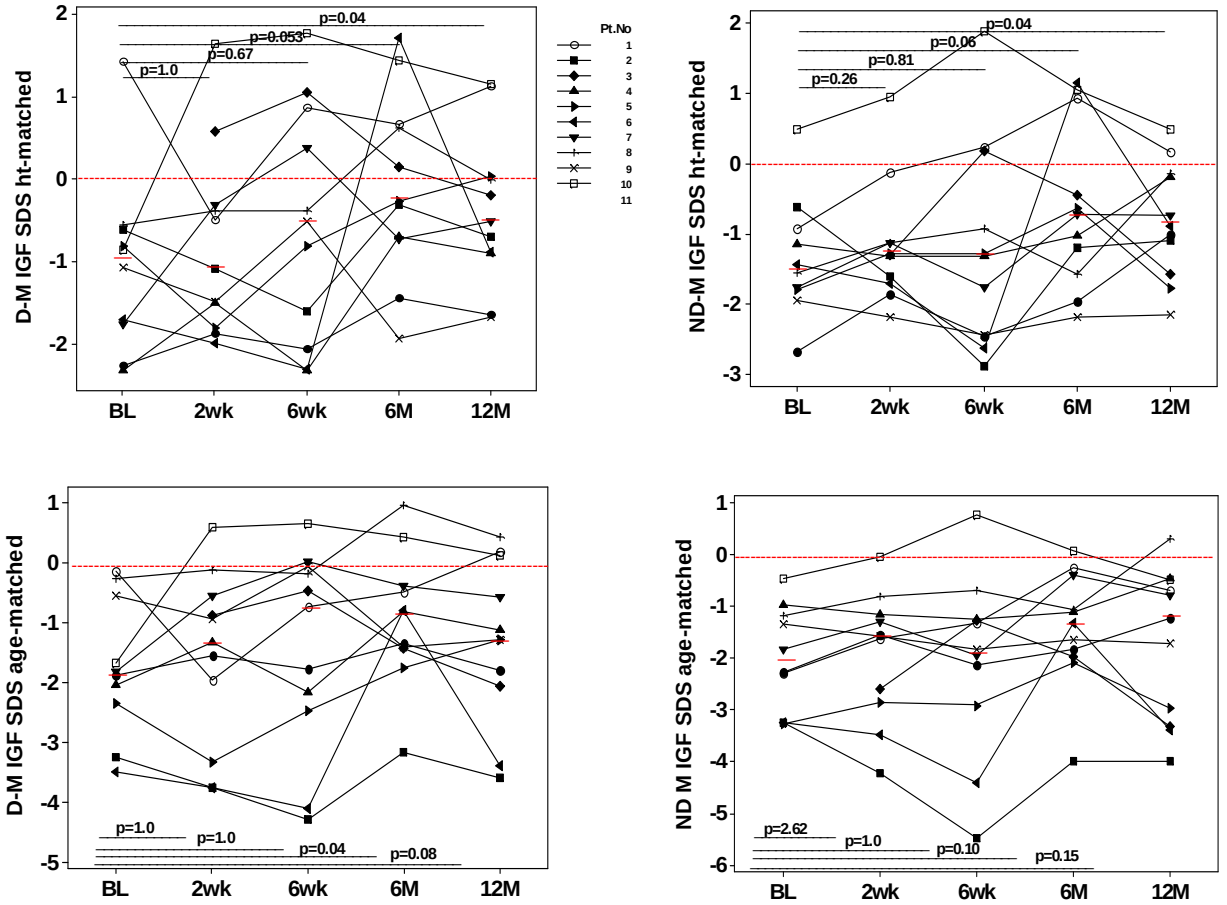
Pt.No	1	2	3	4	5	6	7	8	9	10	11
Dominant hand MIGF (kg)											
BL	18	10	*	20	10	16	13	25	24	20	22
2wk	12	9	35	24	8	15	18	26	22	35	24
6wk	16	8	39	20	10	14	21	26	27	36	23
6M	18	11	32	29	13	34	20	36	20	36	27
12M	22	*	30	29	15	19	21	35	21	36	26
Dominant Ht-matched MIGF SDS											
BL	1.4	-0.6	*	-2.3	-0.8	-1.7	-1.8	-0.6	-1.1	-0.8	-2.2
2wk	-0.5	-1.1	0.6	-1.5	-1.8	-2.0	-0.3	-0.4	-1.5	1.6	-1.9
6wk	0.9	-1.6	1.1	-2.3	-0.8	-2.3	0.4	-0.4	-0.5	1.8	-2.1
6M	0.7	-0.3	0.1	-0.7	-0.3	1.7	-0.7	0.6	-1.9	1.4	-1.4
12M	1.1	-0.7	-0.2	-0.9	0.0	-0.9	-0.5	0.0	-1.7	1.2	-1.6
Dominant Age-matched MIGF SDS											
BL	-0.1	-3.3	*	-2.0	-2.4	-3.5	-1.8	-0.3	-0.6	-1.7	-1.9
2wk	-2.0	-3.7	-0.9	-1.3	-3.3	-3.8	-0.6	-0.1	-0.9	0.6	-1.5
6wk	-0.7	-4.3	-0.5	-2.2	-2.5	-4.1	0.0	-0.2	-0.1	0.7	-1.8
6M	-0.5	-3.2	-1.4	-0.8	-1.8	-0.8	-0.4	0.9	-1.4	0.4	-1.3
12M	0.2	-3.6	-2.1	-1.1	-1.3	-3.4	-0.6	-1.3	-1.3	0.1	-1.8
Non-dominant hand MIGF (kg)											
BL	11	10	*	26	8	17	13	20	20	27	20
2wk	13	8	23	25	9	16	15	22	19	30	24
6wk	14	6	32	25	9	13	13	23	18	37	21
6M	19	9	28	27	12	30	20	22	19	33	24
12M	18	*	22	34	10	19	20	34	19	31	30
Non-dominant Ht-matched MIGF SDS											
BL	-0.9	-0.6	*	-1.1	-1.8	-1.4	-1.8	-1.5	-1.9	0.5	-2.7
2wk	-0.1	-1.6	-1.3	-1.3	-1.3	-1.7	-1.1	-1.1	-2.2	1.0	-1.9
6wk	0.2	-2.9	0.2	-1.3	-1.3	-2.6	-1.8	-0.9	-2.4	1.9	-2.5
6M	0.9	-1.2	-0.4	-1.0	-0.6	1.1	-0.7	-1.6	-2.2	1.1	-2.0
12M	0.2	-1.1	-1.6	-0.2	-1.8	-0.9	-0.7	-0.1	-2.1	0.5	-1.0
Non-dominant Age-matched MIGF SDS											
BL	-2.3	-3.3	*	-1.0	-3.3	-3.2	-1.8	-1.2	-1.3	-0.5	-2.3
2wk	-1.6	-4.2	-2.6	-1.2	-2.8	-3.5	-1.3	-0.8	-1.6	0.0	-1.5
6wk	-1.3	-5.5	-1.3	-1.2	-2.9	-4.4	-1.9	-0.7	-1.8	0.8	-2.1
6M	-0.3	-4.0	-2.0	-1.1	-2.1	-1.3	-0.4	-1.1	-1.6	0.1	-1.8
12M	-0.7	-4.0	-3.3	-0.5	-3.0	-3.4	-0.8	0.3	-1.7	-0.5	-1.2

Table 5.4.8: Changes in maximum isometric grip force (MIGF) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)

						p-Value			
Value	**BL N,11**	**2wk N,11**	**6wk N,11**	**6M N,11**	**12M N,11**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
D- hand MIGF(kg)	19 (10,25)	22 (8,35)	21 (8,39)	27 (11,36)	22 (11,36)	0.68	0.44	**0.02**	**0.01**
D- hand Ht-matched MIGF SDS	-1.0 (-2.3,1.4)	-1.1 (-2.0,1.6)	-0.5 (-2.3,1.8)	-0.3 (-1.9,1.7)	-0.5 (-1.7,1.2)	1.0	0.67	0.05	**0.04**
D- hand Age-matched MIGF SDS	-1.9 (-3.5,-0.1)	-1.3 (-3.8,0.6)	-0.7 (-4.3,0.7)	-0.8 (-3.2,0.9)	-1.3 (-3.6,0.4)	1.0	1.0	**0.04**	0.08
ND-hand MIGF(kg)	18 (8,27)	19 (8,30)	18 (6,37)	22 (9,33)	20 (10,34)	0.18	0.90	**0.01**	**0.01**
ND hand Ht-matched MIGF SDS	-1.5 (-2.7,0.5)	-1.3 (-2.2,1.0)	-1.3 (-2.9,1.9)	-0.7 (-2.2,1.1)	-0.9 (-2.1,0.5)	0.26	0.81	0.06	**0.04**
ND hand Age-matched MIGF SDS	-2.1 (-3.3,-0.5)	-1.6 (-4.2,0.0)	-1.8 (-5.5,0.8)	-1.3 (-4.0,0.1)	-1.2 (-4.0,0.3)	0.26	1.0	0.10	0.15

D-(dominant), ND- (non-dominant)

Figure 5.4.4: Changes in maximum MIGF age-adjusted and height-adjusted z-score (SD) for dominant and non-dominant hand at BL, 2wks, 6wks, 6M & 12M during treatment with biologic therapy in paediatric CD patients



5.4.8 Mechanograph

The Leonardo mechanograph was used to measure lower limb muscle force, power, velocity, jumping height and efficiency of the movement. Individual patient measurements for jump height (m), maximum-velocity (V-max (m/s), Esslinger fitness index (EFI (%), maximum - force (F-max (kN), maximum-power (P-max (kW), efficiency (%) of the movement are shown in (Table 5.4.10;Figure 5.4.5). Median (range) jump height (m) changed from 0.29(0.20,0.43) at BL to 0.34(0.24,0.50) at 2wk(p=0.54), 0.35(0.20,0.75) at 6wk(p=20), 0.35(0.32,0.53) 6M (p=10) and 0.36(0.27,0.52) at 12M (p=0.12). Median maximum-velocity (Vmax (m/s) changed from 1.96(1.42,2.46) at BL to 2.05(1.61,2.73) at 2wk (p=0.72), 2.19(1.57,2.70) at 6wk (p=0.52), 0.22(2.03,2.79) 6M (p=0.09) and 2.30(1.91,2.87) at 12M (p=0.05). Median Esslinger fitness index (EFI%) changed from 73(50,108) at BL to 80(55,116) at 2wk (p=0.91), 92(63,105) at 6wk(p=0.72), 94(81,108) 6M (p=0.14) and 96(65,109) at 12M (p=0.05). Median maximum-force (F-max (kN) changed from 1.01(0.60,1.41) at BL to 1.09(0.56,1.64) at 2wk (p=0.91), 1.15(0.24,1.48) at 6wk(p=1.0), 1.27(0.69,1.41) 6M (p=0.10) and 1.28(0.68,1.79) at 12M(p=0.03). Median maximum-power (P-max (kW) changed from 1.35(0.74,2.70) at BL to 1.95(0.66,3.48) at 2wk (p=0.61),2.16(0.66,3.17) at 6wk(p=0.29), 2.31(1.19,3.11) 6M (p=0.08) and at 2.26(0.99,3.68) 12M (p=0.03). Median efficiency % also changed from 87(46,105) at BL to 91(53,107) at 2wk (p=0.51), 92(64,102) at 6wk (p=1.0), 95(85,104) 6M (p=0.14) and 96(65,109) at 12M (p=0.10) (Table 5.4.10). Despite the fact that the improvement in efficiency% of the movement was not significant, but the change was likely to be through improvements in jump height and velocity thereby indicating higher muscular flexibility.

5.4.8.1 Association of mechanograph and disease activity

A significant negative association was observed between jump height (m), V-max (m/s and EFI% and PCDAI score at BL (r,0.51;p=0.01), (r,0.60;p=0.008),(r,0.63;p=0.006) and with efficiency (%) of the movement at BL (r,0.51;p=0.02) and 6wk(r,0.52;p=0.03) respectively (Figure 5.4.6).

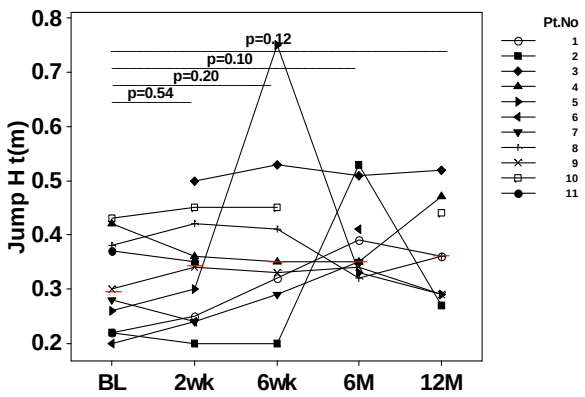
Table 5.4.9: Individual changes in maximum isometric grip force (MIGF) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients

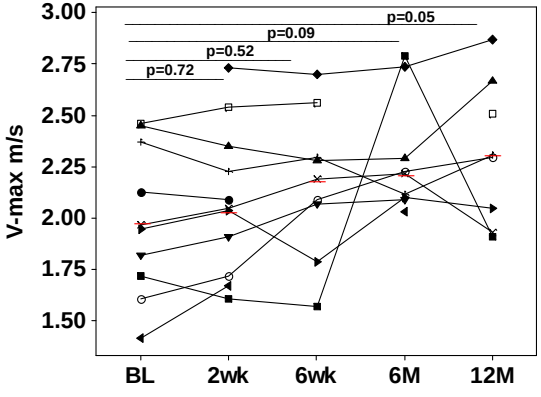
Pt.No	1	2	3	4	5	6	7	8	9	10	11
Jump Ht(m)											
BL	0.22	0.22	*	0.42	0.36	0.20	0.28	0.38	0.30	0.43	0.37
2wk	0.35	0.20	0.50	0.36	0.30	0.24	0.24	0.42	0.34	0.45	0.35
6wk	0.32	0.20	0.53	0.35	0.75	*	0.29	0.41	0.33	0.45	*
6M	0.40	0.55	0.51	0.35	0.33	0.41	0.35	0.32	0.34	*	*
12M	0.36	0.27	0.52	0.47	0.29	*	*	0.36	0.29	0.44	*
V-max(m/s)											
BL	1.61	1.72	*	2.45	1.95	1.42	1.82	2.37	1.97	2.46	2.13
2wk	1.72	1.61	2.73	2.35	2.04	1.67	1.91	2.23	2.15	2.54	2.09
6wk	2.19	1.57	2.70	2.28	1.79	*	2.07	2.30	2.24	2.56	*
6M	2.23	2.79	2.74	2.29	2.10	2.07	2.09	2.12	2.22	*	*
12M	2.30	1.91	2.87	2.67	2.05	*	*	2.31	1.93	2.51	*
EFI%											
BL	63	70	*	99	88	50	68	108	64	101	76
2wk	67	62	116	90	95	55	80	91	71	104	74
6wk	92	63	105	80	84	*	92	96	77	103	*
6M	102	108	107	84	99	82	94	81	82	*	*
12M	106	72	108	109	91	*	*	91	65	101	*
F-max(tot)kN											
BL	0.8	0.6	*	1.4	0.6	1.0	0.6	1.4	1.0	1.0	1.2
2wk	0.7	0.5	1.6	1.2	0.7	1.1	0.7	1.3	1.1	1.1	1.2
6wk	1.0	0.5	1.4	1.1	0.2	*	0.8	1.4	1.1	1.2	*
6M	1.2	1.3	1.3	1.3	0.7	0.8	0.9	1.4	1.3	*	*
12M	1.2	0.7	1.2	1.7	0.7	*	*	1.8	1.4	1.1	*
P-max(tot)kW											
BL	0.8	0.7	*	2.7	1.0	0.1	0.9	2.5	1.7	2.1	2.0
2wk	0.9	0.6	3.4	2.4	1.1	1.2	1.1	2.2	1.9	2.2	2.0
6wk	1.4	0.6	3.1	2.2	1.1	*	1.4	2.4	2.2	2.3	*
6M	1.8	3.1	3.1	2.3	1.1	1.3	1.5	2.4	2.4	*	*
12M	2.1	0.1	3.0	3.6	1.1	*	*	2.7	2.1	2.3	*
Efficiency %											
BL	63	70	*	95	99	46	86	97	89	105	85
2wk	75	66	97	92	101	53	88	91	93	107	85
6wk	85	64	97	92	87	*	92	94	100	102	*
6M	86	103	104	85	103	95	95	89	98	*	*
12M	106	72	108	109	91	*	*	75	65	101	*

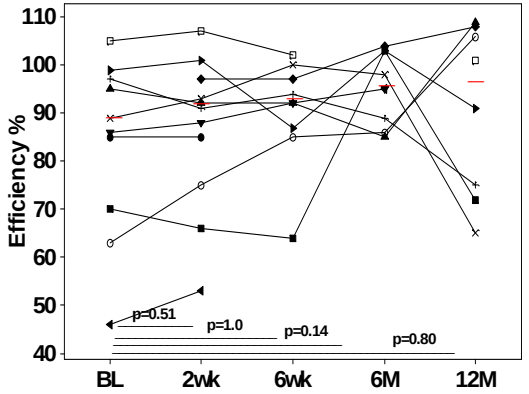
Table 5.4.10: Changes in mechanography jump height (m), maximum-velocity (V-max (m/s), Esslinger fitness index (EFI (%), maximum -force (Fmax(kN), maximum-power (P-max (kW), efficiency (%) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)

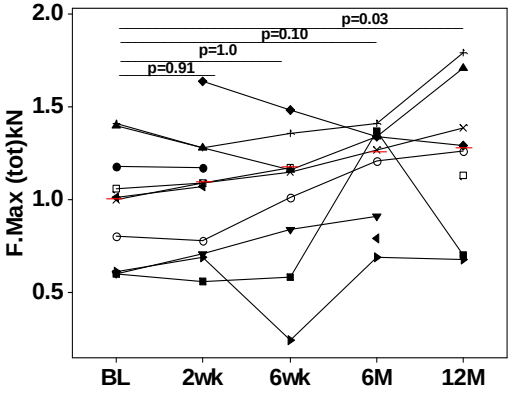
						p-Value			
Value	**BL N,11**	**2wk N,11**	**6wk N,11**	**6M N,11**	**12M N,11**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
Jump height (m)	0.29 (0.20,0.43)	0.34 (0.24,0.50)	0.35 (0.20,0.75)	0.35 (0.32,0.53)	0.36 (0.27,0.52)	0.54	0.20	0.10	0.12
V-max(m/s)	1.96 (1.42,2.46)	2.05 (1.61,2.73)	2.29 (1.57,2.70)	2.22 (2.03,2.79)	2.30 (1.91,2.87)	0.72	0.52	0.09	0.05
EFI%	73 (50,108)	80 (55,116)	92 (63,105)	94 (81,108)	96 (65,109)	0.91	0.72	0.14	0.05
F-max(tot)kN	1.0 (0.6,1.4)	1.1 (0.5,1.6)	1.1 (0.2,1.5)	1.2 (0.6,1.4)	1.2 (0.6,1.7)	0.91	1.0	0.10	**0.03**
P-max(tot)kW	1.3 (0.7,2.7)	1.9 (0.6,3.4)	2.1 (0.6,3.1)	2.3 (1.1,3.1)	2.2 (0.9,3.6)	0.61	0.29	0.08	**0.03**
Efficiency %	87 (46,105)	91 (53,107)	92 (64,102)	95 (85,104)	96 (65,109)	0.51	1.0	0.14	0.10

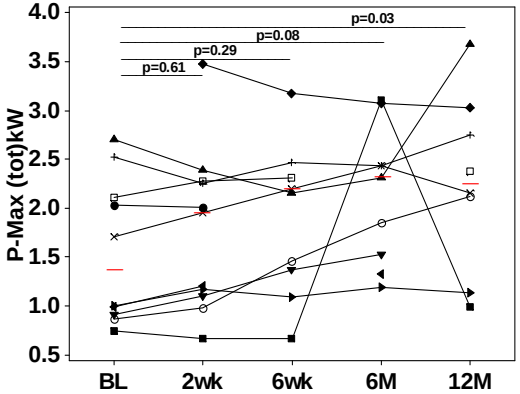
Figure 5.4.5: Changes in Mechanography, jump height (m), maximum-velocity (V-max (m/s), Esslinger fitness index (EFI (%), maximum force (Fmax(kN), maximum-power (P-max (kW), efficiency (%) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)











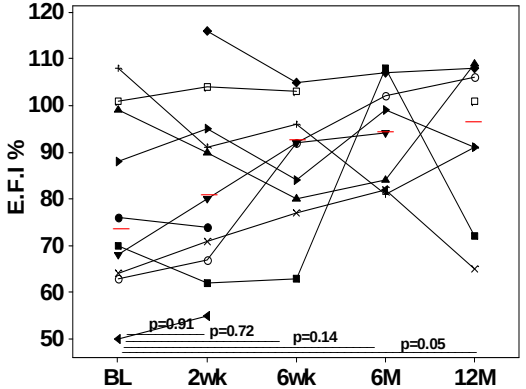
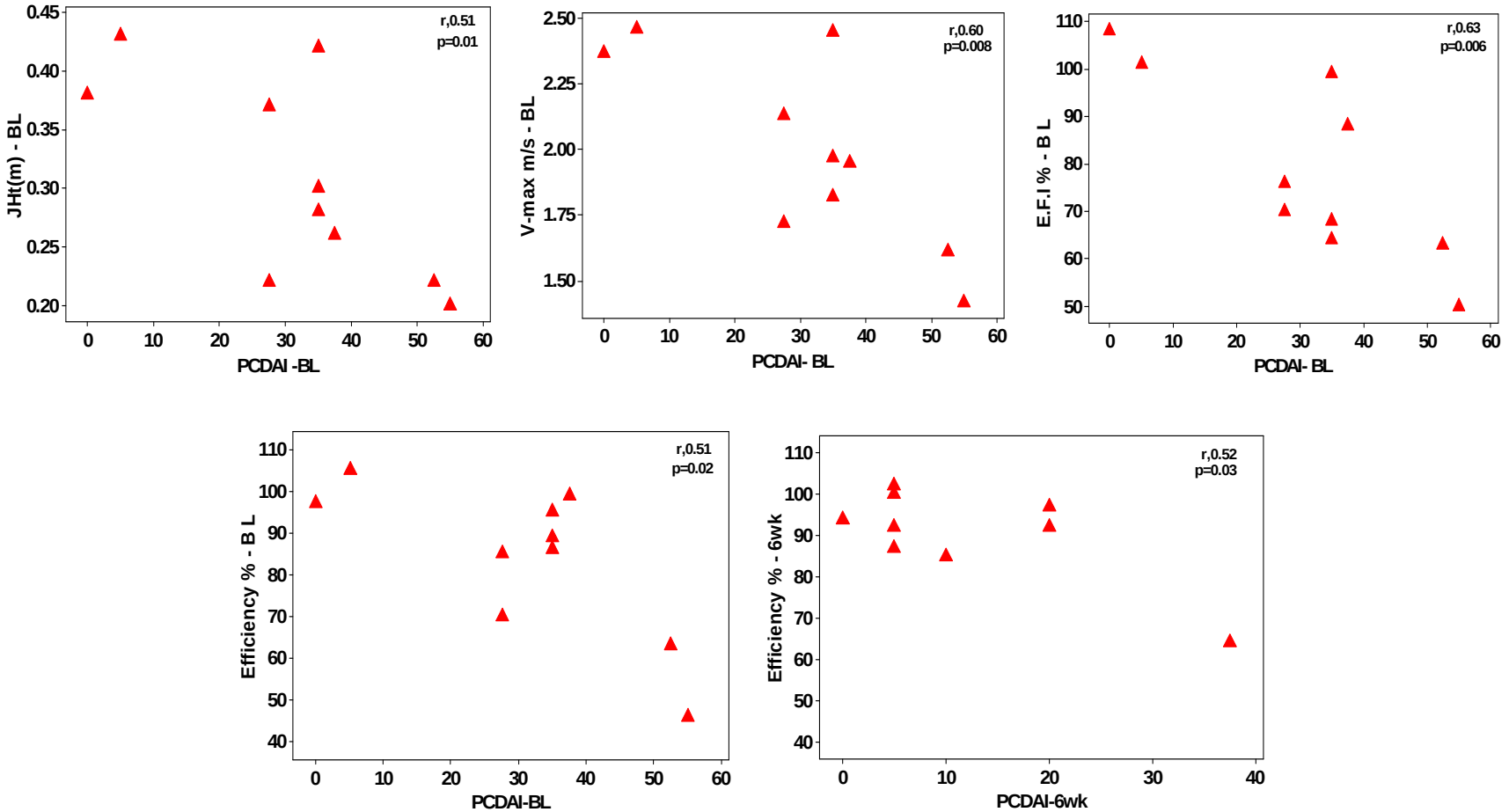


Figure 5.4.6: Association of mechanographic parameters jump height (JHt m), maximum-velocity (V-max (m/s), Esslinger fitness index (EFI (%), efficiency (%) of the movement and paediatric Crohn's disease activity index (PCDAI)



5.4.9 Change in body composition

Measurements of body composition compartments were carried out by two different methods i.e. through foot to foot technique using a TANITA body fat analyzer (TBF-300, Tokyo, Japan) and DXA technique.

5.4.9.1 Changes in body composition measured by DXA

Individual changes in body composition compartments fat mass (FM (kg), fat mass index (FMI (kg/m²), fat free mass (FFM kg), fat free mass index (FMI kg/m²) and percent (%) body fat) measured by DXA are presented in (Table 5.4.11;Figure 5.4.7). Median (range) FM (kg) was 10.7(3.6,16.2) at BL and 10.3(3.7,24.7) at 6M (p=0.23) and 8.5(3.9,31.8) at 12M(p=0.14). Median FMI (kg/m²) was 3.9(2.0,5.4) at BL, and 4.3(2.1,8.2) at 6M(p=0.16) and 3.4(2.1,10.5) at 12M(p=0.50), respectively. Median FFM (kg/m²) changed from 36.7(20.6,47.5) at BL, to 39.1(22.8,49.1) at 6M(p=0.004) and 38.9(23.7,54.8) at 12M(p=0.004). Median FFMI (kg/m²) was also increased from 13.1(11.3,17.1) at BL, to 14.0(11.9,16.8) at 6M(p=0.02) and 14.7(11.9,17.5) at 12M(p=0.009). Total body fat (%) however, remained unchanged from 23.1(14.7,32.2) at BL, to 22.6(14.6,40.4) at 6M(p=0.26) and 20.8(14.3,46.7) at 12M(p=0.96) respectively(Table 5.4.12).

5.4.9.2 Changes in body composition measured by TANITA

Individual changes in body composition compartments measured by TANITA are presented in (Table 5.4.13;Figure 5.4.8). Median (range) FM (kg) changed from 4.9(1.4,17.5) at BL to 5.3(2.0,18.1) at 2wk (p=0.12), 6.4(3.1,18.3) at 6wk(p=0.02), 6.1(2.1,17.5) at 6M(p=0.02) and 5.5(1.7,26.7) at 12M(p=0.04). Median FMI(kg/m²) also changed from 1.9(0.8,5.8) at BL to 2.3(1.2,5.8) at 2wk(p=0.10), 2.1(0.9,8.8) at 6wk(p=0.02), 1.8(1.1,6.0) at 6M(p=0.09) and 2.5(1.4,6.1) at 12M(p=0.12). FFM (kg) changed from 37.7(23.6,53.4) at BL to 40.5(23.2,52.0) at 2wk (p=0.41), 40.8(23.0,53.4) at 6wk(p=0.07), 46.3(24.5,53.7) at 6M(p=0.02) and 46.5(25.8,61.0) at 12M(p=0.01). FFMI (kg/m²) changed from 14.1(12.6,17.7) at BL to 15.4(12.8,17.7) at 2wk(p=0.50), 15.8(13.2,18.2) at 6wk(p=0.07), 16.4(13.2,17.9) at 6M(p=0.03) and 17.2(12.9,22.9) at 12M(p=0.009). Total body (%)fat however, remained unchanged from 10.7(5.5,30.3) a BL, to 10.1(7.9,30.4) at 2wk(p=0.24) and 9.8(2.4,29.2) at 6wk(p=0.47), 11.4(7.7,27.0) at 6M(p=0.95) and 12.1(5.6,37.2) at 12M(p=0.31) respectively (Table 5.4.14). Improvement in FFM seems to be a specific effect of anti-TNF-α therapy.

Table 5.4.11: Individual changes in body composition compartments (fat mass (FM kg), fat mass index (FMI (kg/m²), fat free mass (FFM kg), fat free mass index (FFMI (kg/m²) and percent (%) body fat) measured by DXA at BL,6M & 12M following treatment with biologic therapy in paediatric CD patients

Pt.No	1	2	3	4	5	6	7	8	9	10	11
Fat Mass (kg)											
BL	7.8	3.6	14.4	11.9	5.0	11.1	5.7	8.5	16.2	10.7	13.0
6M	9.2	3.7	10.1	16.2	6.5	7.0	11.1	10.3	24.7	11.9	15.0
12M	11.2	3.9	8.1	12.9	6.0	8.2	8.5	12.8	31.8	8.1	19.6
Fat Mass Index (kg/m^2)											
BL	3.9	2.0	4.9	3.9	2.8	4.4	2.5	3.1	5.4	4.2	4.1
6M	4.2	2.1	3.4	5.2	3.3	2.7	4.6	3.5	8.2	4.4	4.6
12M	4.9	2.0	2.7	4.1	3.0	3.2	3.3	4.1	10.5	2.9	6.0
Fat-free Mass (kg)											
BL	26.5	21.5	42.1	42.5	20.5	32.6	25.8	47.5	36.6	37.4	41.5
6M	32.9	22.8	44.1	48.9	23.4	33.1	28.2	49.1	39.0	39.3	44.8
12M	35.6	24.1	43.1	54.8	23.6	34.5	31.6	48.7	38.9	42.1	49.2
Fat-free Mass Index (kg/m^2)											
BL	13.3	12.3	14.5	14.0	11.5	12.9	11.3	17.1	12.1	14.6	13.1
6M	15.2	12.8	15.1	16.0	12.1	13.1	11.8	16.7	12.9	14.7	13.9
12M	15.6	13.0	14.7	17.5	11.8	13.6	12.5	15.6	12.8	15.2	15.2
Percent (%) Body Fat											
BL	23.4	14.7	26.4	22.8	20.3	26.3	18.9	15.7	32.2	23.1	24.8
6M	22.6	14.6	19.3	25.8	22.3	18.2	29.1	18.1	40.4	24.1	26.2
12M	24.8	14.3	16.5	19.8	20.8	19.9	22.0	21.7	46.7	16.9	29.5

Figure 5.4.7: Individual changes in body composition (DXA) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

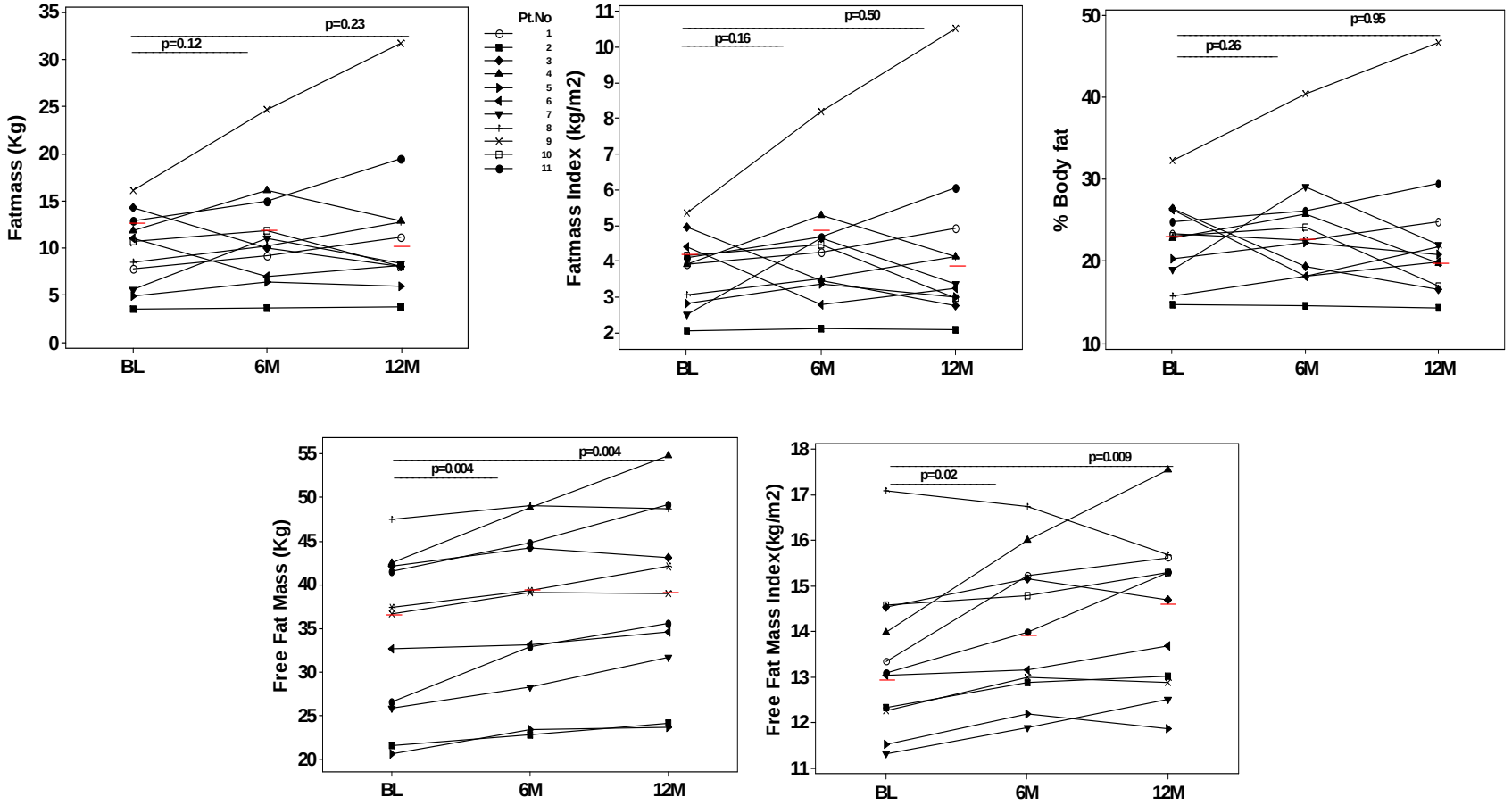


Table 5.4.12: Changes body composition compartments (fat mass (FM kg), fat mass index (FMI (kg/m²), fat free mass (FFM (kg), fat free mass index (FFMI (kg/m²) and percent (%) body fat) measured by DXA at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value BL-6M	 BL-12M
FM (kg)	10.7	10.3	8.5	0.23	0.14
	(3.6,16.2)	(3.7,24.7)	(3.9,31.8)		
FMI (kg/m^2)	3.9	4.3	3.4	0.16	0.50
	(2.0,5.4)	(2.1,8.2)	(2.1,10.5)		
FFM (kg)	36.7	39.1	38.9	**0.004**	**0.004**
	(20.6,47.5)	(22.8,49.1)	(23.7,54.8)		
FFMI (kg/m^2)	13.1	14.0	14.7	**0.02**	**0.009**
	(11.3,17.1)	(11.9,16.8)	(11.9,17.5)		
% Body Fat	23.1	22.6	20.8	0.26	0.95
	(14.7,32.2)	(14.6,40.4)	(14.3,46.7)		

Table 5.4.13: Individual changes in body composition compartments (fat mass (FM (kg), fat mass index (FMI (kg/m²), fat free mass (FFM (kg), fat free mass index (FFMI (kg/m²) and percent (%) body fat) measured by TANITA at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients

Pt.No	1	2	3	4	5	6	7	8	9	10	11
FM (kg)											
BL	5.8	1.4	*	6.1	4.0	2.6	2.7	3.0	17.5	6.6	5.8
2wk	5.3	2.0	5.6	5.5	6.1	3.7	3.2	4.4	18.0	7.5	5.1
6wk	6.4	9.3	5.0	7.5	7.6	4.3	3.1	5.5	18.3	7.2	4.6
6M	5.9	2.1	9.6	13.9	4.7	*	4.6	6.6	17.5	6.5	6.2
12M	10.3	1.7	2.9	6.6	4.0	*	4.3	8.0	26.7	2.9	10.8
FMI (kg/m^2)											
BL	2.9	0.8	*	2.0	2.2	1.0	1.2	1.1	5.8	2.6	1.8
2wk	2.7	1.1	1.9	1.8	3.4	1.5	1.4	1.6	6.0	2.9	1.6
6wk	3.2	5.3	1.7	2.5	4.3	1.7	1.4	2.0	6.1	2.8	1.5
6M	2.7	1.2	1.6	4.5	2.4	*	1.9	2.3	5.8	2.4	1.9
12M	4.5	0.9	1.0	2.1	2.0	*	1.7	2.6	8.8	1.1	3.4
FFM (kg)											
BL	27.8	23.6	*	50.5	24.5	36.1	29.0	49.4	40.4	39.2	53.4
2wk	31.0	23.2	51.5	49.2	24.0	38.9	29.2	49.3	42.2	40.5	52.0
6wk	33.3	23.0	52.4	48.3	24.6	40.2	32.0	50.5	44.2	40.8	53.4
6M	38.1	24.5	49.6	49.4	25.3	*	33.8	52.6	47.4	45.2	53.7
12M	36.4	27.8	48.9	61.0	25.8	*	35.8	51.1	45.3	47.6	58.0
FFMI (kg/m^2)											
BL	13.9	13.5	*	16.6	13.7	14.3	12.7	17.8	13.4	15.3	16.8
2wk	15.5	13.3	*	16.2	13.4	15.4	12.8	17.7	14.0	15.8	16.4
6wk	16.7	13.2	*	15.9	13.8	15.9	14.0	18.2	14.6	15.9	16.8
6M	17.6	13.9	17.0	16.2	13.2	*	13.4	17.9	15.7	17.0	16.7
12M	15.9	15.0	16.7	19.5	12.9	*	14.1	16.4	15.0	17.3	18.0
% Body Fat											
BL	17.1	5.5	*	10.8	13.5	6.7	8.6	5.7	30.3	14.3	10.5
2wk	14.6	7.9	9.8	10.1	21.9	8.7	10.1	8.2	30.4	15.6	9.0
6wk	16.1	2.4	8.7	13.4	23.6	9.6	8.7	9.8	29.2	14.9	8.0
6M	11.1	7.7	8.4	8.0	15.7	*	11.9	11.6	27.0	12.5	10.3
12M	22.0	5.6	5.6	9.7	13.4	*	10.8	13.6	37.2	5.8	15.7

Figure 5.4.8: Individual changes in body composition (TANITA) at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients

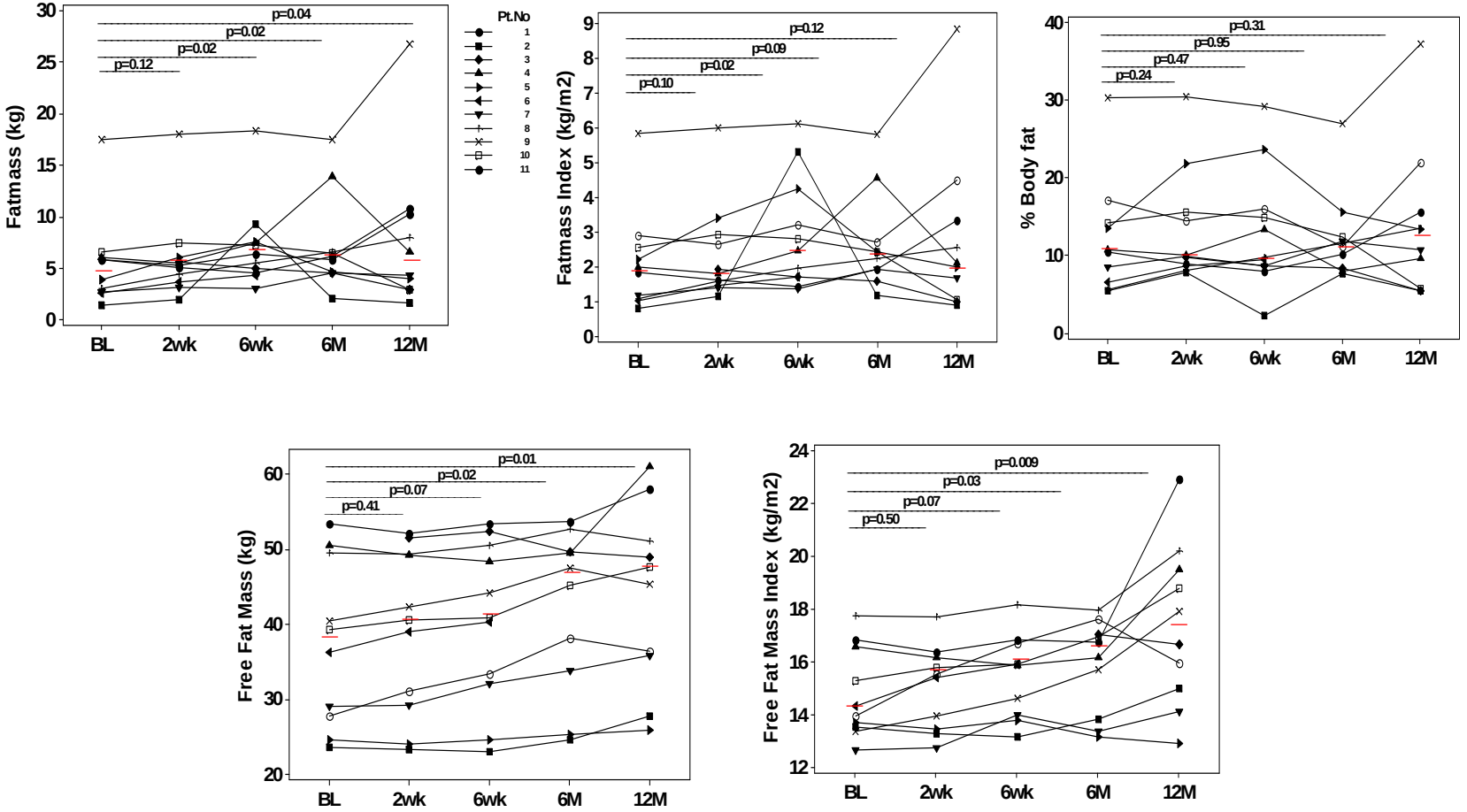


Table 5.4.14: changes in body composition compartments (fat mass (FM (kg), fat mass index (FMI (kg/m²), fat free mass (FFM (kg), fat free mass index (FFMI (kg/m²) and percent (%) body fat) measured by TANITA at BL, 2wks, 6wks, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)

							p-Value		
Value	**BL N,11**	**2wk N,11**	**6wk N,11**	**6M N,11**	**12M N,11**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
FM (kg)	4.9	5.3	6.4	6.1	5.5	0.12	**0.02**	**0.02**	**0.04**
	(1.4,17.5)	(2.0,18.1)	(3.1,18.3)	(2.1,17.5)	(1.7,26.7)				
FMI (kg/m^2)	1.9	2.3	2.1	1.8	2.5	0.10	**0.02**	0.09	0.12
	(0.8,5.8)	(1.2,5.8)	(0.9,8.8)	(1.1,6)	(1.4,6.1)				
FFM (Kg)	37.7	40.5	40.8	46.3	46.5	0.41	0.07	**0.02**	**0.01**
	(23.6,53.4)	(23.2,52)	(23,53.4)	(24.5,53.7)	(25.8,61)				
FFMI(kg/m^2)	14.1	15.4	15.8	16.4	17.2	0.50	0.07	**0.03**	**0.009**
	(12.6,17.7)	(12.8,17.7)	(13.2,18.2)	(13.2,17.9)	(12.9,22.9)				
% Body Fat	10.7	10	9.8	11.4	12.1	0.24	0.47	0.95	0.31
	(5.5,30.3)	(7.9,30.4)	(2.4,29.2)	(7.7,27)	(5.6,37.2)				

5.4.9.3 Comparison between DXA and TANITA

All body composition compartments (FM(kg), FFM(kg), %body fat) measured by BIA were found to be positively and highly correlated with those by DXA at BL, 6M and 12M (Table 5.4.15). FM, FFM and % body fat calculated by DXA were linearly regressed against those predicted by TANITA. There were no significant correlations between FM, FMI, %fat, FFM or FFMI and disease duration and markers of inflammation.

Table 5.4.15: Spearman rank correlation (R-coefficient and p-values) for body composition variables between DXA and TANITA at BL, 6M & 12M following biologic therapy

Parameters	R coefficient	p-value
FM (kg)		
BL	0.45	0.03
6M	0.60	0.008
12M	0.84	0.0001
FFM (kg)		
BL	0.85	0.0001
6M	0.87	0.0001
12M	0.99	0.0001
(%) Body Fat		
BL	0.58	0.01
6M	0.63	0.006
12M	0.90	0.0001

5.4.10 Densitometric changes

5.4.10.1 Total body

Individual changes in total body bone mineral content (TB-BMC (g), total body bone area (TB BA (cm^2), total body bone mineral content z-score (TB-BMC z-score (SD), total body bone mineral density (TB-BMD (g/cm^2) are presented in (Table 5.4.16; Figure 5.5.9). Median (range) BMC (g) increased from 1833(815,2548) at BL to 1954(876,2629) at 6M (p=0.045) and 2025(901,2650) at 12M(p=0.009). The median BMC z-scores (SD) remained unchanged from -0.1(-0.7,0.4) at BL to -0.1(-0.8,0.2)(p=0.44) at 6M and -0.2(-0.8,0.1) at 12M(p=0.07) and remained below zero from BL to 6M & 12M. Median TB-BMD (g/cm^2) remained unchanged from 0.99(0.78,1.16) at BL to 1.01(0.79,1.13) at 6M (p=0.26), and 1.04(0.79,1.13) at 12M (p=0.16)(Table 5.4.17). The median percentage change of TB-BMC (g) from BL to 6M was 3.2%(-2.9,20.1) (p=0.03) and BL to 12M was 10.5%(-2.6,40.3) (p=0.009). The median percentage change of TB-BMD (g/cm^2) from BL to 6M was 0.9%(-2.1,3.9) (p=0.26) and BL to 12M was 0.8%(-2.8,10.0) (p=0.19) (Table 5.4.17; Figure 5.4.10).

5.4.10.2 Lumbar spine

Individual changes in lumbar spine bone mineral content (LS-BMC (g), lumbar spine bone mineral content z-score (LS-BMC z-score (SD), lumbar spine bone mineral density (LS-BMD (cm^2) lumbar spine bone area (LS-BA (cm^2), Lumbar spine volumetric bone mineral apparent density (BMAD (g/cm^3), lumbar spine volumetric bone mineral apparent density z-score (LS-BMAD z-scores (SD) are presented in (Table 5.4.18; Figure 5.4.11-11). Median (range) LS-BMC (g) remained unchanged from 32(11,41) at BL to 29(12,53) at 6M (p=0.10) and 30(15,52) at 12M(p=0.06). The median LS-BMC z-scores (SD) was -0.6(-1.3,0.2) at BL and -0.4(- 1.6,0.3)(p=0.51) at 6M and -0.4(-1.3,0.2) at 12M(p=0.88) and remained below zero from BL to 6M & 12M. Median LS-BMD (g/cm^2) remained unchanged from 0.8(0.7,1.3) at BL to 0.8(0.7,1.2) at 6M (p=0.62), and 0.9(0.7,1.2) at 12M (p=0.10)(Table 5.4.19).

The median percentage change of LS-BMC (g) from BL to 6M was 8.9%(13.1,29.4) (p=0.54) and BL to 12M was 13.5%(-28.2,80.9)(p=0.06). The median percentage change of LS-BMD (g/ cm^2) from BL to 6M was 0.9%(-9.2,16.9) (p=0.62) and BL to 12M was 5.4%(-6.7,29.4) (p=0.05). Median LS-BMAD (g/cm^3) did not change from 0.13(0.11,0.19) at BL to 0.13(0.18,0.16) at 6M (p=0.82), and 0.14(0.11,0.16) at 12M (p=0.12). Median LS-BMAD z-scores (SD) also remained unchanged from -1.6(-2.7,1.7) at BL to -1.8(-3.1,0.2) at 6M (p=0.35), and -1.5(-2.4,-0.2) at 12M (p=0.68) and remained below zero throughout the study interval. The median percentage change of LS-BMAD (g/cm^3) from BL to 6M was 0.9%(-9.2,16.9) (p=0.62) and BL to 12M was 5.4%(-6.7,29.4) (p=0.05) (Table 5.4.19;Figure 5.4.13).

Table 5.4.16: Individual changes in densitometric evaluations of total body (bone mineral content (BMC (g), bone mineral content z-score (BMC z-score (SD), bone mineral density (BMD (g/cm²) and bone area (BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

Pt.No	1	2	3	4	5	6	7	8	9	10	11
BA(cm²)											
BL	1333	1075	2040	2143	1041	1722	1418	2004	2200	1803	2162
6M	1541	1079	1963	2220	1108	1694	1415	2138	2318	1890	2213
12M	1700	1182	1971	2308	1141	1714	1617	2217	2357	1925	2401
BMC(g)											
BL	1090.4	867.7	2073.8	2340.6	815.4	1555.7	1317.9	1970.0	2547.8	1833.4	2225.8
6M	1309.6	876.7	2012.7	2379.0	876.1	1514.3	1325.4	2178.9	2628.9	1954.1	2356.5
12M	1530.0	958.9	2045.2	2488.1	901.2	1515.5	1501.3	2329.9	2650.6	2024.8	2598.7
BMD(g/cm²)											
BL	0.818	0.807	1.017	1.092	0.783	0.904	0.930	0.983	1.158	1.017	1.029
6M	0.580	0.813	1.026	1.071	0.791	0.894	0.937	1.019	1.134	1.034	1.065
12M	0.900	0.812	1.040	1.080	0.790	0.884	0.928	1.051	1.125	1.052	1.082
BMC for BA SDS											
BL	-0.7	0.2	-0.3	0.0	0.1	-0.7	0.0	-0.5	0.4	-0.1	-0.3
6M	-0.8	0.2	-0.2	-0.1	-0.1	-0.7	0.0	-0.4	0.2	-0.1	-0.2
12M	-0.8	-0.2	-0.1	-0.2	-0.2	-0.8	-0.4	-0.2	0.1	0.0	-0.2

Figure 5.4.9: Individual changes in densitometric evaluations of total body (bone mineral content (BMC (g), bone mineral content z-score (BMC z-score (SD), bone mineral density (BMD (g/cm²) and bone area (BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

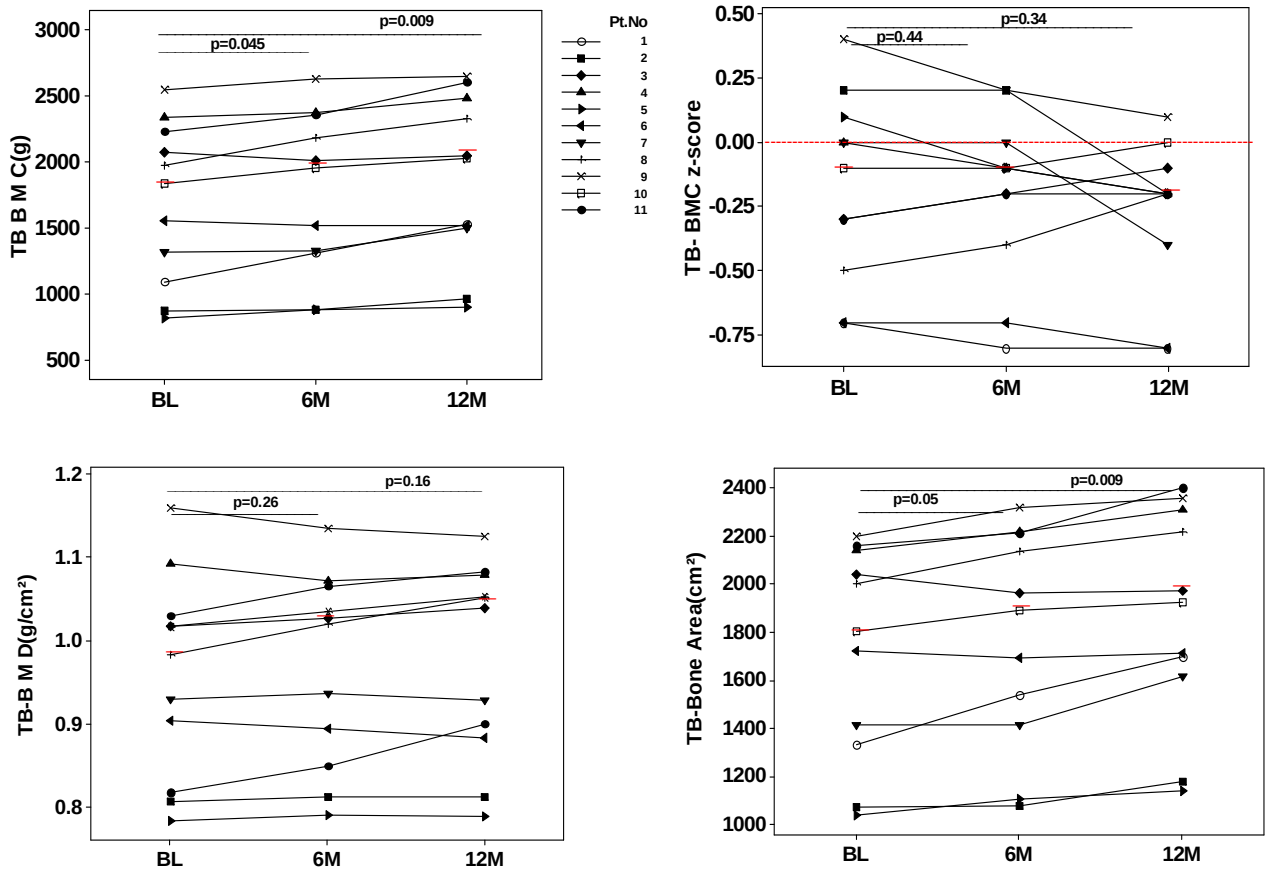


Table 5.4.17: Individual changes in densitometric evaluations of total body (bone mineral content (BMC (g), bone mineral content z-score (BMC z-score (SD), bone mineral density (BMD (g/cm²) and bone area (BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients (median-range)

Value	BL	6M	12M	p-Value		%change		p-Value	
	N,11	N,11	N,11	BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Bone area(cm²)	1803	1890	1925	0.05	**0.009**	3.6	9.6	**0.045**	**0.009**
	(1041,2200)	(1079,2318)	(1141,2401)			(-3.8,15.6)	(-3.4,24.5)		
BMC(g)	1833	1954	2025	**0.045**	**0.009**	3.2	10.5	**0.03**	**0.009**
	(815,2548)	(876,2629)	(901,2650)			(-2.9,20.1)	(-2.6,40.3)		
BMD(g/cm²)	0.99	1.01	1.04	0.26	0.16	0.9	0.8	0.26	0.19
	(0.78,1.16)	(0.79,1.13)	(0.79,1.13)			(-2.1,3.9)	(-2.8,10.0)		
BMC for Bone Area SDS	-0.1	-0.1	-0.2	0.44	0.07				
	(-0.7,0.4)	(-0.8,0.2)	(-0.8,0.1)						

Figure 5.4.10: Percentage (%) change total body (bone mineral content (BMC (g), bone mineral density (BMD (g/cm²) and bone area (BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD

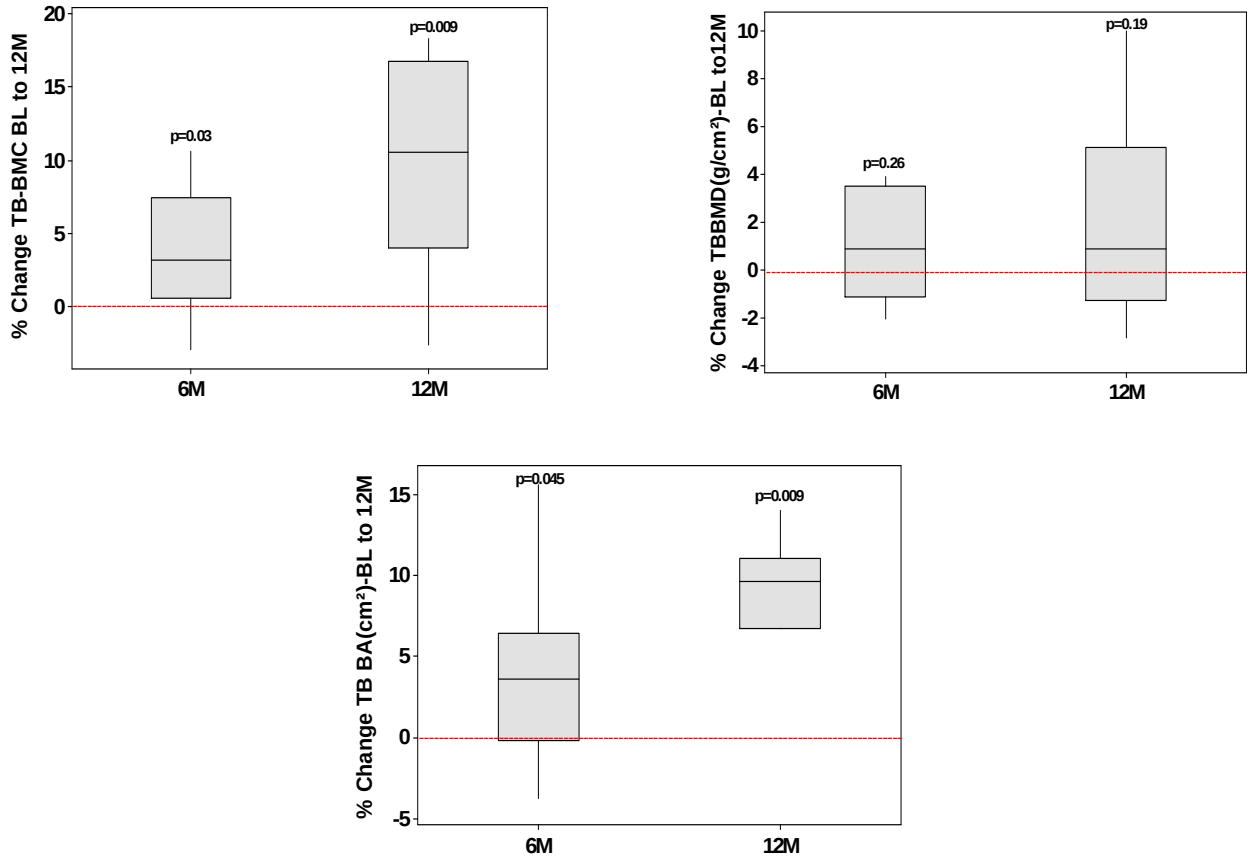


Table 5.4.18: Individual changes in densitometric evaluations of lumbar spine bone mineral content (LS-BMC (g), lumbar spine bone mineral content z-score (LS-BMC z-score(SD), lumbar spine bone mineral density (LS-BMD (g/cm²) lumbar spine bone area (LS-BA (cm²), lumbar spine volumetric bone mineral apparent density (BMAD(g/cm³), lumbar spine volumetric bone mineral apparent density z-score (LS-BMAD z-scores (SD) at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
Bone area(cm²)											
BL	26.6	25.2	37.1	42.0	16.5	37.0	26.7	39.5	32.3	27.8	40.2
6M	29.3	25.0	37.2	40.2	18.0	37.5	27.9	41.1	44.6	30.4	41.6
12M	31.5	19.3	38.8	30.7	28.1	37.7	30.15	41.5	44.1	31.4	43.4
BMC(g)											
BL	19.8	17.5	32.7	33.3	10.8	31.6	21.0	32.3	40.8	23.1	37.8
6M	25.5	17.5	34.4	28.9	12.4	31.1	20.4	35.9	52.8	25.2	42.6
12M	30.4	14.8	37.1	23.9	19.5	31.8	23.7	35.2	52.0	27.5	49.1
BMD(g/cm²)											
BL	0.74	0.69	0.88	0.79	0.65	0.85	0.78	0.81	1.26	0.83	0.94
6M	0.87	0.70	0.92	0.71	0.68	0.82	0.73	0.87	1.18	0.82	1.02
12M	0.96	0.76	0.95	0.77	0.69	0.84	0.78	0.85	1.17	0.87	1.13
BMC for Bone Area SDS											
BL	-0.6	-0.4	-0.6	-1.3	-0.5	-0.7	-0.1	-1.0	0.2	0.0	-0.5
6M	-0.3	-0.3	-0.4	-1.6	-0.7	-0.7	0.3	-0.9	0.2	-0.3	-0.2
12M	-0.1	-0.3	-0.3	-1.3	-0.8	-0.8	-0.5	-1.0	0.0	-0.2	0.2
BMAD(g/cm³)											
BL	0.12	0.12	0.12	0.10	0.13	0.12	0.13	0.11	0.19	0.14	0.13
6M	0.14	0.12	0.13	0.09	0.13	0.11	0.12	0.12	0.15	0.13	0.14
12M	0.15	0.15	0.13	0.12	0.13	0.12	0.12	0.11	0.15	0.14	0.15
BMAD SDS											
BL	-1.79	-1.20	-1.74	-2.74	-1.13	-1.89	-1.43	-2.39	1.66	-1.13	-1.15
6M	-0.99	-1.99	-1.38	-3.13	-1.08	-2.14	-2.04	-2.09	-0.19	-1.53	-1.03
12M	-0.49	-0.49	-1.28	-1.94	-2.39	-2.04	-1.79	-2.28	-0.19	-1.18	-0.44

Figure 5.4.11: Individual changes in densitometric evaluations of lumbar spine bone mineral content (LS-BMC (g), lumbar spine bone mineral content z-score (LS-BMC z-score (SD), lumbar spine bone mineral density (LS-BMD (g/cm²), lumbar spine bone area (LS-BA (cm²) at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD

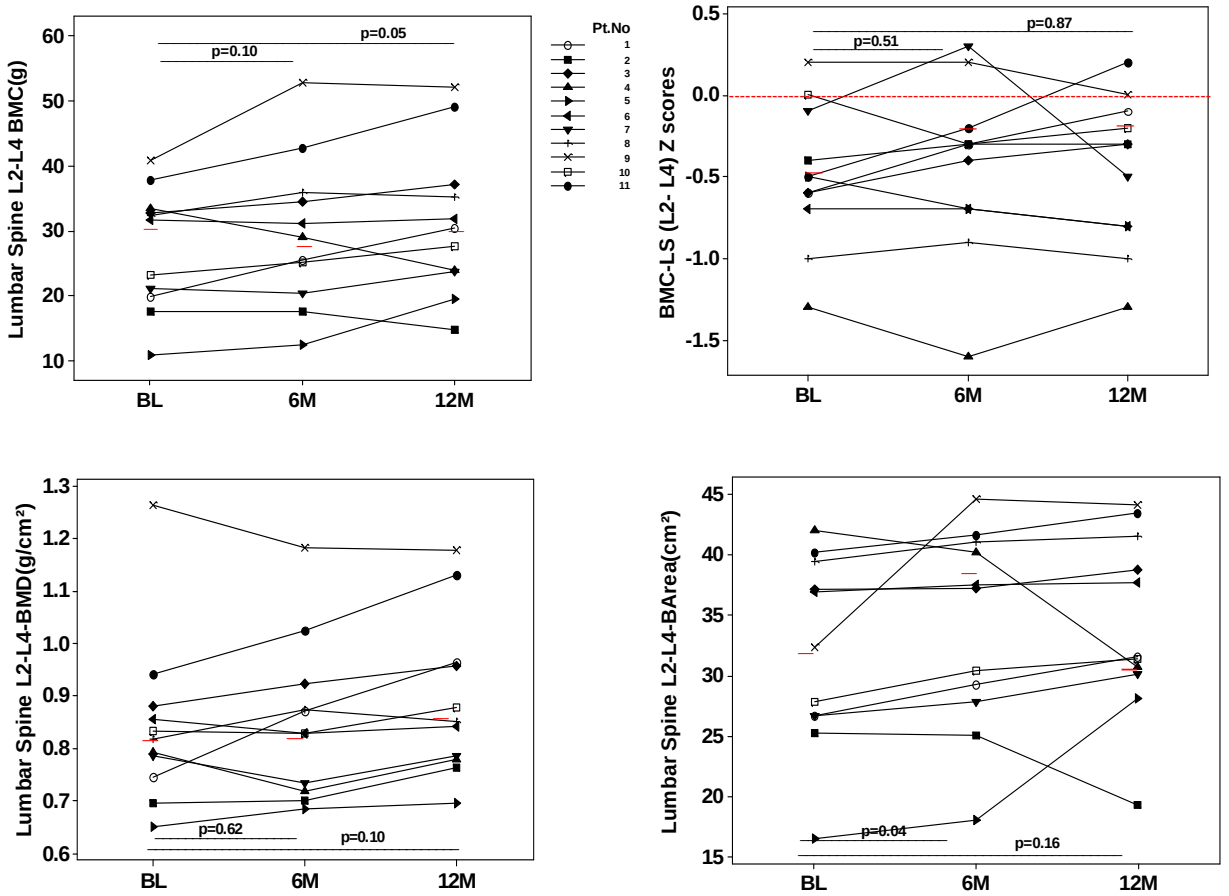


Figure 5.4.12: Individual changes in lumbar spine volumetric bone mineral apparent density (BMAD (g/cm³), lumbar spine volumetric bone mineral apparent density z-score (LS-BMAD z-scores (SD) at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD

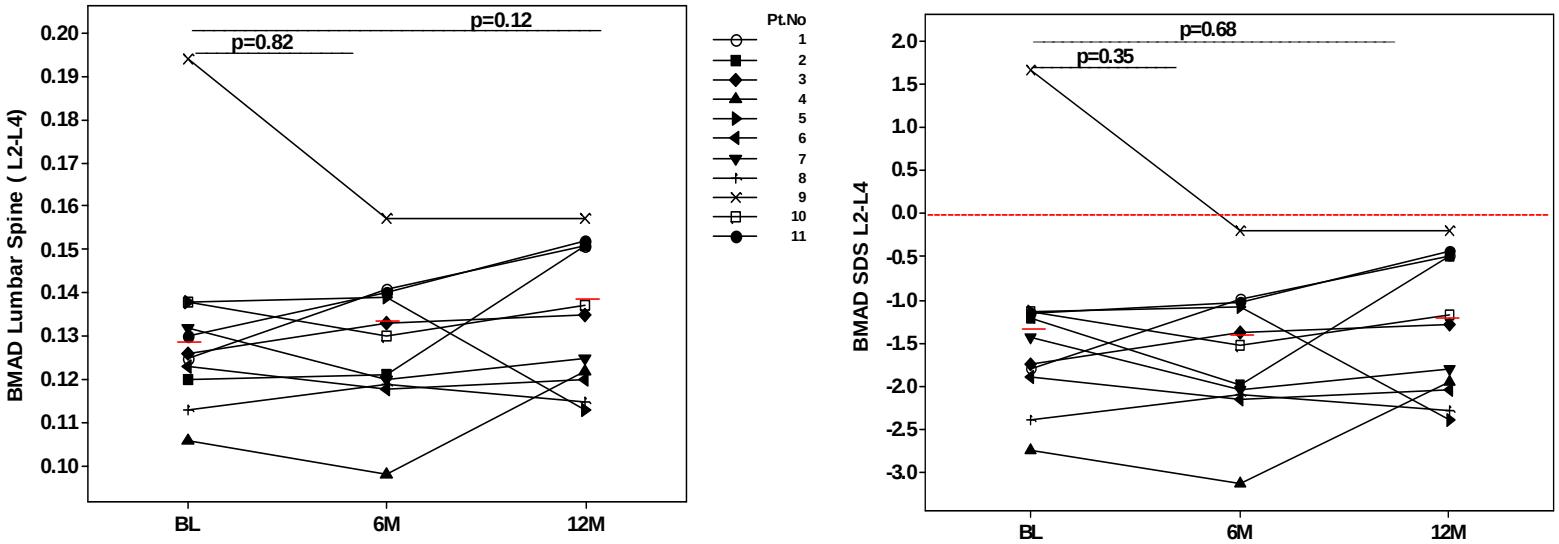
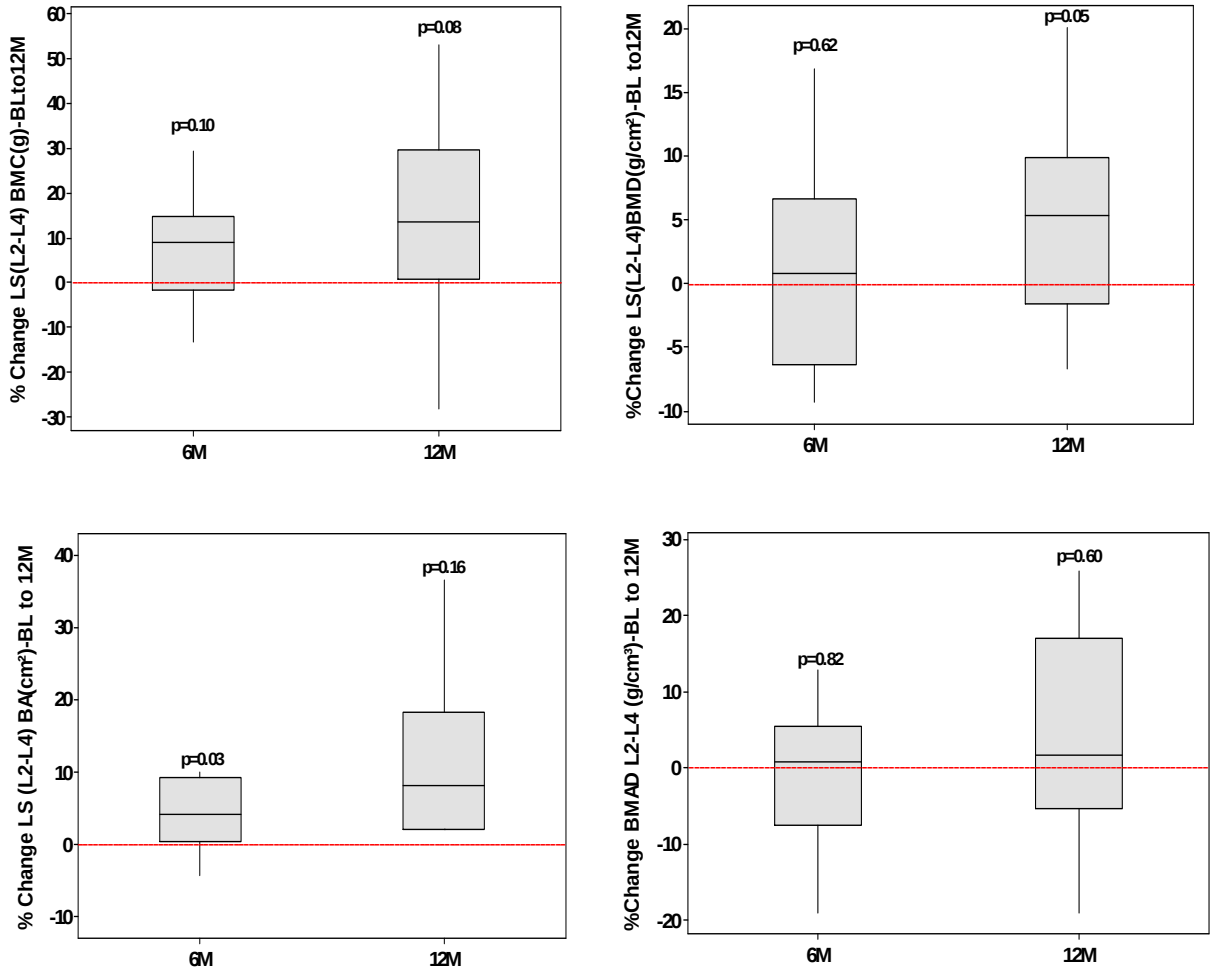


Table 5.4.19: Changes in densitometric evaluations of lumbar spine bone mineral content (LS-BMC (g), lumbar spine bone mineral content z-score (LS-BMC z-score(SD), lumbar spine bone mineral density (LS-BMD (g/cm²) lumbar spine bone area (LS-BA (cm²), lumbar spine volumetric bone mineral apparent density (BMAD(g/cm³), lumbar spine volumetric bone mineral apparent density z-score (LS-BMAD z-scores (SD) at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value		%change		p-Value	
				BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Bone area(cm²)	32.3	37	32	**0.04**	0.16	4.1	8.1	**0.03**	0.16
	(17,42)	(18,45)	(19,44)			(-4.3,38.2)	(-26.8,69.6)		
BMC(g)	32	29	30	0.10	0.06	8.9	13.5	0.54	0.06
	(11,41)	(12,53)	(15,52)			(-13.1,29.4)	(-28.2,80.9)		
BMD(g/cm²)	0.8	0.8	0.9	0.62	0.10	0.9	5.4	0.62	0.05
	(0.7,1.3)	(0.7,1.2)	(0.7,1.2)			(-9.2,16.9)	(-6.7,29.4)		
BMC for Bone Area SDS	-0.6	-0.4	-0.4	0.51	0.88				
	(-1.3,0.2)	(-1.6,0.3)	(-1.3,0.2)						
BMAD(g/cm³)	0.13	0.13	0.14	0.82	0.12	-0.7	-1.7	0.82	0.60
	(0.11,0.19)	(0.18,0.16)	(0.11,0.16)			(-1.9,12.8)	(-19.0,25.8)		
BMAD SDS	-1.6	-1.8	-1.5	0.35	0.68				
	(-2.7,1.7)	(-3.1,0.2)	(-2.4,-0.2)						

Figure 5.4.13: Percentage (%) change Lumbar spine (bone mineral content (LS-BMC (g), bone mineral density (LS-BMD (g/cm²) and bone area (LS-BA(cm²) and lumbar spine volumetric bone mineral apparent density (LS-BMAD (g/cm³) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD



5.4.10.3 Proximal femur

Individual changes in proximal femur bone mineral content (PF-BMC (g), proximal femur one mineral density (PF-BMD (g/cm²) and proximal femur bone area (PF-BA (cm²) are presented in (Table 5.4.20; Figure 5.4.14). Median (range) PF-BMC (g) was 23(12,33) at BL and 24(12,37) at 6M (p=0.75) and 27(13,36) at 12M(p=0.06). Median PF-BMD (g/cm²) was 0.77(0.65,1.06) at BL and 0.81(0.63,1.11) at 6M (p=0.68), and 0.88(0.66,1.07) at 12M(p=0.11) (Table 5.4.21). The median percentage change of PF-BMC (g) from BL to 6M was -0.3%(-7.5,18.5)(p=0.75) and BL to 12M was 5.2%(-7.1,40.1)(p=0.08). The median percentage change of PF-BMD (g/cm²) from BL to 6M was 1.3%(-8.3,8.9)(p=0.62) and BL to 12M was 4.4%(-8.3,23.7) (p=0.12) (Table 5.4.21;Figure 5.4.15).

5.4.10.4 Femoral neck

Individual changes in femoral neck bone mineral content (FN-BMC (g), femoral neck bone area (FN-BA), femoral neck bone mineral density (FN-BMD (g/ cm²), femoral neck volumetric bone mineral apparent density (FN-BMAD (g/cm³), lumbar spine volumetric bone mineral apparent density z-score (FN-BMAD z-score (SD) are presented in (Table 5.4.22; Figure 5.4.16-16). Median (range) FN-BMC (g) was 3.9(2.2,5.2) at BL, 3.8(2.2,5.5) at 6M (p=0.68) and 4.1(2.2,5.4) at 12M(p=0.14). Median FN-BMD (g/cm²) was 0.76(0.61,1.05) at BL, 0.78(0.61,1.09) at 6M (p=0.45), and 0.86(0.66,1.06) at 12M (p=0.06) (Table 5.4.23).

The median percentage change of FN-BMC (g) from BL to 6M was 1.3%(- 7.6,16.3)(p=0.75) and BL to 12M was 4.4%(-7.9,31.9) (p=0.23). The median percentage change of FN-BMD (g/ cm²) from BL to 6M was 3.1%(-8.5,12.6) (p=0.45) and BL to 12M was 4.3%(-7.8,24.6) (p=0.05). Median FN-BMAD (g/cm³) changed from 0.14(0.12,0.18) at BL to 0.15(0.12,0.18) at 6M (p=0.26), and 0.16(0.13,0.18) at 12M (p=0.47). Median FN-BMAD z-scores (SD) changed from -1.1(-1.8,0.3) at BL to -0.8(-1.8,0.3) at 6M (p=0.14), and -0.4(-1.6,- 0.2) at 12M (p=0.42) but stayed below zero through out the study interval. The median percentage change of FN-BMAD (g/cm³) from BL to 6M was 4.0%(-10.1,19.5) (p=0.54) and BL to 12M was 6.7%(-7.9,18.4) (p=0.75)(Table 5.4.23;Figure 5.4.18).

Table 5.4.20: Individual changes in densitometric evaluations of proximal femur bone mineral content (PF-BMC (g), proximal femur bone mineral density (PF-BMD (g/cm²) proximal femur bone area (PF-BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
Bone area(cm²)											
BL	24.4	19.8	33.5	31.3	17.0	30.4	24.0	31.3	20.2	28.4	34.3
6M	26.5	20.4	32.8	32.3	16.4	29.9	23.3	32.8	30.5	28.9	35.3
12M	27.6	18.8	33.6	31.6	17.0	30.1	26.0	33.3	30.6	30.5	35.5
BMC(g)											
BL	17.6	12.9	28.2	29.2	12.0	21.7	17.2	33.2	31.7	23.0	26.7
6M	20.9	13.0	28.0	28.3	11.9	20.7	17.2	36.6	29.3	23.6	29.5
12M	24.7	12.5	29.6	29.4	12.5	20.4	21.01	35.7	29.5	26.6	32.4
BMD(g/cm²)											
BL	0.72	0.65	0.84	0.93	0.70	0.71	0.71	1.06	1.05	0.81	0.77
6M	0.78	0.63	0.85	0.87	0.72	0.69	0.73	1.11	0.96	0.81	0.83
12M	0.89	0.66	0.88	0.92	0.73	0.67	0.80	1.07	0.96	0.87	0.91

Figure 5.4.14: Individual changes in densitometric evaluations of proximal femur bone mineral content (PF-BMC (g), proximal femur bone mineraldensity (PF-BMD (g/cm²) proximal femur bone area (PF-BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

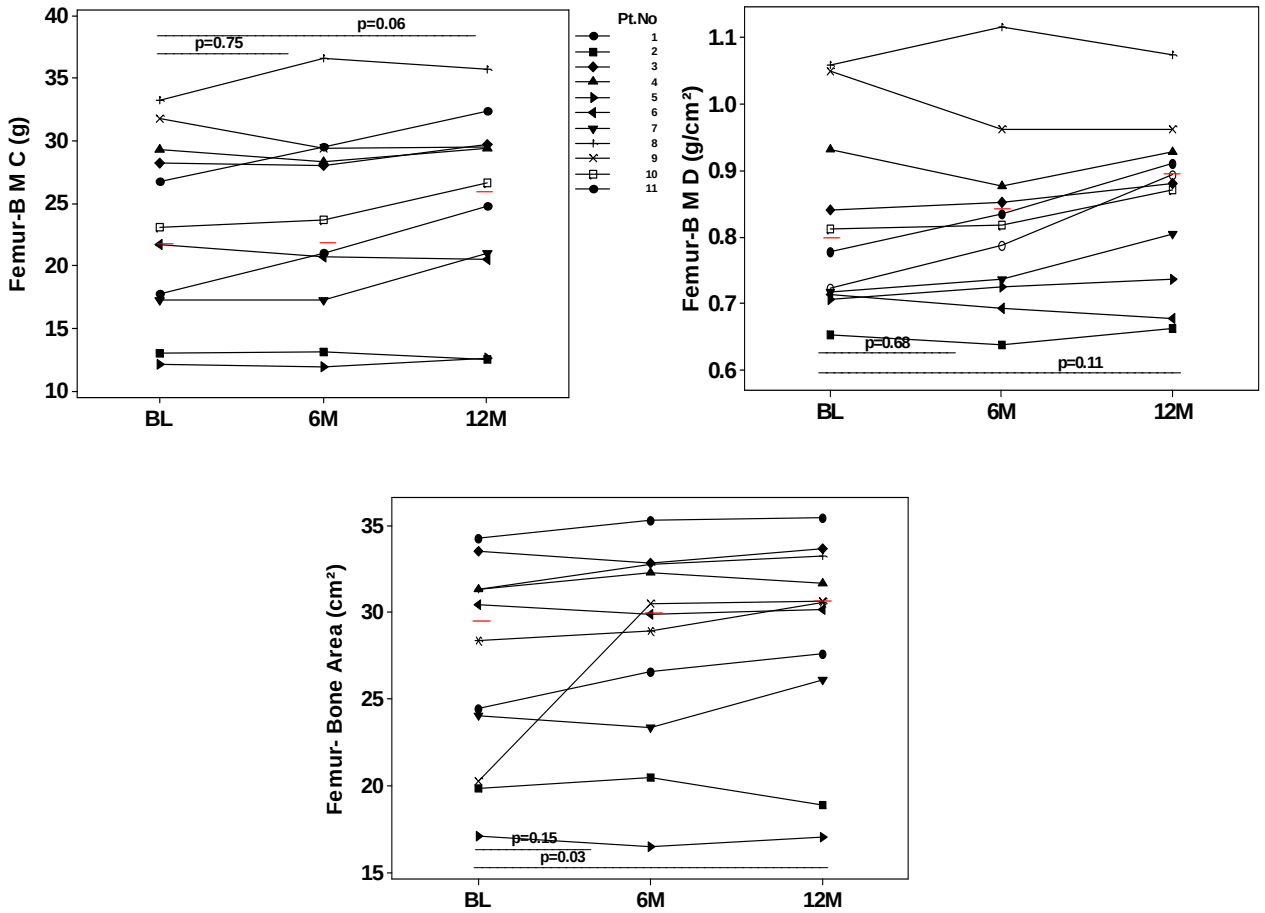
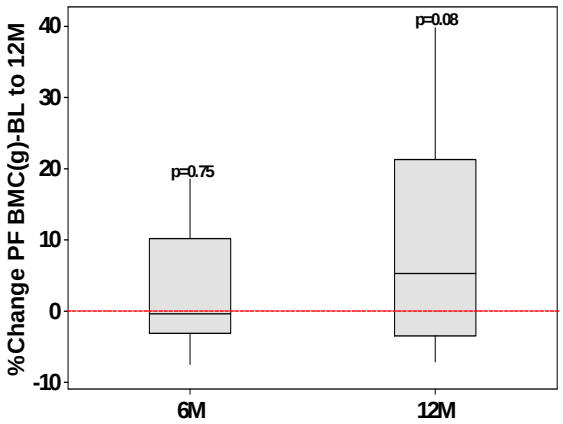
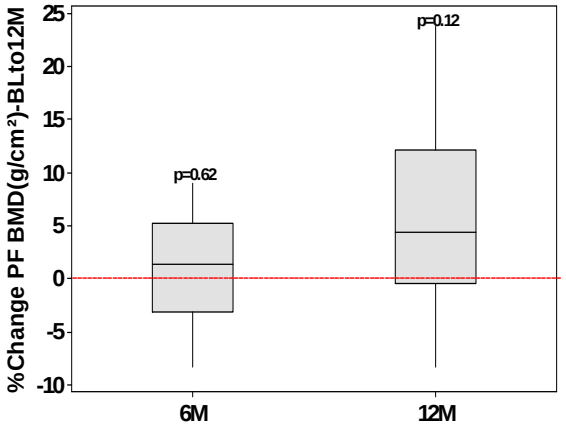


Table 5.4.21: Changes in densitometric evaluations of proximal femur bone mineral content (PF-BMC (g), proximal femur bone mineral density (PF-BMD (g/cm²) proximal femur bone area (PF-BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value		%change		p-Value	
				BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Bone area(cm²)	28	30	31	0.15	**0.03**	2.9	3.5	0.23	**0.04**
	(17,34)	(17,35)	(17,36)			(-3.5,50.8)	(-4.9,51.2)		
BMC(g)	23	24	27	0.75	0.06	-0.3	5.2	0.75	0.08
	(12,33)	(12,37)	(13,36)			(-7.5,18.5)	(-7.1,40.1)		
BMD(g/ cm²)	0.77	0.81	0.88	0.68	0.11	1.3	4.4	0.62	0.12
	(0.65,1.06)	(0.63,1.11)	(0.66,1.07)			(-8.3,8.9)	(-8.3,23.7)		

Figure 5.4.15: Percentage (%) change in proximal femur bone mineral content (PF-BMC (g), proximal femur bone mineral density (PF-BMD (g/cm²) proximal femur bone area (PF-BA (cm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD





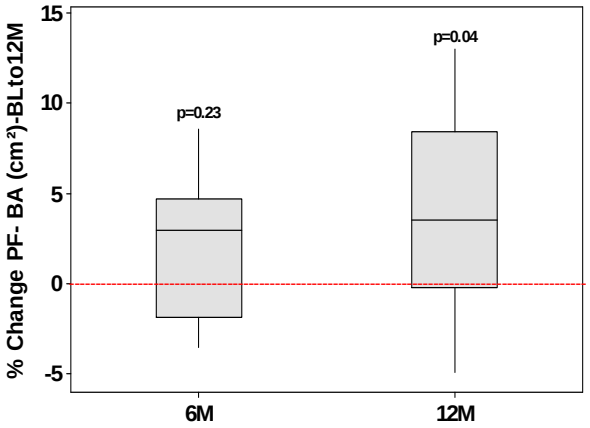


Table 5.4.22: Individual changes in femoral neck bone mineral content (FN-BMC (g), femoral neck bone mineral density (FN-BMD (g/cm²), femoral neck bone area (FN-BA (cm²), femoral neck volumetric bone mineral apparent density (BMAD (g/cm³), femoral neck volumetric bone mineral apparent density z-score (FN-BMAD z-score (SD) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
Bone area(cm²)											
BL	4.2	3.5	4.9	4.5	3.7	4.5	4.1	4.8	4.5	5.0	5.5
6M	4.3	3.6	5.0	4.6	3.4	4.2	4.0	5.0	4.5	5.0	5.4
12M	4.4	3.2	5.1	4.5	3.4	4.4	4.3	5.0	4.5	4.7	5.5
BMC(g)											
BL	2.9	2.1	4.2	4.4	2.6	3.1	3.1	5.1	4.5	3.8	4.4
6M	3.4	2.2	4.5	4.1	2.4	3.3	3.0	5.4	4.1	3.8	4.6
12M	3.8	2.1	4.6	4.4	2.4	3.1	3.6	5.3	4.1	4.0	5.0
BMD(g/cm²)											
BL	0.72	0.65	0.84	0.93	0.70	0.71	0.71	1.06	1.05	0.81	0.77
6M	0.78	0.63	0.85	0.87	0.72	0.69	0.73	1.11	0.96	0.81	0.83
12M	0.89	0.66	0.88	0.92	0.73	0.67	0.80	1.07	0.96	0.87	0.91
BMAD(g/cm³											
BL	0.13	0.14	0.14	0.17	0.14	0.12	0.14	0.17	0.17	0.12	0.12
6M	0.14	0.13	0.15	0.16	0.17	0.15	0.15	0.18	0.16	0.12	0.13
12M	0.16	0.16	0.14	0.18	0.16	0.12	0.15	0.17	0.16	0.14	0.13
BMAD SDS											
BL	-1.36	-1.14	-1.07	-0.25	-0.89	-1.57	-0.79	0.29	0.21	-1.71	-1.82
6M	-0.86	-1.36	-0.79	-0.39	0.07	-0.75	-0.57	0.32	-0.07	-1.82	-1.46
12M	-0.43	-0.36	-1.07	0.18	-0.07	-1.61	-0.43	0.11	-0.29	-0.93	-1.21

Figure 5.4.16: Individual changes in femoral neck bone mineral content (FN-BMC (g), femoral neck bone mineral density (FN-BMD (g/cm²), femoral neck bone area (FN-BA (cm²), at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

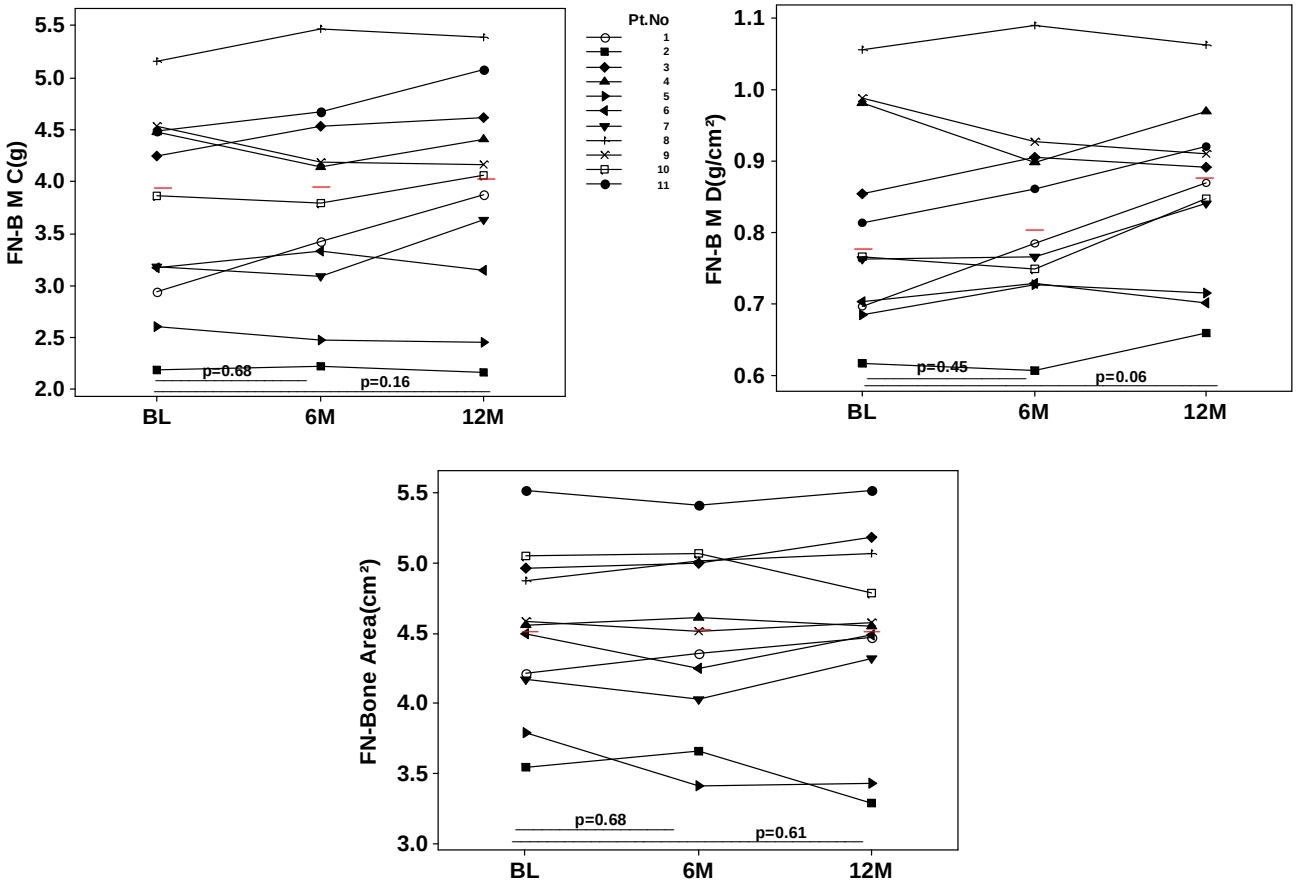


Figure 5.4.17: Individual changes in femoral neck volumetric bone mineral apparent density (BMAD (g/cm³), femoral neck volumetric bone mineral apparent density z-score (FN-BMAD z-score (SD) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

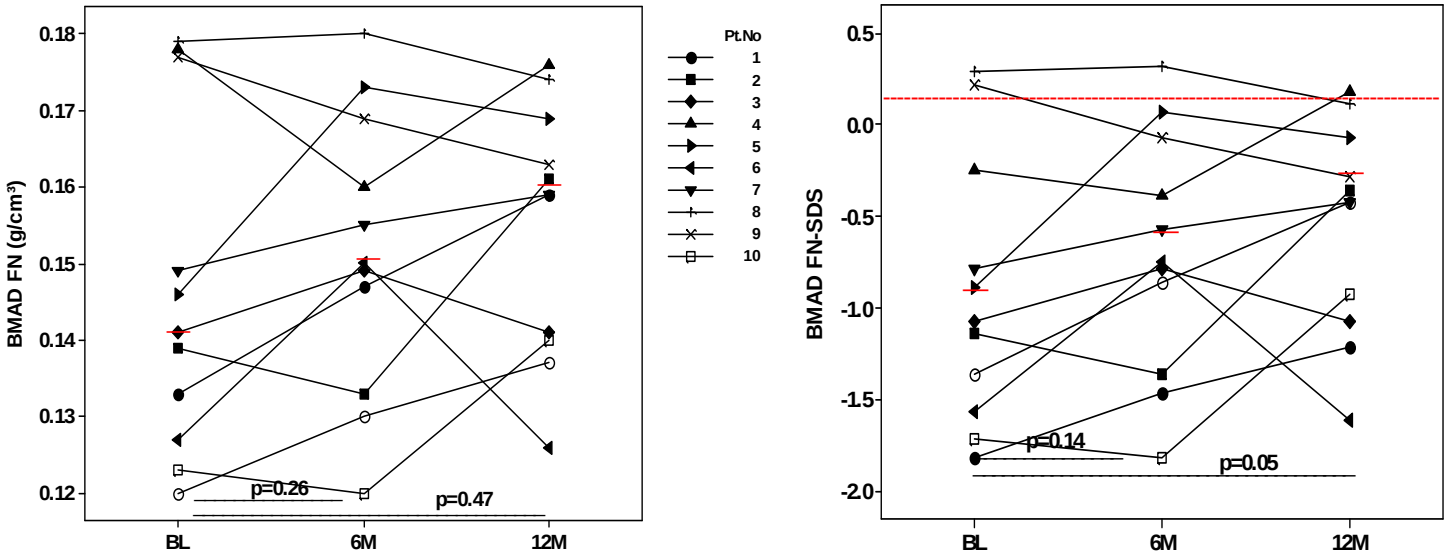
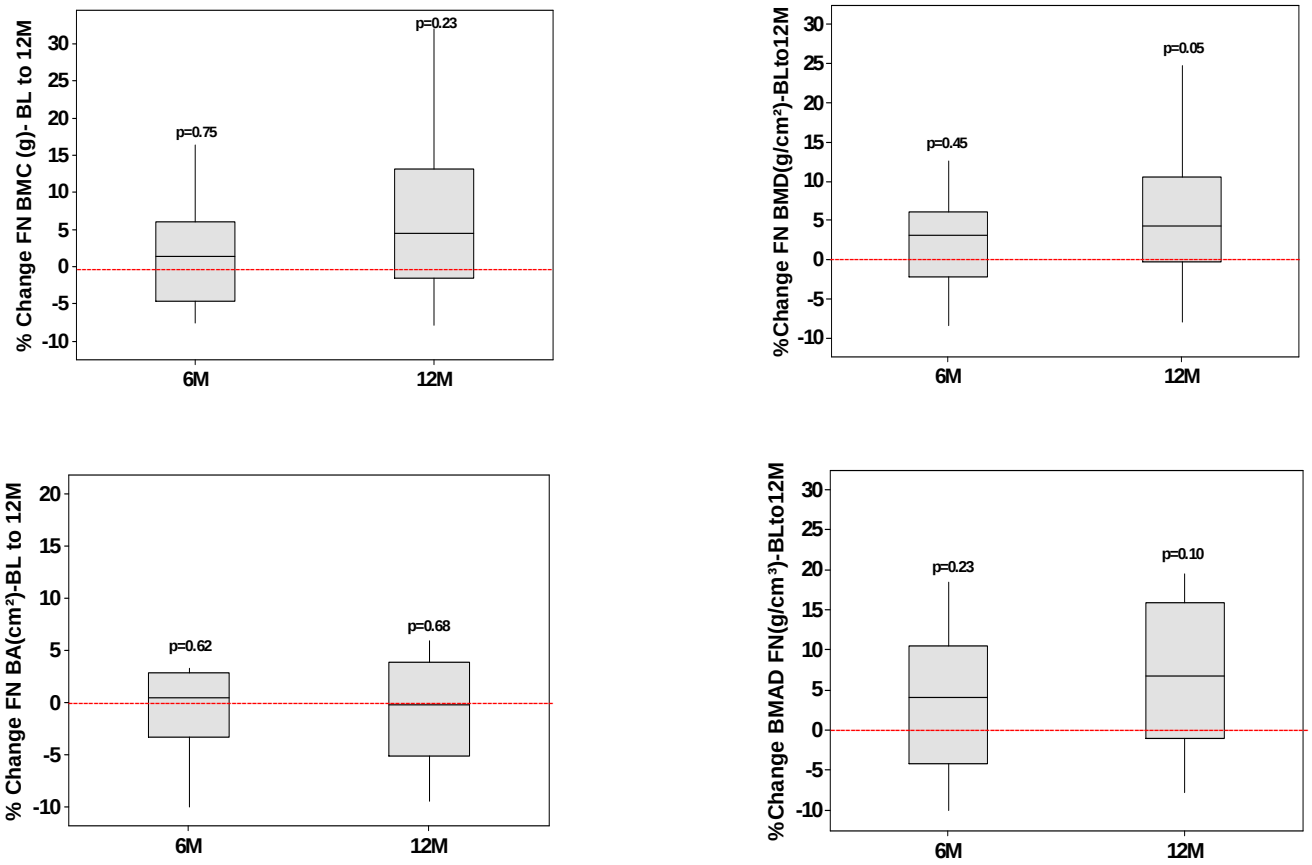


Table 5.4.23: changes in femoral neck bone mineral content (FN-BMC (g), femoral neck bone mineral density (FN-BMD (g/cm²), femoral neck bone area (FN-BA (cm²), femoral neck volumetric bone mineral apparent density (BMAD (g/cm³), femoral neck volumetric bone mineral apparent density z-score (FN-BMAD z-score (SD) at BL, 6M & 12M following treatment with biologic therapy in paediatric patients with CD (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value		%change		p-Value	
				BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Bone area(cm²)	4.5	4.5	4.6	0.68	0.42	0.4	-0.2	0.62	0.68
	(3.6,5.5)	(3.4,5.4)	(3.3,5.5)			(-10.1,3.3)	(-9.4,5.9)		
BMC(g)	3.9	3.8	4.1	0.68	0.14	1.3	4.4	0.75	0.23
	(2.2,5.2)	(2.2,5.5)	(2.2,5.4)			(-7.6,16.3)	(-7.9,31.9)		
BMD(g/cm²)	0.76	0.78	0.86	0.45	0.06	3.1	4.3	0.45	0.05
	(0.61,1.05)	(0.61,1.09)	(0.66,1.06)			(-8.5,12.6)	(-7.8,24.6)		
BMAD (g/cm³)	0.14	0.15	0.16	0.26	0.47	4.0	6.7	0.54	0.75
	(0.12;0.18)	(0.12,0.18)	(0.13,0.18)			(-10.1,19.5)	(-7.9,18.4)		
BMAD SDS	-1.1	-0.8	-0.4	0.14	0.05				
	(-1.8,0.3)	(-1.8,0.3)	(-1.6,0.2)						

Figure 5.4.18: Percentage (%) change femoral neck (bone mineral content (FN-BMC (g), bone mineral density (FN-BMD (g/cm²) and bone area (FN-BA (cm²) and volumetric bone mineral apparent density (FN-BMAD (g/cm³) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD



5.4.11 Musculoskeletal changes

5.4.11.1 Tibia bone and muscle parameters

As mentioned in methods bone and muscle measures in left tibia were measured by QCT. Individual changes in total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³), at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) at 38%, muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of tibial length are presented in (Table 5.4.24;

Figure 5.4.19-19). Median (range) tibia Tot BMD changed from 245(181,313) at BL to 260(209,316) at 6M (p=0.03) and 260(208,317) at 12M(p=0.10). The median TrbBMD (mg/cm³) remain unchanged from 190(140,251) at BL, to 189(173,249) at 6M(p=0.30) and 187(150,249) at 12M (p=0.75). Median SSI (mm³) changed from 1125(411,1711) at BL to 1182(517,1809) at 6M (p=0.22) and 1189(581,1777) at 12M(p=0.04). Median CrtBMD (mg/cm³) was 1104(1088,1187) at BL, 1129(1036,1181) at 6M(p=0.47) and 1120(966,1183) at 12M(p=0.10). Median Mus-CSA mm²) increased from from 4665(3018,6184) at BL to 5356(3210,6959) at 6M (p=0.02) and 5392(3373,7055) at 12M(p=0.04). Median Fat-CSA (mm²) changed from 1809(892,2763) at BL to 1971(986,3505) at 6M(p=0.04) and 1899(1026,3475) at 12M(p=0.08). Median Crt-BA (mm²) was 283(138,374) at BL, 298(134,380) at 6M(p=0.35) and 281(131,376) at 12M(p=0.96), respectively (Table 5.4.25).

The median percentage change of TotBMD from BL to 6M was 5.8%(-4.1,15.1)(p=0.03) and from BL to 12M was 5.8%(-10.6,26.2)(p=0.06) (Table 5.4.25). Median percentage change of TrbBMD (mg/cm³) from BL to 6M was 0.9%(-7.4,24.1)(p=0.30) and from BL to 12M was -0.8%(- 9.2,23.6)(p=0.75). Median percentage change of SSI from BL to 6M was 1.4%(-11.8,42.2)(p=0.22) and from BL to 12M was 6.5%(5.2,41.2)(p=0.01). Median percentage change of CrtBMD from BL to 6M was -0.3%(-6.0,2.2)(0.47) and from BL to 12M was - 0.5%(-11.4,2.7)(p=0.47). Median percentage of Mus-CSA from BL to 6M was 7.4%(-3.1,23.3)(p=0.02) and from BL to 12M was 11.8%(-6.8,38.9)(p=0.04). Median percentage of Fat-CSA from BL to 6M was 10.6%(-16.8,42.4)(p=0.04) and from BL to12M was 7.5%(-15.8,105.5)(p=0.10). Median percentage of Crt-BA from BL to 6M was 2.1%(-5.9,8.9)(p=0.96) and from BL-12M was -0.1%(-24.0,10.6)(p=0.47), respectively (Table 5.4.25;Figure 5.4.21-21).

Table 5.4.24: Individual change tibia total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) at 38%, muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of tibial length at BL,6M &12M following biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
TotBMD(mg/cm³)											
BL	1.81.3	211.9	277.5	245.1	208.2	251.6	201.9	274.6	312.9	278.1	226.1
6M	208.7	227.9	287.6	245.1	235.5	262.2	-	302.9	316.0	265.3	257.2
12M	207.9	227.3	281.1	259.5	257.7	224.9	212.7	312.9	317.2	261.4	285.5
TrbBMD(mg/cm³)											
BL	151.8	189.9	229.1	174.4	139.7	190.2	165.2	210.1	251.0	217.2	183.9
6M	182.6	189.6	233.4	173.8	173.3	188.3	*	235.7	249.2	201.0	189.7
12M	149.9	172.4	223.6	182.8	172.6	187.0	176.7	212.4	248.9	204.2	209.6
SSI(mm³)											
BL	566.5	411.4	843.8	1710.5	566.3	1170.9	1125.4	1401.1	1500.6	1150.9	1116.8
6M	516.5	554.7	1200.2	1808.5	566.4	1163.4	*	1427.6	1322.9	1161.1	1346.1
12M	684.1	581.0	873.6	1621.2	612.3	1189.3	1205.9	1776.8	1504.3	1225.8	1116.8
CrtBMD(mg/cm³)											
BL	1102.1	1094.4	1153.5	1135.3	1104.2	1091.2	1090.3	1088.4	1135.7	1158.3	1187.1
6M	1035.6	1083.7	1162.8	1148.3	1075.9	1114.9	*	1064.1	1142.5	1157.7	1181.0
12M	1024.3	1083.7	1182.7	1129.9	1050.2	1120.2	966.0	1114.3	1147.3	1161.3	1151.8
Mus-CSA(mm²)											
BL	4210.5	3017.5	4948.7	5781.0	3675.2	4665.2	3740.5	6183.5	5360.1	5483.5	4430.2
6M	5158.5	3210.0	5370.7	6958.5	3790.5	4520.7	*	6303.2	6007.2	5340.5	5460.5
12M	5454.5	3373.2	5578.7	7055.2	4028.5	4587.0	4238.2	6492.7	5392.0	4228.5	6152.5
Fat-CSA(mm²)											
BL	2204.2	892.0	1818.7	1931.0	1365.5	2152.2	1497.5	1808.5	2763.0	1509.0	1755.5
6M	2061.8	985.5	1992.8	2138.0	1621.7	1790.0	*	1905.7	3505.0	2149.2	1984.7
12M	2074.5	1026.0	1898.7	2075.0	1540.2	1812.5	1366.2	1994.5	3475.0	3101.2	1828.7
Crt-BA(mm²)											
BL	207.7	137.7	290.2	373.7	157.2	283.0	223.7	339.2	367.0	268.5	345.5
6M	215.0	134.0	316.3	380.0	161.5	280.5	*	367.7	355.0	275.0	325.0
12M	207.5	131.0	318.2	376.2	172.2	275.5	170.0	375.2	362.0	281.2	324.0

Figure 5.4.19: Individual changes in pQCT tibial total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) at 38% at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

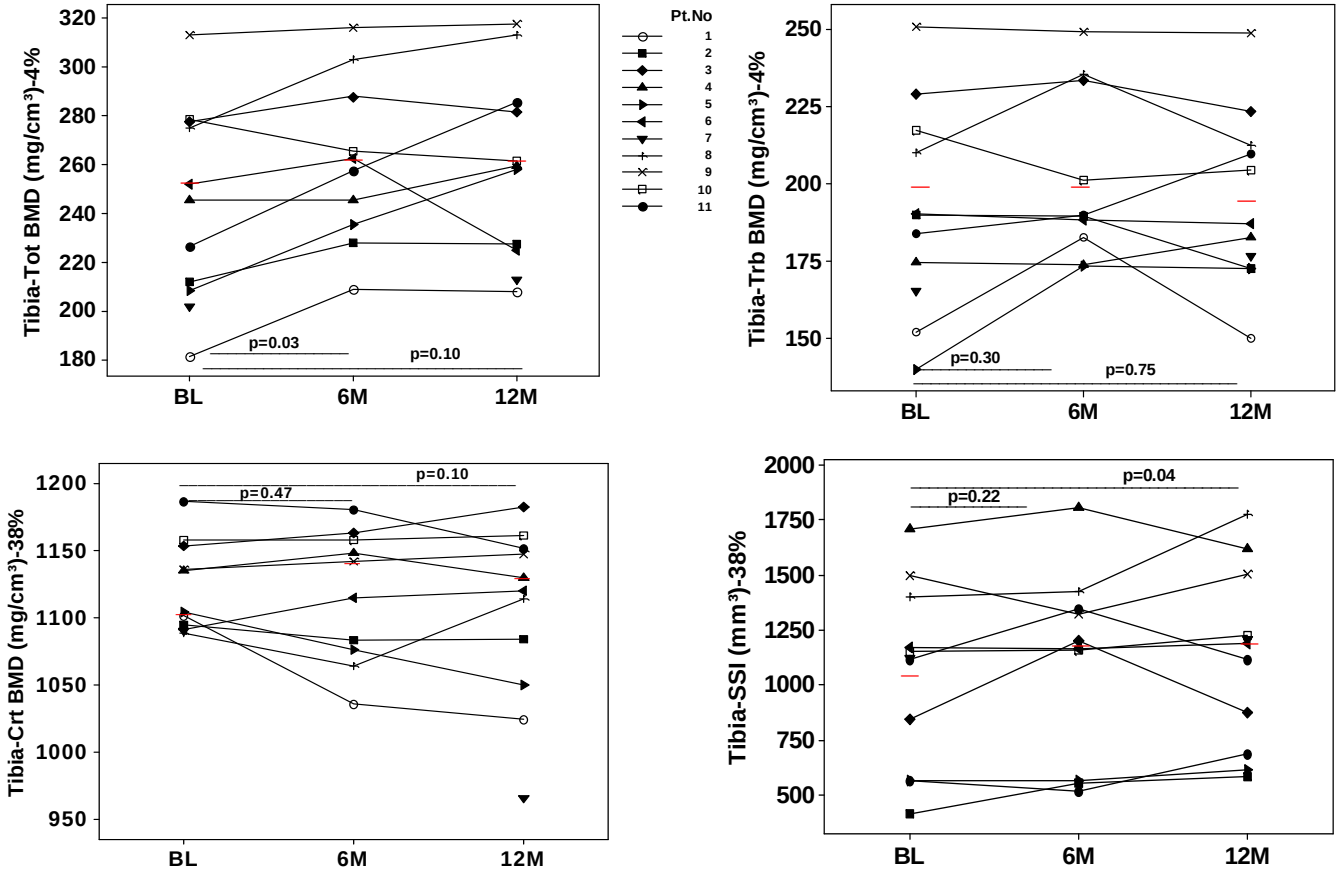


Figure 5.4.20: Individual changes in pQCT tibial muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

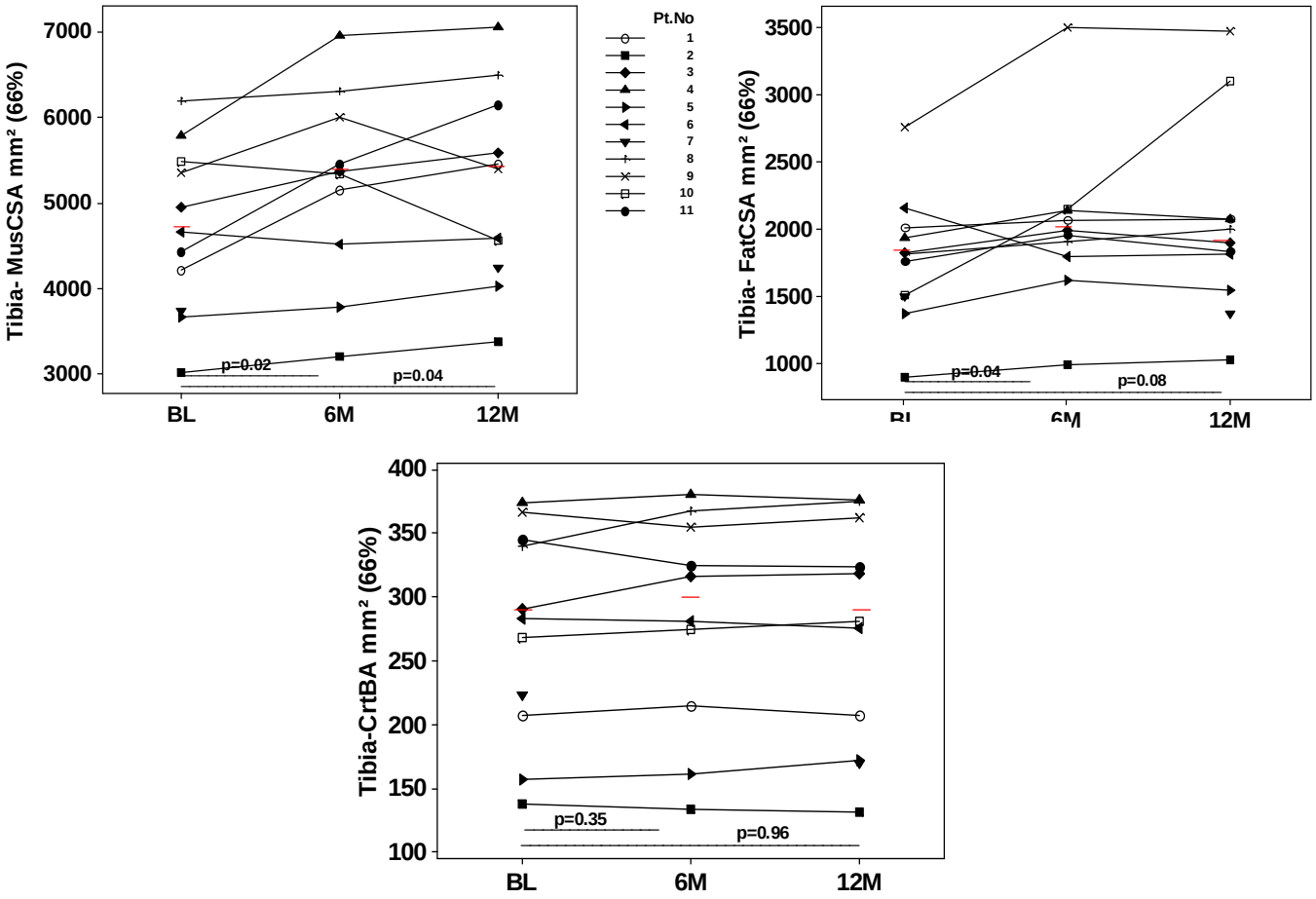


Table 5.4.25: Change tibia total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) at 38%, muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of tibial length at BL,6M &12M following biologic therapy in paediatric patients with CD (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value		%change		p-Value	
				BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Tibia									
TotBMD(mg/cm³)	245	260	260	**0.03**	0.10	5.8	5.8	**0.03**	0.06
	(181,313)	(209,316)	(208,317)			(-4.6,15.1)	(-10.6,26.2)		
TrbBMD(mg/cm³)	190	189	187	0.30	0.75	0.9	-0.8	0.30	0.75
	(140,251)	(173,249)	(150,249)			(-7.4,24.1)	(-9.2,23.6)		
SSI(mm³)	1125	1182	1189	0.22	**0.04**	1.4	6.5	0.22	**0.01**
	(411,1711)	(517,1809)	(581,1777)			(-11.8,42.2)	(5.2,41.2)		
CrtBMD(mg/cm³)	1104	1129	1120	0.47	0.10	-0.3	-0.5	0.47	0.39
	(1088,1187)	(1036,1181)	(966,1183)			(-6.0,2.2)	(-11.4,2.7)		
Mus-CSA(mm²)	4665	5356	5392	**0.02**	**0.04**	7.4	11.8	**0.02**	**0.04**
	(3018,6184)	(3210,6959)	(3373,7055)			(-3.1,23.3)	(-16.8,38.9)		
Fat-CSA(mm²)	1809	1971	1899	**0.04**	0.08	10.6	7.5	**0.04**	0.10
	(892,2763)	(986,3505)	(1026,3475)			(-16.8,42.4)	(-15.8,105.5)		
Crt-BA(mm²)	283	298	281	0.35	0.96	2.1	-0.1	0.96	0.47
	(138,374)	(134,380)	(131,376)			(-5.9,8.9)	(-24.0,10.6)		

Figure 5.4.21: Percentage (%) change in tibia tibial total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) at 38% of tibial length at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD (median-range)

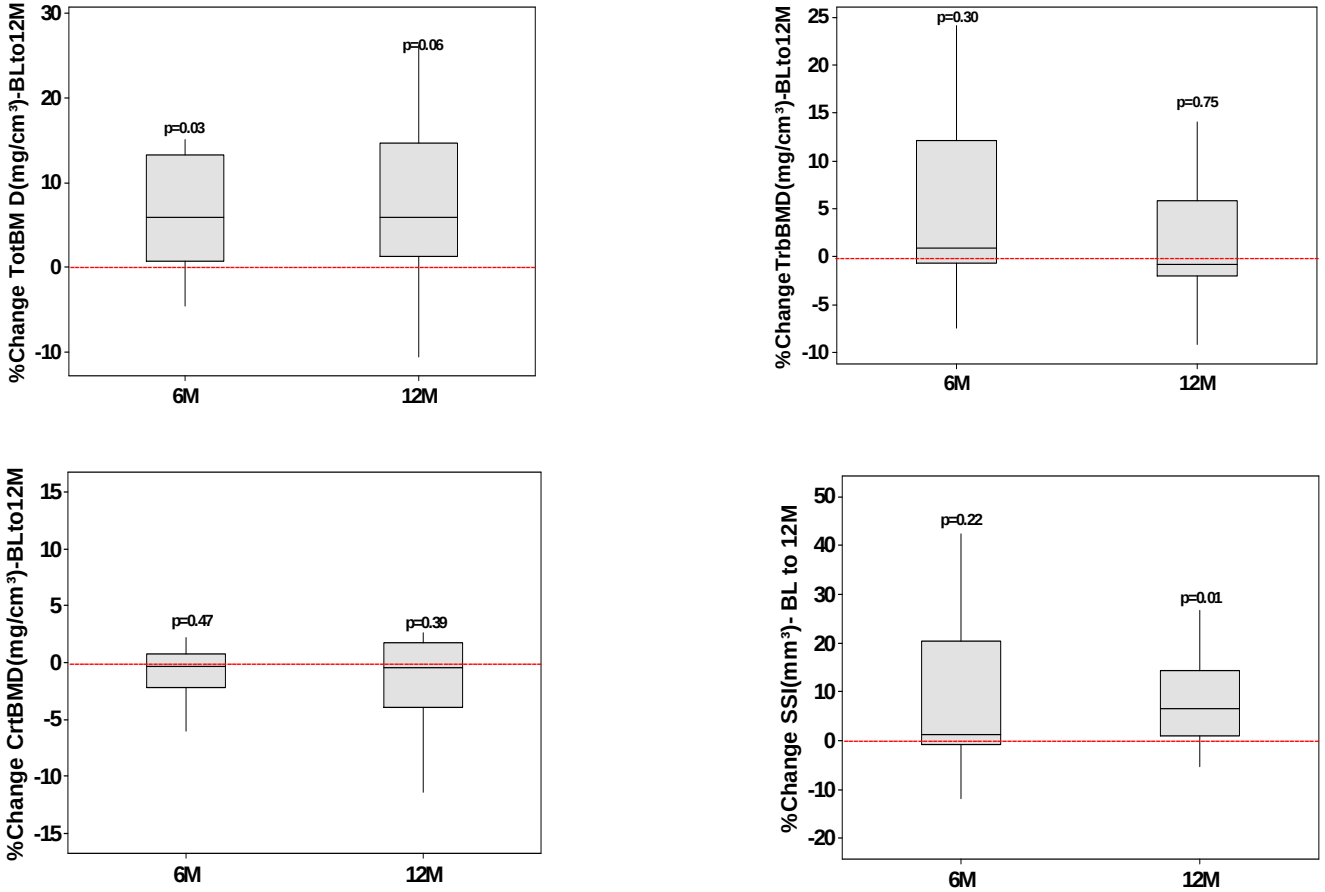
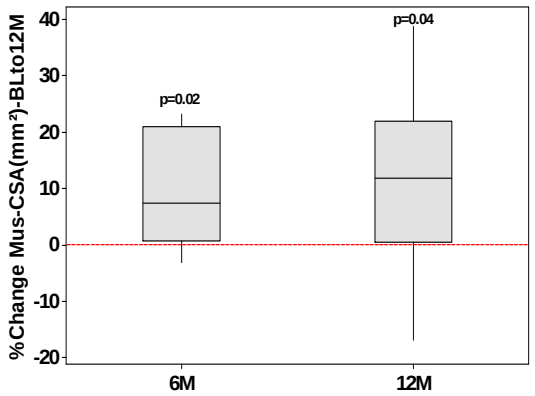
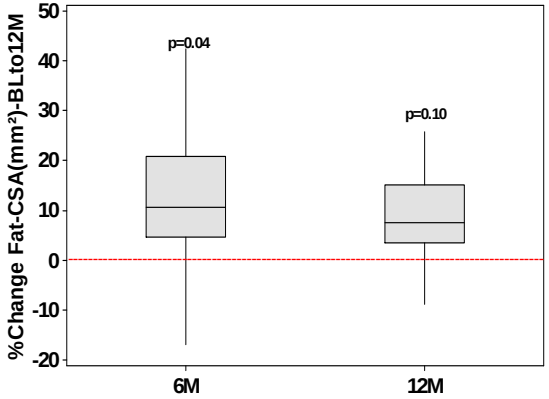
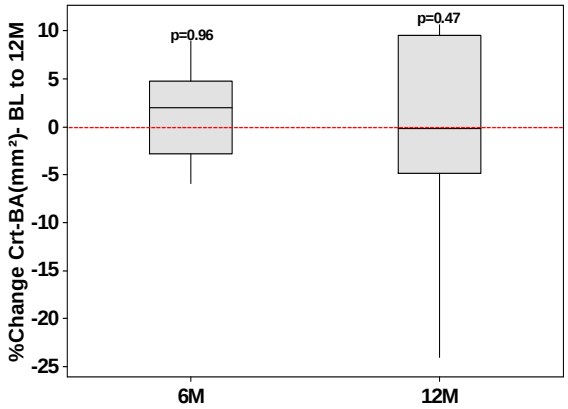


Figure 5.4.22:Percentage (%) change in tibia muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of tibial length at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD (median-range)







5.4.11.2 Radius bone and muscle parameters

As mentioned in methods bone and muscle parameters of non-dominant radius were also measured by pQCT. Individual changes in total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stressstrain index (SSI (mm²) , muscle cross-sectional area (Mus-CSA mm²), fat cross-sectional area (Fat-CSA mm²) and cortical bone area (Crt-BA (mm²) at 66% of tibial length are presented in (Table 5.4.26; Figure 5.5.23-24). Median (range) radius TotBMD (mg/cm³) remained unchanged from 254(183,296) at BL to 253(195,353) at 6M (p=0.12) and 255(190,290) at 12M(p=0.16). The median TrbBMD (mg/cm³) also remain unchanged from 147(93,209) at BL, to 145(93,202) at 6M(p=0.68) and 143(113,184) at 12M(p=0.89). Median SSI (mm²) was 156(89,330) at BL, 184(73,326) at 6M(p=0.68) and 159(89,323) at 12M(p=0.75). Median CrtBMD (mg/cm³) was 1020(947,1155) at BL, 1058(946,1171) at 6M(p=0.50) and 1023(852,1167) at 12M(p=0.82). Median Mus-CSA (mm²) changed from 1827(1216,2848) at BL to 2219(1369,3094) at 6M (p=0.01) and 2539(1203,4238) at 12M(p=0.08). Median Fat-CSA (mm²) was 695(684,1231) at BL, 871(600,1817) at 6M(p=0.26) and 758(366,2204) at 12M(p=0.68). Median Crt-BA (mm²) was 106(68,164) at BL, 131(59,156) at 6M(p=0.62) and 133(26,170) at 12M (p=0.59), respectively (Table 5.4.27). The median percentage change of TotBMD (mg/cm³) from BL to 6M was 5.5%(-15.7,37.9)(p=0.06) and from BL to 12M was 2.0%(-3.9,29.2)(p=0.16).

Median percentage change of TrbBMD (mg/cm³), from BL to 6M was 0.1%(-15.1,37.4)(p=0.56) and from BL to 12M was 2.5%(- 24.1,20.6)(p=0.89). Median percentage change of SSI (mm³) from BL to 6M was 0.7%(- 40.2,46.3)(p=0.68) and from BL to 12M was 5.1%(-31.2,17.9)(p=0.68). Median percentage change of CrtBMD (mg/cm³) from BL to 6M was 1.4%(-7.2,11.2)(p=0.56) and from BL to 12M was 1.1%(-16.1,10.3)(p=0.89). Median percentage of Mus-CSA (mm²) from BL to 6M was 14.6%(-6.4,37.1)(p=0.01) and from BL to 12M was 16.8%(-25.7,184)(p=0.08). Median percentage of Fat-CSA from BL to 6M was 9.1%(-48.7,223)(p=0.14) and from BL to12M was 2.6%(-64.2,149)(p=0.35). Median percentage of Crt-BA (mm²) changed from BL to 6M was 1.4%(- 13.9,27.1)(p=0.50) and from BL-12M was 0.3%(-61.9,12)(p=0.50), respectively (Table 5.4.27;Figure 5.4.25-6).

Table 5.4.26: Individual change radius total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³), muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of radius length at BL,6M &12M following biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
TotBMD(mg/cm³)											
BL	183.2	262.3	256.1	231.0	253.8	191.1	200.1	245.0	275.5	296.0	258.5
6M	194.6	253.1	253.2	240.1	267.7	206.5	216.5	315.0	261.7	301.2	243.6
12M	236.8	282.2	255.4	235.7	245.3	189.9	222.3	289.5	272.9	284.4	288.6
TrbBMD(mg/cm³)											
BL	108.7	165.5	141.9	146.7	147.2	93.3	143.7	188.2	146.9	209.1	118.2
6M	115.2	176.9	138.6	131.7	202.3	93.4	176.6	164.3	145.1	177.3	137.6
12M	116.5	178.8	130.5	150.4	143.2	112.6	151.9	142.9	146.9	184.0	131.9
SSI(mm³)											
BL	166.6	121.9	189.5	329.6	88.5	160.2	184.0	191.4	175.7	202.1	134.8
6M	167.9	72.8	218.1	326.1	87.9	186.2	177.0	151.9	183.7	206.2	197.3
12M	149.0	89.3	207.6	323.4	94.8	128.8	126.6	200.9	192.5	214.4	159.0
CrtBMD(mg/cm³)											
BL	1020.0	947.1	1073.9	1129.4	990.6	973.8	1052.1	954.0	1154.5	1020.3	1096.2
6M	945.8	957.1	1103.4	1081.9	975.6	1082.4	1030.5	991.1	1170.5	1058.4	1128.7
12M	862.6	851.5	1130.5	1071.5	988.5	1074.8	882.5	1042.3	1167.3	1032.5	1116.5
Mus-CSA(mm²)											
BL	1619.0	1359.0	2480.0	2291.5	1216.0	1826.5	1494.7	2847.7	1589.0	2670.7	2141.0
6M	2219.0	1368.8	2512.8	3094.0	1449.0	1932.5	1731.2	2967.7	1937.2	2498.5	2453.5
12M	1202.5	1588.5	2539.0	3393.5	1437.0	2052.2	4238.2	2762.7	2000.2	2591.0	2649.7
Fat-CSA(mm²)											
BL	1023.5	661.0	1230.7	676.7	744.0	1170.5	689.7	694.5	1195.2	284.0	605.5
6M	966.3	695.0	984.5	871.0	812.2	600.2	879.2	740.5	1817.2	915.2	793.5
12M	365.7	552.5	766.7	801.5	757.7	649.2	1334.5	712.7	2204.2	706.0	977.7
Crt-BA(mm²)											
BL	105.2	68.2	102.7	164.0	73.7	129.2	99.0	125.7	150.0	105.5	125.5
6M	99.0	58.7	130.5	155.7	73.5	131.2	105.0	127.5	146.0	132.5	134.2
12M	58.5	26.0	132.7	164.5	57.5	129.0	170.0	139.5	150.2	120.7	143.0

Figure 5.4.23: Individual change radius total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD(mg/cm³), Stress-strain index (SSI (mm³) 66%, at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

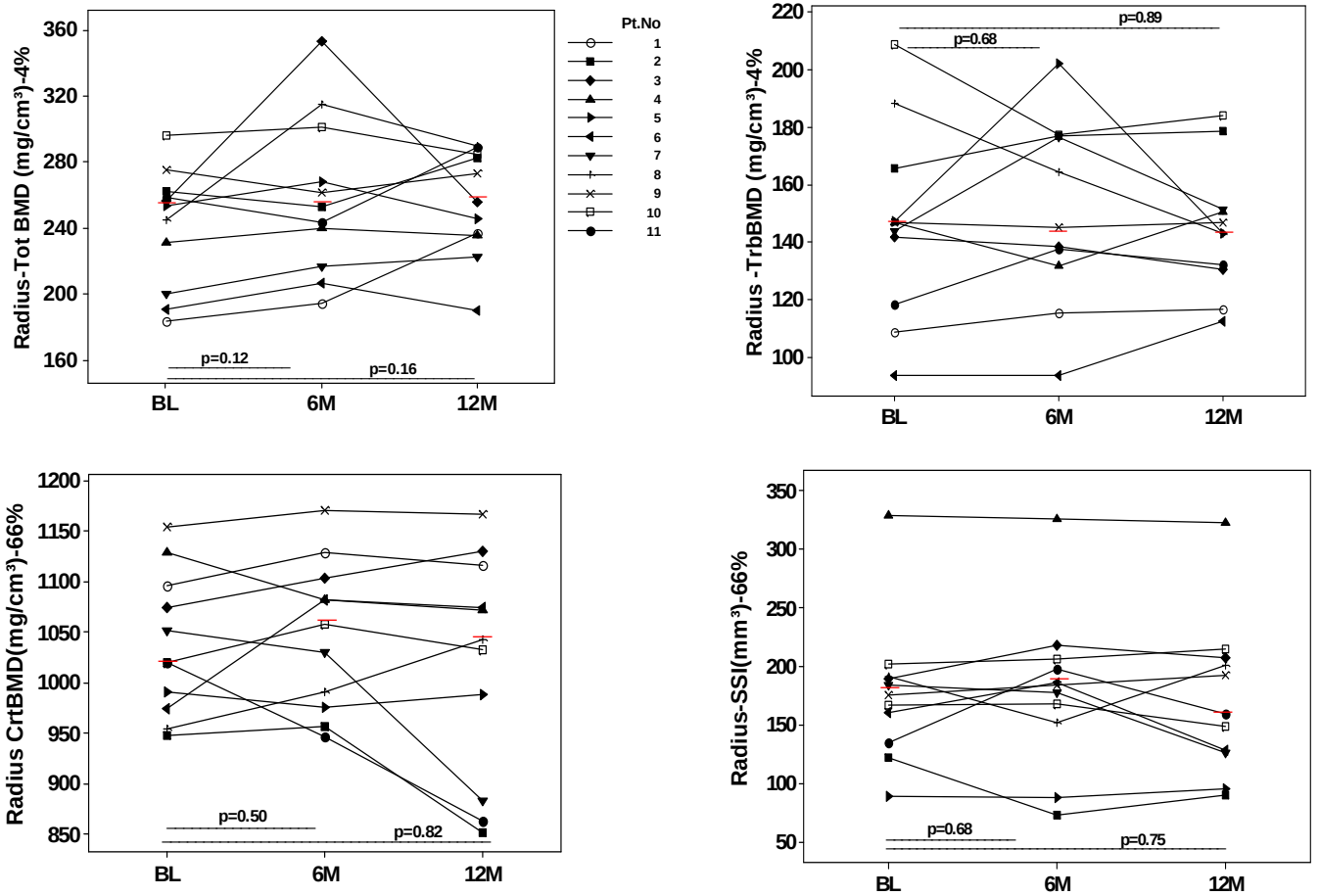


Figure 5.4.24: Individual changes in pQCT radius muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) 66% site, at BL, 6M & 12M following treatment with biologic therapy in paediatric CD patients

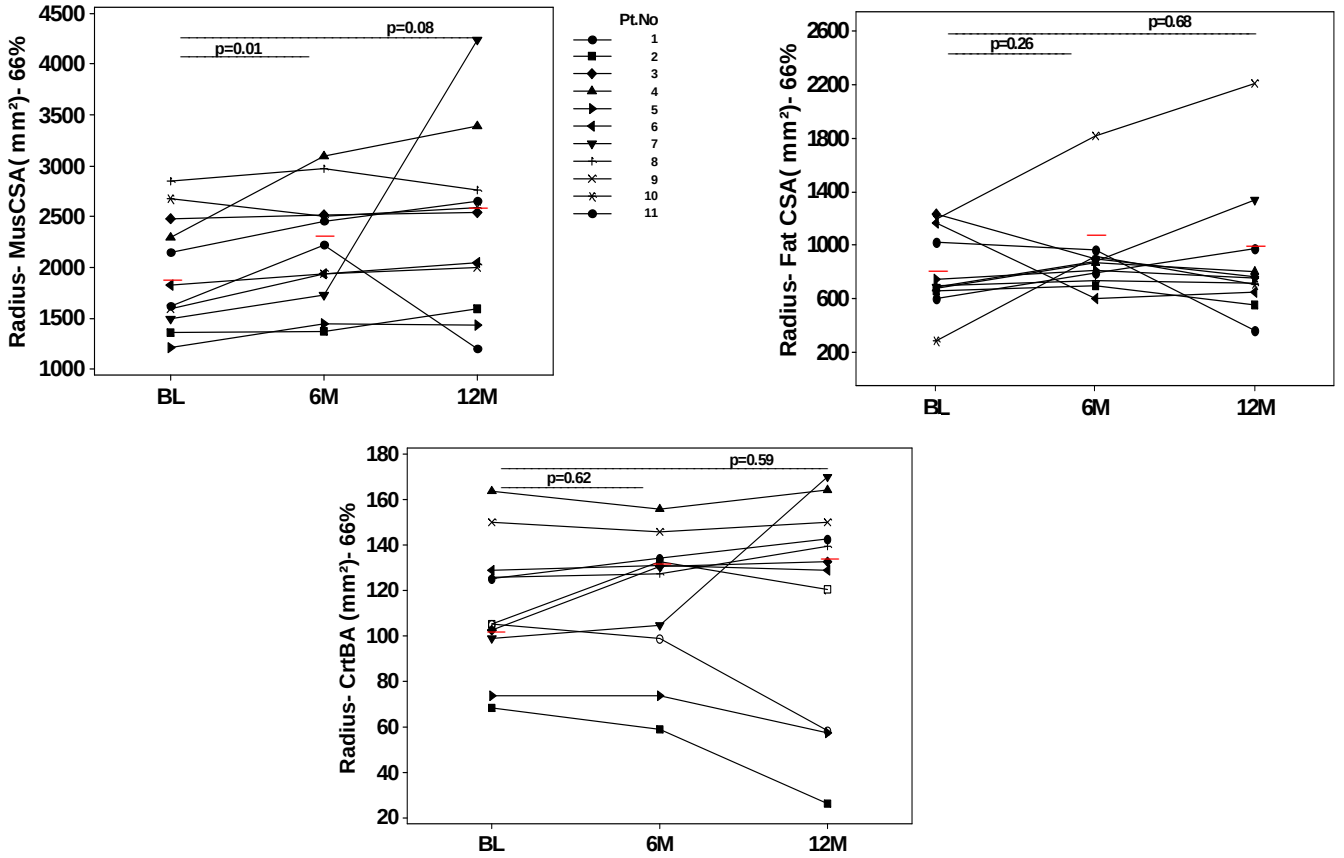


Table 5.4.27: change radius total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³), muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of radius length at BL,6M &12M following biologic therapy in paediatric patients with CD (median-range)

Value	BL N,11	6M N,11	12M N,11	p-Value		%change		p-Value	
				BL-6M	BL-12M	BL-6M	BL-12M	BL-6M	BL-12M
Radius									
TotBMD(mg/cm³)	354	253	355	0.12	0.16	5.5	2.0	0.06	0.16
	(183,296)	(195,353)	(190,290)			(-15.7,37.9)	(-3.9,29.2)		
TrbBMD(mg/cm³)	147	145	143	0.68	0.89	0.1	2.5	0.56	0.89
	(93,209)	(93,202)	(113,184)			(-15.1, 37.4)	(-24.1,20.6)		
SSI(mm³)	156	184	159	0.68	0.75	0.7	5.1	0.68	0.68
	(89,330)	(73,326)	(89,323)			(-40.2,46.3)	(-31.2,17.9)		
CrtBMD(mg/cm³)	1020	1058	1023	0.50	0.82	1.4	1.1	0.56	0.89
	(947,1155)	(946,1171)	(852,1167)			(-7.2,11.2)	(-16.1,10.3)		
Mus-CSA(mm²)	1827	2219	2539	**0.01**	0.08	14.6	16.8	**0.01**	0.08
	(1216,2848)	(1369,3094)	(1203,4238)			(-6.4,37.1)	(-25.7,184)		
Fat -CSA(mm²)	695	871	758	0.26	0.68	9.1	2.6	0.14	0.35
	(284,1231)	(600,1817)	(366,2204)			(-48.7,223)	(-64.2,149)		
Crt-BA(mm²)	106	131	133	0.62	0.59	1.4	0.3	0.50	0.68
	(68,164)	(59,156)	(26,170)			(-13.9,27.1)	(-61.9,12)		

Figure 5.4.25: Percentage (%) change in radius total bone mineral density (TotBMD (mg/cm³), trabecular bone mineral density (TrbBMD (mg/cm³) at 4%, cortical bone mineral density (CrtBMD (mg/cm³), Stress-strain index (SSI (mm³) 66% site at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD (median-range)

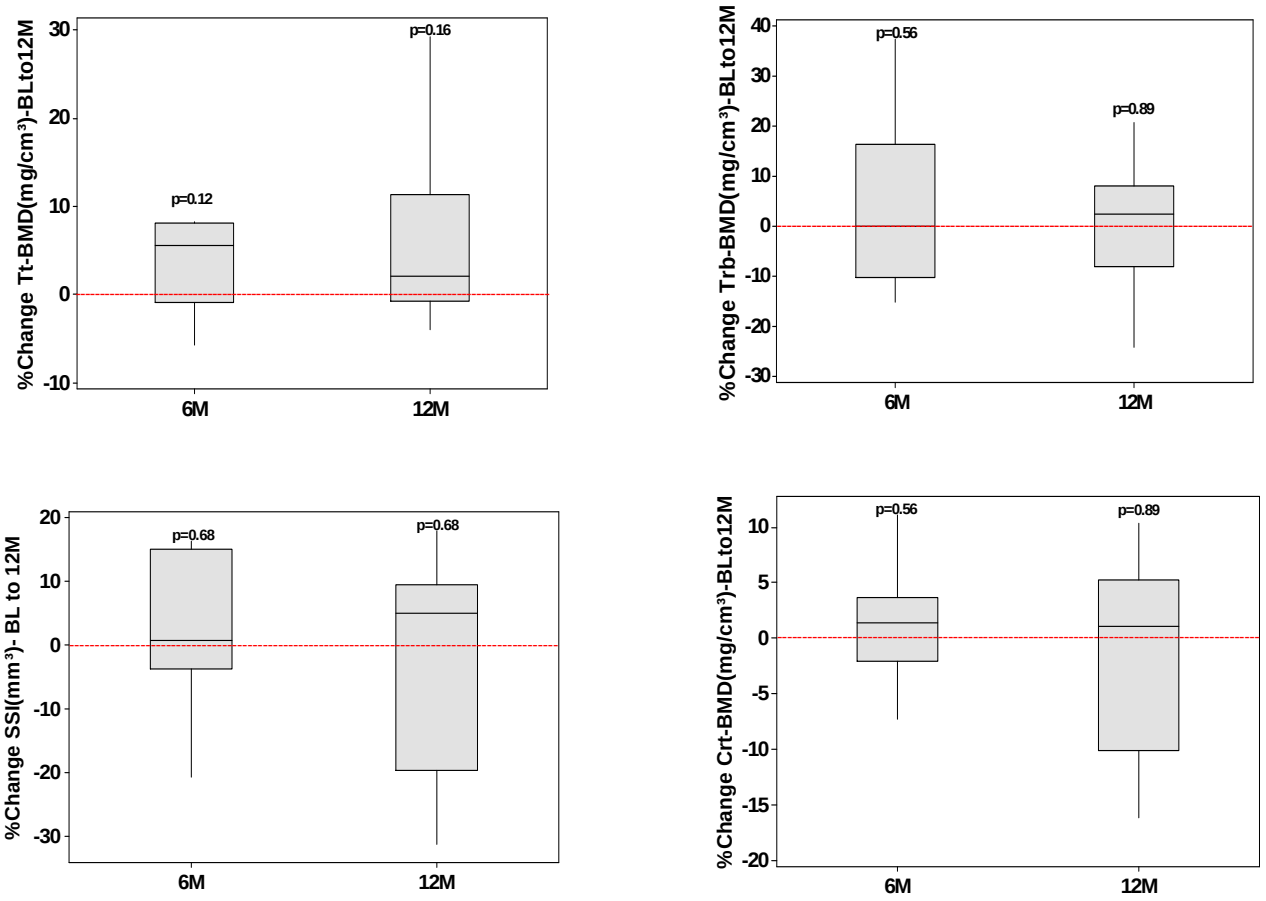
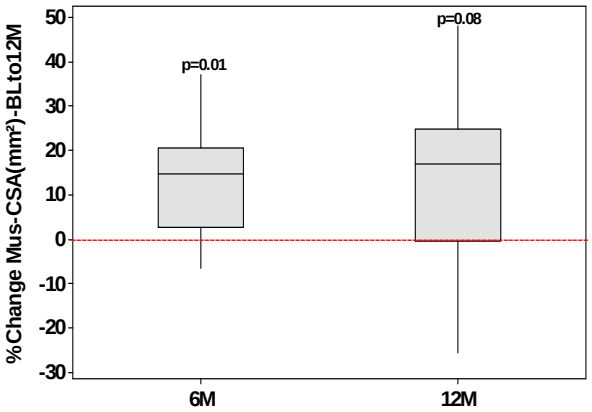
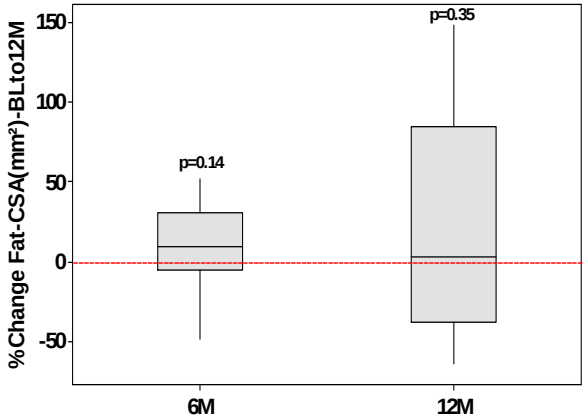
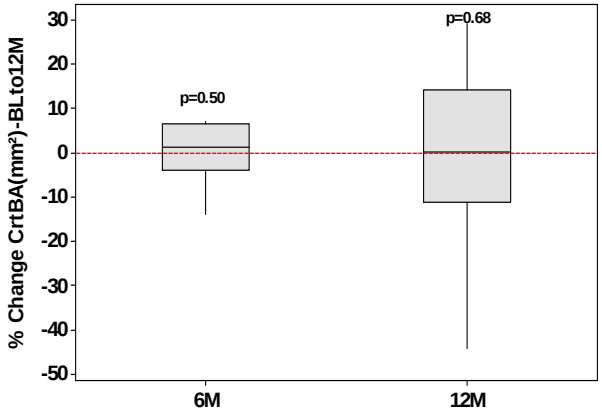


Figure 5.4.26: Percentage (%) change in radius muscle cross-sectional area (Mus-CSA (mm²), fat cross-sectional area (Fat-CSA (mm²) and cortical bone area (Crt-BA (mm²) at 66% of radius length at BL, 6M &12M following treatment with biologic therapy in paediatric patients with CD (median-range)







5.4.11.3 Height corrected radius pQCT parameters

The radius pQCT outcome measure (bone and muscle) were adjusted for height. Individual changes in height-adjusted z-scores for radius total bone mineral density (TotBMD z-score (SD), trabecular bone mineral density (TrbBMD z-score (SD) at 4%, cortical bone mineral density (CrtBMD z-score (SD), Stress-strain index (SSI z-score (SD), muscle cross-sectional area (Mus-CSA z-score (SD), fat cross-sectional area (Fat-CSA z-score (SD) and cortical bone area (Crt-BA z-score (SD) and muscle bone cross-sectional area (Mus-bone CSA z-score (SD) at 66% of radius length at BL,6M &12M are presented in (Table 5.4.28;Figure 5.4.27-7).The height-adjusted z-scores for radius pQCT bone and muscle parameters did not change significantly and remained below zero throughout the study interval. The median (range) TotBMD z-score (SD) were -1.6(-2.8,-0.5) at BL, -2.0(-2.9,0.3) a6M (p=0.83) and -1.5(-3.3,-0.3) at12M(p=0.75). Median TrbBMD z-score (SD) were -1.6(- 3.9,0.0) at BL,-1.6(-3.5,0.1) at 6M (p=0.68) and -2.0(-2.8,-0.7) at 12M (p=0.89). Median CrtBMD z-score (SD) were -0.5(-2.3,2.0) at BL, 0.0(-2.2,2.5) at 6M(p=0.56) and -0.5(-4.8,2.0) at 12M(p=0.35). Median SSI z-score (SD) were -1.2(-2.6,1.2) at BL, -1.8(-3.0,0.2) at 6M(p=0.06) and -2.0(-3.4,0.0) at 12M(p=0.18). Median Mus-CSA z-score (SD) were -2.4(- 4.3,-0.9) at BL, -2.2(-3.2,-0.5) at 6M(p=0.45) and -1.9(-4.3,-0.8) at 12M(p=0.68). Median Fat-CSA z-score (SD) were -0.5(-1.5,-0.8) at BL, -0.4(-2.4,-2.0) at 6M (p=0.82) and -0.2(- 1.0,1.08) at 12M(p=0.50). Median Crt-BA z-score (SD) were -2.2(-3.9,-0.4) at BL, -1.9(-4.1,- 0.7) at 6M(p=0.76) and -2.8(-6.9,-0.8) at 12M(p=0.10). Median Mus-bone CSA z-score (SD) were -0.3(-1.9,3.9) at BL, -0.5(-2.4,1.7) at 6M(p=0.32) and -0.4(-6.2,1.7) at 12M(p=0.12) respectively (Table 5.4.29).

5.4.11.4 Association between musculoskeletal parameters & disease activity

5.4.11.4.1 Radius musculoskeletal parameters & disease activity

A significant negative association was observed between greater PCDAI score and radius TotBMD (mg/cm^3) and TrbBMD z-scores at BL only (r,0.54;p=0.01),(r,0.45;p=0.02) and with Crt-BA z-scores at 6M (r,0.48;p=0.01). No significant correlation was observed between CrtBMD, Mus-CSA, Fat-CSA, Mus-bone-CSA z-scores with PCDAI at BL, 6M and 12M. Association between radius musculoskeletal parameters and inflammatory markers were also observed. TotBMD Zscore and TrbBMD Zscore also had a significant negative association with CRP at BL (0.39;p=0.03), (r,0.36;p=0.048) and a significant positive association with ALB at BL (r,0.38;p=0.04),(0.36;p=0.048) and SSI z-scores with ALB at at BL(r,0.36;p=0.049). No

significant correlation was observed between CrtBMD, Mus-CSA, Fat-CSA and Mus bone-CSA z-scores with ALB, ESR and CRP at BL, 6M and 12M.

5.4.11.4.2 Tibia musculoskeletal parameters & disease activity

No significant association was observed between tibia TotBMD (mg/cm³), TrbBMD (mg/cm³), CrtBMD (mg/cm³), SSI (mm³), Crt-BA (mm²), Mus-CSA (mm²) and Fat-CSA (mm²) with PCDAI at BL, 6M and 12M. No association was observed between TotBMD (mg/cm³), CrtBMD (mg/cm³), Crt-BA (mm²), SSI (mm³), Mus-CSA (mm²) with ALB, ESR and CRP at BL, 6M and 12M. However, positive association was observed between TrbBMD (mg/cm³), and ALB at BL(r,0.40;p=0.03) and Fat-CSA (mm²) with ALB at 6M(r,0.52;p=0.04).

5.7.2.2.3 Radius musculoskeletal parameters & growth

Association between radius musculoskeletal parameters and growth in terms of HV (cms/yr) and HtSDS and BMISDS were also observed at BL, 6M and 12M. No significant association was observed between radius TotBMD(mg/cm³),, TrbBMD(mg/cm³),, SSI (mm³), Crt-BA (mm²), and Mus-CSA z-scores with BMISDS, HtSDS and HV at BL, 6M and 12M. A negative significant association was observed between CrtBMD z-scores and HV(cms/yr) at 6M (r,0.37;p=0.04), and 12M (r,0.45;p=0.02) and a positive association between HtSDS at BL (r,0.61;p=0.004) and 6M (r,0.36;p=0.049). Fat-CSA-z-scores also had a positive significant association with HtSDS at 6M(r,0.39;p=0.03) but not with BMISDS and HV(cms/yr). A positive association was observed between Mus-bone CSA z-scores with BMISDS at 12m (r,0.31;p=0.049) and with HtSDS at BL (r,0.41;p=0.03) and 12M(r,0.42;p=0.03) respectively.

5.7.2.2.4 Tibia musculoskeletal parameters & growth

Association between tibia musculoskeletal parameters and growth in terms of HV (cms/yr) and HtSDS and BMISDS were also observed at BL, 6M and 12M.Tibia TotBMD (mg/cm³), was associated positively with HtSDS at only 12M (r,0.37;p=0.04). TrbBMD (mg/cm³) and Fat-CSA (mm²) did not show any association with HV (cms/yr), HtSDS and BMISDS. A positive significant association was observed between SSI (mm³) and HtSDS at BL(r,0.52;p=0.01) and 12M(r,0.42;p=0.03). A negative significant association was observed between CrtBMD (mg/cm³) and HV (cms/yr) at 6M (r,0.49;p=0.02) and 12M(r,0.39;p=0.03). A significant positive association was observed between CrtBMD (mg/cm³) and HtSDS at BL

(r,0.59;p=0.005), 6M(r,0.57;p=0.01) and 12M(r,0.39;p=0.03). A positive significant association was observed between Mus-CSA (mm^2) and BMISDS at 12M (r,0.49;p=0.01) and HtSDS at 6M (r,0.65;p=0.005)and 12M(r,0.46;p=0.02) respectively.

Table 5.4.28: Individual change radius height-adjusted z-scores (SD) for total bone mineral density (TotBMD), trabecular bone mineral density (TrbBMD) at 4%, cortical bone mineral density (CrtBMD), Stress-strain index (SSI), muscle cross-sectional area (Mus-CSA), fat cross-sectional area (Fat-CSA) and cortical bone area (Crt-BA) and muscle bone cross-sectional area (Mus-Bone CSA) at 66% of radius length at BL,6M &12M following biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
Radius											
TotBMD SDS											
BL	-2.8	-0.8	-2.2	-2.0	-1.1	-2.8	-2.4	-1.4	-1.2	-0.5	-1.6
6M	-2.6	-1.1	-2.3	-2.0	-0.6	-2.9	-2.0	0.3	-1.6	-0.6	-2.0
12M	-1.6	-0.3	-2.5	-2.2	-1.2	-3.3	-1.9	-0.8	-1.5	-1.1	-1.4
TrbBMD SDS											
BL	-2.0	-1.0	-2.0	-2.1	-1.5	-3.9	-1.6	-0.4	-1.5	0.0	-3.2
6M	-2.6	-0.7	-2.0	-2.8	0.1	-3.5	-0.7	-1.2	-1.6	-1.1	-2.5
12M	-2.5	-0.7	-2.0	-2.1	-1.6	-2.8	-1.5	-2.2	-1.5	-1.0	-2.5
SSI SDS											
BL	-0.5	-2.1	-2.5	0.5	-2.6	-1.4	-0.7	-1.1	1.2	-1.2	-2.5
6M	-0.6	-3.0	-2.2	0.2	-2.8	-2.3	-1.0	-1.8	-1.6	-1.3	-1.8
12M	-1.1	-2.6	-2.6	0.0	-2.7	-3.4	-2.0	-1.4	-1.3	-1.3	-2.6
CrtBMD SDS											
BL	-0.3	-1.7	-0.5	1.9	-0.8	-2.3	0.8	-1.6	2.0	-0.7	0.8
6M	-2.2	-1.5	0.2	0.3	-1.0	-0.1	0.2	-1.1	2.5	0.0	1.7
12M	-4.8	-3.6	0.9	-0.2	-0.7	-0.5	-3.1	-0.4	2.0	-1.0	1.0
Mus-CSA SDS											
BL	-1.9	-2.6	-1.6	-2.4	-3.0	-1.4	-2.5	-0.9	-4.3	-0.9	-3.2
6M	-0.5	-2.5	-1.7	-1.0	-2.2	-2.2	-2.2	-0.9	-3.2	-1.3	-2.8
12M	-4.3	-1.8	-1.6	-0.8	-2.2	-1.9	-3.2	-1.8	-2.8	-1.3	-2.4
Fat-CSA SDS											
BL	-0.2	-0.5	0.8	-0.4	-0.4	-0.5	-1.0	-0.5	-0.5	-1.5	-0.6
6M	-2.4	-0.9	-0.2	-0.2	-0.6	-0.9	-0.2	-0.4	2.0	-0.5	0.2
12M	-0.8	-0.5	0.1	0.0	-0.2	-1.0	-0.5	-0.3	1.0	-0.1	-0.2
Crt-BA SDS											
BL	-0.4	-3.4	-3.9	-1.5	-3.0	-0.7	-2.2	-2.3	-0.8	-2.1	-3.7
6M	-1.5	-4.1	-2.8	-1.9	-3.0	-0.7	-1.9	-2.9	-1.1	-1.3	-3.4
12M	-3.9	-6.9	-2.7	-1.8	-4.6	-0.8	-3.7	-2.8	-0.8	-2.4	-3.0
Mus-bone CSA SDS											
BL	0.7	-1.5	-1.9	1.4	-0.3	-0.3	0.6	-0.9	3.9	-1.6	-0.1
6M	-1.8	-2.4	-0.5	0.0	-1.5	1.2	0.0	-1.1	1.7	-0.5	-0.4
12M	-1.1	-6.2	-0.4	-0.4	-2.9	0.5	-2.6	0.1	1.7	-0.9	0.3

Figure 5.4.27:Change in radius height-adjusted z-score(SD) for total bone mineral density (TotBMD), trabecular bone mineral density (TrbBMD) at 4%,cortical bone mineral density (CrtBMD), Stress-strain index (SSI) 66% at BL,6M &12M following biologic therapy in paediatric patients with CD

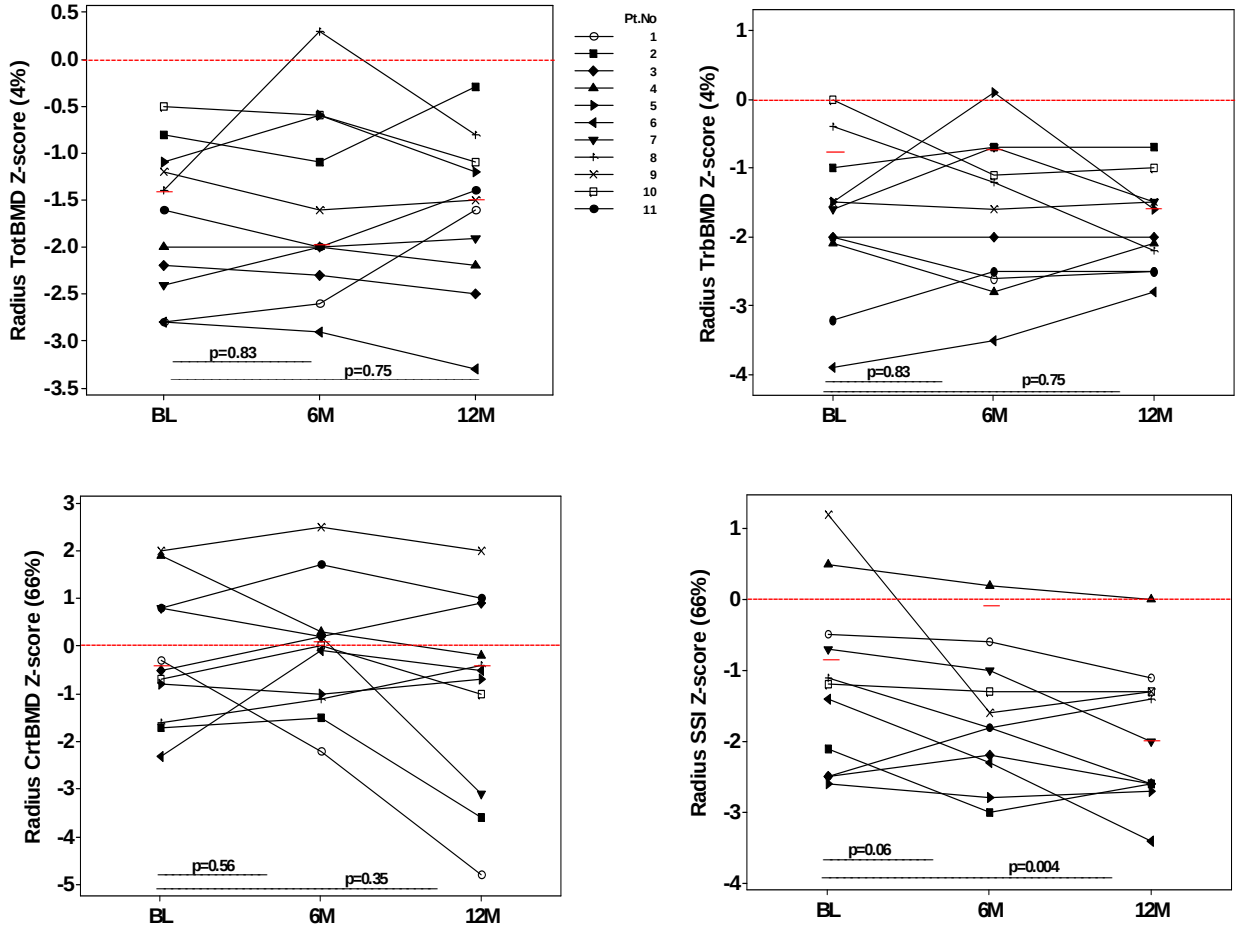


Figure 5.4.28:Change in radius height-adjusted z-scores (SD) for muscle cross-sectional area (Mus-CSA), fat cross-sectional area (Fat-CSA) and cortical bone area (Crt-BA) and muscle bone area (Mus-BoneCSA) at 66% of tibial length at BL,6M &12M following biologic therapy in paediatric patients with CD (median-range)

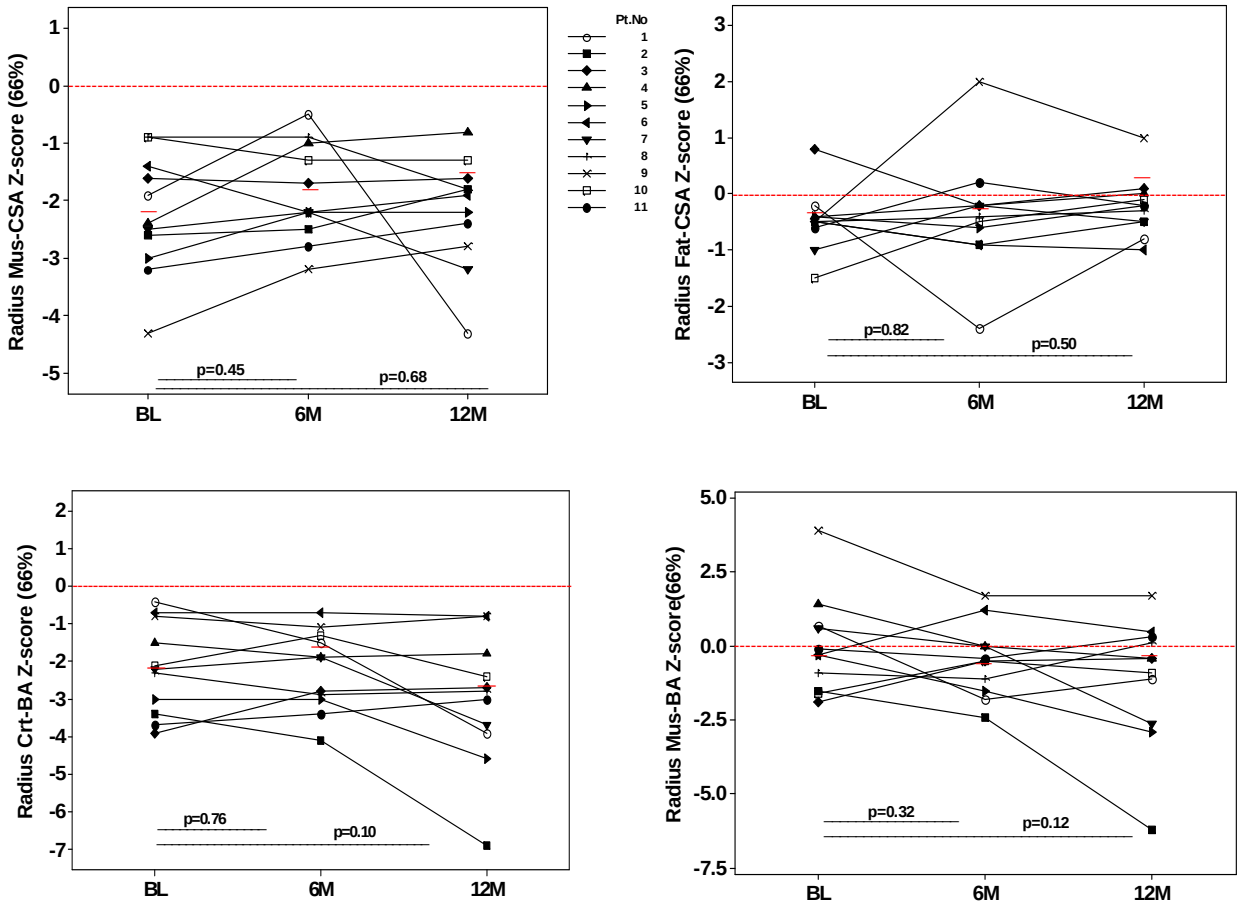


Table 5.4.29: Change in radius height-adjusted z-score(SD) total bone mineral density (TotBMD), trabecular bone mineral density (TrbBMD) at 4%, cortical bone mineral density (CrtBMD), Stress-strain index (SSI), muscle cross-sectional area (Mus-CSA), fat cross-sectional area (Fat-CSA),cortical bone area (Crt-BA) and muscle bone cross-sectional area (Mus-Bone CSA)at 66% of tibial length at BL,6M &12M following biologic therapy in paediatric patients with CD (median-range)

Value	BL	6M	12M	p-Value	
				BL-6M	BL-12M
Tot density z-score (SD)	-1.6 (-2.8,-0.5)	-2.0 (-2.9,0.3)	-1.5 (-3.3,-0.3)	0.83	0.75
Trb density z-score (SD)	-1.6 (-3.9,0.0)	-1.6 (-3.5,0.1)	-2.0 (-2.8,-0.7)	0.68	0.89
SSIPOL3 z-score (SD)	-1.2 (-2.6,1.2)	-1.8 (-3.0,0.2)	-2.0 (-3.4,0.0)	0.06	0.18
Crt density z-score (SD)	-0.5 (-2.3,2.0)	0.0 (-2.2,2.5)	-0.5 (-4.8,2.0)	0.56	0.35
Muscle Area z-score (SD)	-2.4 (-4.3,-0.9)	-2.2 (-3.2,-0.5)	-1.9 (-4.3,-0.8)	0.45	0.68
Fat Area z-score (SD)	-0.5 (-1.5,0.8)	-0.4 (-2.4,2.0)	-0.2 (-1.0,1.0)	0.82	0.50
Bone Area z-score (SD)	-2.2 (-3.9,-0.4)	-1.9 (-4.1,-0.7)	-2.8 (-6.9,-0.8)	0.76	0.10
MUS Bone Area z-score (SD)	-0.3 (-1.9,3.9)	-0.5 (-2.4,1.7)	-0.4 (-6.2,1.7)	0.32	0.12

5.4.12 Changes in bone metabolism

5.4.12.1 Changes in bone specific alkaline phosphatse

Individual changes in serum bone specific alkaline phosphatase (BALP (µg/l) are presented in (Table 5.4.30;Figure 5.4.29). Median (range) BALP (µg/l) changed from 21.3(9.2,70.5) at BL to 16.9 (7.9,77.3) at 2wk (p=0.56), 26.7(7.6,88.7) at 6wk(p=0.045), 28.1(5.5,117.5) at 6M(p=0.01) and 38.3(10.9,110.5) at 12M(p=0.03) (Table 5.8.2). The median percentage change of BALP(g/l)from BL to 2wk was -1.5%(-30.8,63.7) (p=0.62), from BL to 6wk was 14.8%(-9.6,248.3)(p=0.02), from BL to 6M was 68.1%(-40.5,210.5) (p=0.01) and from BL to 12M was 128.7%(-67.2,592.5)(p=0.01),respectively (Table 5.4.32;Figure 5.4.30).

5.4.12.2 Changes in cross linked C-telopeptide of type I collagen

Individual changes in serum cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) are presented in (Table 5.4.30;Figure 5.4.29). Median (range) CTX-1(ng/ml) was 0.73(0.22,2.41) at BL, 0.87(0.22,2.29) at 2wk (p=0.82), 0.95(0.36,2.29) at 6wk(p=0.30), 1.21(0.73,2.35) at 6M(p=0.04) and 1.08(0.29,2.01) at 12M(p=0.50) (Table 5.8.2). The median percentage change of CTX-1(ng/ml) from BL to 2wk was -7.9%(-65.4,271.3) (p=0.96), from BL to 6wk was 13.6%(-35.1,124.9)(p=0.14), from BL to 6M was 62.1%(-17.4,263.9) (p=0.03) and from BL to 12M was 19.9%(-50.9,823.7)(p=0.26) respectively, (Table 5.4.32;Figure 5.4.30).

5.4.12.3 Association of bone markers and disease activity

A significant negative association was observed between BALP(µg/l) and PCDAI at BL (r,0.46;p=0.02) only and there was no significant association between BALP(µg/l) and ESR, CRP and ALB at BL, 2wk, 6wk 6M and 12M. No significant association was observed between CTX-1(ng/ml) and PCDAI, ESR,CRP and ALB at BL, 2wk, 6wk 6M and 12M.

5.4.12.4 Association of bone markers and growth

A significant positive association was observed between BALP (µg/l) and HV (cms/yr) at 6M (r,0.55;p=0.01) and 12M (r,0.35;p=0.049). No significant association was observed between CTX-1(ng/ml) and HV (cms/yr) or HtSDS at BL, 6M and 12M.

Table 5.4.30: Individual changes in bone-specific alkaline phosphatase (BALP(µg/l) and cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) at BL, 2wk,6wk, 6M &12M during biologic therapy in paediatric patients with CD

Pt.No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
BALP(µg/l)											
BL	12.8	24.5	12.6	9.24	25.1	9.76	28.6	70.4	10.3	21.2	25.1
2wk	14.7	16.9	16.9	7.9	24.7	8.6	46.8	77.3	9.4	18.7	30.6
6wk	51.2	16.8	19.1	7.6	26.7	22.8	84.6	88.7	8.5	29.9	45.8
6M	-	27.4	21.4	5.5	28.7	16.2	88.7	117.7	24.1	48.2	42.6
12M	88.8	56.1	13.0	43.2	38.2	25.2	103.9	23.1	10.8	110.5	35.1
CTX-1(ng/ml)											
BL	1.27	0.85	0.47	1.66	0.73	0.56	2.40	0.61	0.59	0.21	0.98
2wk	0.95	0.29	0.83	0.86	0.85	0.88	1.01	2.29	0.47	0.22	0.91
6wk	1.82	0.64	0.94	0.80	0.72	2.04	2.29	1.48	0.98	0.35	0.78
6M	*	0.73	1.26	1.37	1.37	1.16	2.35	1.03	0.82	0.79	1.55
12M	1.52	1.41	1.29	0.96	0.68	0.80	1.69	1.07	0.29	2.00	0.90

Figure 5.4.29: Individual changes in bone-specific alkaline phosphatase (BALP(µg/l) and cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) at BL, 2wk,6wk, 6M &12M during biologic therapy in paediatric patients with CD

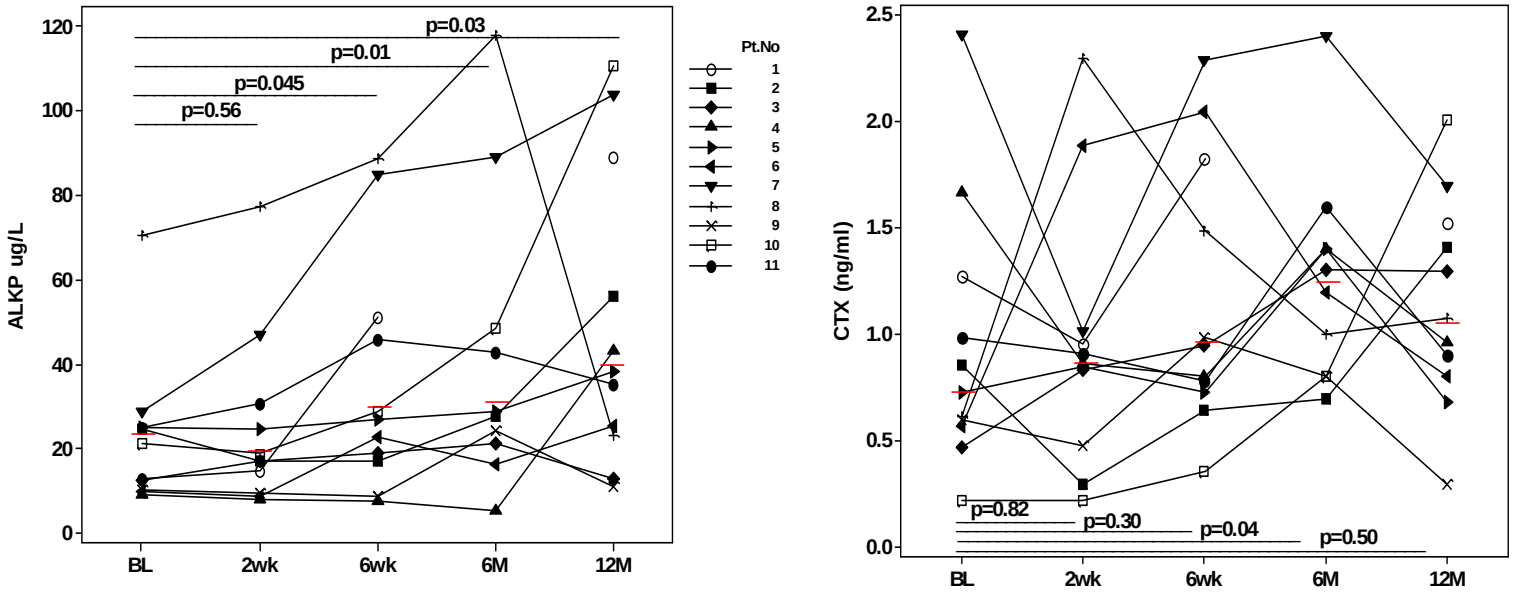


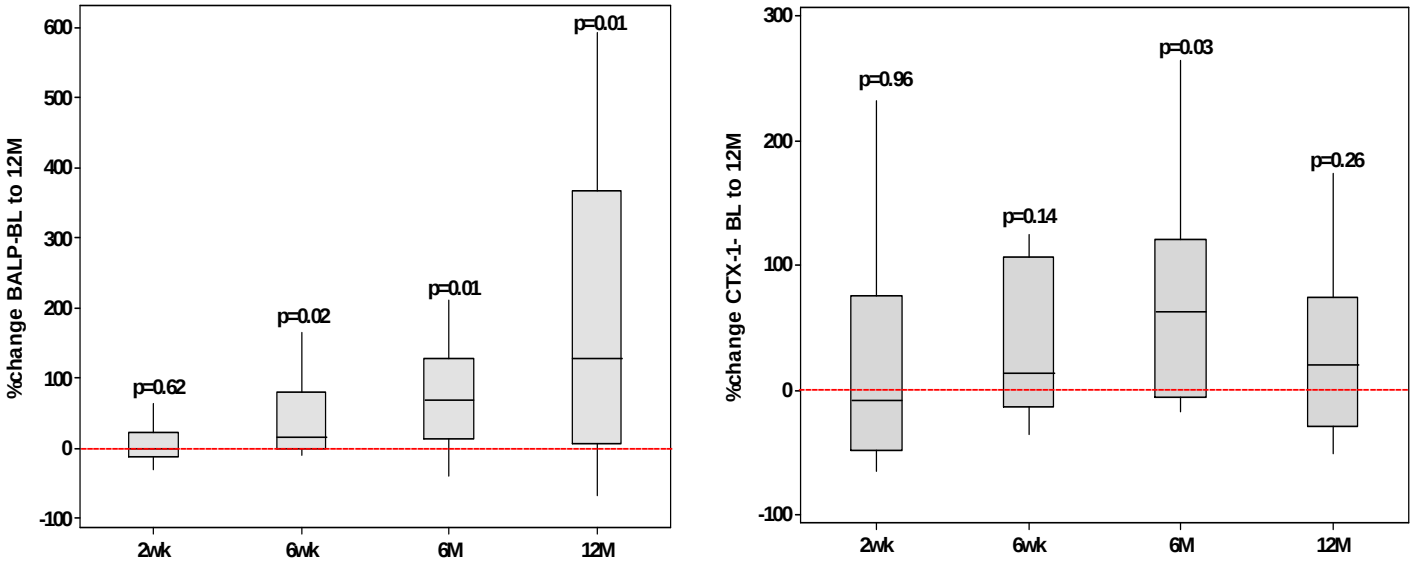
Table 5.4.31: Change in bone-specific alkaline phosphatase (BALP(µg/l) and cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) at BL, 2wk,6wk, 6M &12M during biologic therapy in paediatric patients with CD during biologic therapy in paediatric patients with CD (median-range)

						p-Value			
Value	**BL N,11**	**2wk N,11**	**6wk N,11**	**6M N,11**	**12M N,11**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
BALP(µg/L)	21.3 (9.2,70.5)	16.9 (7.9,77.3)	26.7 (7.6,88.7)	28.1 (5.5,117.7)	38.3 (10.9,110.5)	0.56	**0.045**	**0.01**	**0.03**
CTX-1(ng/ml)	0.73 (0.22,2.41)	0.87 (0.22,2.29)	0.95 (0.36,2.29)	1.21 (0.73,2.35)	1.08 (0.29,2.01)	0.82	0.30	**0.04**	0.50

Table 5.4.32: Percentage (%) change in bone-specific alkaline phosphatase (BALP(µg/l) and cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) at BL, 2wk, 6wk, 6M &12M during biologic therapy in paediatric patients with CD during biologic therapy in paediatric patients with CD (median-range)

	%Change				p-Value			
Value	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
BALP(µg/L)	-1.5 (-30.8,63.7)	14.8 (-9.6,248.3)	68.1 (-40.5,210.5)	128.7 (-67.2,592.5)	0.62	**0.02**	**0.01**	**0.01**
CTX-1(ng/ml)	-7.9 (-65.4,271.3)	13.6 (-35.1,124.9)	62.1 (-17.4,263.9)	19.9 (-50.9,823.7)	0.96	0.14	**0.03**	0.26

Figure 5.4.30: Percentage (%) changes in bone-specific alkaline phosphatase (BALP(µg/l) and cross linked C-telopeptide of type I collagen (CTX-1(ng/ml) from BL to 2wk,6wk, 6M &12M during biologic therapy in paediatric patients with CD



5.4.13 Changes in markers of growth hormone secretion

5.4.13.1 Changes in Insulin like growth factor-I (IGF-1)

Individual changes in IGF-I ng/ml and IGF-1SDS height-age and chronological-age-matched are presented in (Table 5.4.33; Figure 5.5.31). Median (range) IGF-I ng/ml remained unchanged from 286(68,682) at BL to 300(82,649) at 2wk (p=0.56), 258(50,623) at 6wk(p=0.35), 259(151,624) at 6M(p=0.68) and 303(141,524) at 12M (p=0.56). IGF-1 was converted to height-age and chronological-age-matched standard deviation scores (IGF-1SDS). Median IGF-1SDS ht-age-matched was also unchanged at -0.3(-2.4,2.3) at BL , -0.4(-2.8,1.9) at 2wk(p=0.91), -0.3(-3.2,1.2) at 6wk(p=0.48), -0.2(-2.6,1.8) at 6M(p=0.90) and -0.5(-3.3,2.1) at 12M (p=0.68). Median IGF-1SDS chronological age-matched did not change from -0.2(-4.2,1.7) at BL to -1.0(-3.6,1.5) at 2wk(p=0.89), -0.8(-5.2,1.4) at 6wk(p=0.44), -0.7(-2.3,1.2) at 6M(p=0.87) and -1.1(-3.6,1.0) at 12M (p=0.62)(Table 5.4.34).The median percentage change of IGF-I (ng/ml) from BL to 2wk was -4.8%(-32.7,122.6) (p=0.82), from BL to 6wk was -3.9%(-42.2,90.6)(p=0.82), from BL to 6M was -0.5%(-56.5,251.9)(p=0.83) and from BL to 12M was -13.7%(-61.8,413.1)(p=0.89)(Table 5.4.35; Figure 5.4.35).

5.4.13.2 Association of IGF-1 and disease activity

A significant negative association was observed between IGF-I(ng/ml) and PCDAI score at 2wk(r,0.60;p=0.005) and a positive association was observed between IGF-I ng/ml and ALB at BL (r,0.59;p=0.005), at 2wk(r,0.36;p=0.49) and 12M(r,0.44;p=0.02).

5.4.13.3 Association of IGF-1 and growth

A significant negative association was observed between IGF-I(ng/ml) and HtSDS at BL(r,0.37;p=0.04). IGF-1 ng/ml levels were lower in patients who had low HtSDS (Figure 5.4.33).

5.4.13.4 Association of IGF-ISDS height-age-matched and disease activity

A significant negative association was observed between IGF-ISDS ht-age-matched and PCDAI score BL(r,0.38;p=0.04), at 2wk(r,0.43;p=0.02) and at 6wk(r,0.41;p=0.03). A significant negative association was also observed between IGF-ISDS ht-age-matched and ESR at BL(r,0.57;p=0.01), at 2wk(r,0.51;p=0.01) and at 6wk(r,0.46;p=0.02), IGF-ISDS ht-age-

matched and CRP at 2wk(r,0.47;p=0.01) and a significant positive association was observed between IGF-ISDS ht-age-matched and ALB at BL(r,0.44;p=0.02).

5.4.13.5 Association of IGF-ISDS height-age-matched and growth

A significant negative association was observed between IGF-ISDS ht-age-matched and change in HtSDS (ΔHtSDS) at BL(r,0.66;p=0.004). However, no significant association was observed between IGF-ISDS ht-age-matched with HV(cms/yr) at BL, 6M or 12M.

5.4.13.6 Association of IGF-ISDS age-matched and disease activity

A significant negative association was observed between IGF-ISDS chronological-age-matched and PCDAI score at 2wk(r,0.58;p=0.006) and at 6wk(r,0.51;p=0.01). A significant negative association was also observed between IGF-ISDS chronological-age-matched and ESR at BL(r,0.54;p=0.01) and at 2wk(r,0.48;p=0.01) and with CRP at BL(r,0.036;p=0.049)and a significant positive association was observed between IGF-ISDS chronological-age-matched and ALB at BL(r,0.57;p=0.007), at 2wk(r,0.48;p=0.02) and at 6wk(r,0.36;p=0.048).

5.9.1.4 Association of IGF-ISDS age-matched and growth

A significant negative association was observed between IGF-ISDS chronological-age-matched and change is HtSDS (ΔHtSDS) at BL(r,0.43;p=0.03). However, no significant association was observed between IGF-ISDS chronological-age-matched with HV(cms/yr) at BL, 6M or 12M.

5.4.13.7 Changes in Insulin like growth factor binding proteins

Individual changes in insulin like growth factor binding protein 3 (IGFBP-3 (ng/ml), IGFBP-3 SDS height-age and chronological-age-matched and insulin like growth factor binding protein 2 (IGFBP-2 (ng/ml) are presented in (Table 5.4.33;Figure 5.4.33-34). Median (range) IGFBP-3 (ng/ml) did not change with 3.8(1.7,5.9) at BL to 3.3(2.4,4.2) at 2wk(0.32), 3.3(2.1,4.5) at 6wk(p=0.18), 3.4(2.1,4.5) at 6M(p=0.50) and 3.3(2.5,5.8) at 12M (0.79). Median IGFBP-3-SDS ht-age-matched did not change from 0.1(-2.8,2.4) at BL to 0.0(-0.7,1.2) at 2wk(p=0.47), 0.0(-0.9,1.5) at 6wk(p=0.23), 0.1(-2.1,2.6) at 6M (p=0.81), and 0.0(-1.3,2.0) at 12M(p=0.82). Median IGFBP-3-SDS chronological-age-matched remained unchanged from 0.5(-2.9,2.2) at BL to -0.2(-1.4,1.1) at 2wk(p=0.39),-0.2(-1.9,1.4) at 6wk(p=0.18), 0.0(-2.0,1.1) at 6M (p=0.68), and 0.0(-1.2,2.3) at 12M(p=0.82)(Table 5.4.34). The median percentage change of IGFBP-3

(ng/ml) from BL and 2wk was -5.7(-44.1,64.7) (p=0.50), from BL to 6wk was -8.7%(-44.1,76.5)(p=0.56), from BL to 6M was -6.5%(-58.0,70.6) (p=0.83) and from BL to 12M was -11.7%(-50.0,64.7)(p=0.89)(Table 5.9.3;Figure 5.9.4). Median (range) IGFBP-2 (ng/ml) did not change from 713.1(237.5, 1460) at BL to 764.1(153,1307) at 2wk(p=0.90), 561.8(242.4,864.1) at 6wk(p=0.16), 606.5(106.3,877.3) at 6M(p=0.12) and 374.1(198.3,1089) at 12M (p=0.04) (Table 5.4.34; Figure 5.9.34). The median percentage change of IGFBP-2 (ng/ml) from BL and 2wk was 0.7(-35.6,25.1) (p=0.75), from BL to 6wk was -7.2%(-59.2,58.5)(p=0.39), from BL to 6M was -16.3%(-65.4,91.9) (p=0.15) and from BL to 12M was -46.3%(- 65.4,47.1)(p=0.045)(Table 5.4.35;Figure 5.4.35).

5.4.13.8 Association of IGFBPs and disease activity

A significant negative association was observed between IGFBP-3-SDS ht-age-matched and PCDAI score at BL(r,0.59;p=0.006) and CRP at BL(r,0.42;p=0.01) and significant positive association with ALB at BL(r,0.49;p=0.01). A significant positive association was observed between IGFBP-2 and PCDAI score at 2wk(r,0.61;p=0.004) and 12M(r,0.36;p=0.048) and a negative association between IGFBP-2 (ng/ml) and ALB at 12M(r,0.68;p=0.002).

5.4.13.9 Association of IGFBPs and growth

A significant positive association was observed between IGFBP-3-SDS ht-age-matched and HV(cms/yr) at 12M only (r,0.47; p=0.01). A significant negative association was observed between IGFBP-2 (ng/ml) and HV (cms/yr) at 6M only (r,0.52; p=0.01).

5.4.13.10 Changes in acid labile subunits

Individual changes in acid labile subunits (ALS (ng/ml) are presented in (Table 5.4.33; Figure 5.4.34). The median (range) ALS (ng/ml) was 1607(612,4291) at BL, 1633(724,8516) at 2wk(p=0.89), 1910(527,3366) at 6wk(p=0.75), 1732(736,2633) at 6M(p=0.26), 1693(766,2530) at 12M(p=0.39)(Table 5.4.34). The median percentage change of ALS (ng/ml) from BL and 2wk was -0.8%(-83.1,123) (p=1.0), from BL to 6wk was -9.3%(-235.9,365)(p=0.89), from BL to 6M was -8.4%(-47.3,105.4)(p=0.35) and from BL to 12M was -10.9%(-49.1,84.3)(p=0.045)(Table 5.4.35; Figure 5.4.35).

5.9.3.1 Association of acid labile subunits and disease activity

No significant association was observed between ALS (ng/ml) and PCDAI score at anytime point. However a significant positive association was observed with ALB at BL(r,0.67;p=0.002) and at 12M(r,0.41;p=0.03).

5.9.3.2 Association of acid labile subunits and growth

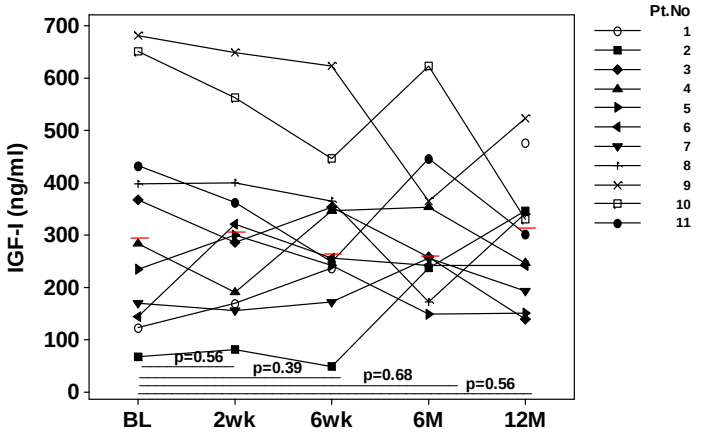
A significant positive association was observed between ALS (ng/ml) and HtSDS at BL(r,0.49; p=0.01), 6M(r,0.66;p=0.004) and 12M(r,0.61;p=0.004). No significantly association was observed between ALS (ng/ml) and HV(cms/yr at BL,6M and 12M.

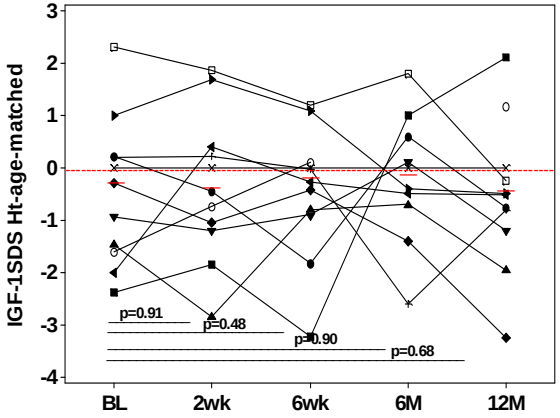
Table 5.4.33: Individual change in Insulin like growth factor-I (IGF-1 (ng/ml), Insulin like growth factor-I height-age and chronological-age-matched standard deviation scores (IGF-ISDS ht-age-matched and IGF-1SDS age-matched), Insulin like growth factor binding protein-3 (IGFBP-3(ng/ml), Insulin like growth factor binding protein-3 ht-age-matched and chronological-age-matched standard deviation scores (IGFBP-3SDS ht-age-matched IGFBP-3SDS age matched), Insulin like growth factor binding protein-2 (IGFBP-2 (ng/ml), and acid labile subunits (ALS (ng/ml) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

Pt.No	1	2	3	4	5	6	7	8	9	10	11
IGF-1(ng/ml)											
BL	12.49	67.6	368.5	285.5	236.4	144.8	171.2	397.9	682	652.5	433.3
2wk	171.6	82.3	286.8	192.2	300.2	322.3	157.8	400.9	649.1	564.6	363.7
6wk	238.0	49.6	354.1	347.0	242.9	257.8	172.9	366.7	623.8	448.4	250.3
6M	*	238	260.4	354.1	151.0	242.9	257.8	172.9	366.7	623.8	448.4
12M	477.1	347	140.8	248.7	152.8	242.3	193.2	343.3	523.5	331.7	303.4
IGF-1 Ht-age-matched SDS											
BL	-1.6	-2.4	-0.3	-1.5	1.0	-2.0	-0.9	0.2	0.0	2.3	0.2
2wk	-0.7	-1.8	-1.1	-2.8	1.7	0.4	-1.2	0.2	0.0	1.9	-0.4
6wk	0.1	-3.2	-0.4	-0.8	1.1	-0.3	-0.9	0.0	0.0	1.2	-1.8
6M	*	1.0	-1.4	-0.7	-0.4	-0.5	0.1	-2.6	0.0	1.8	0.6
12M	1.2	2.1	-3.3	-2.0	-0.5	-0.5	-1.2	-0.8	0.0	-0.3	-0.8
IGF-1 age-matched SDS											
BL	-2.4	-4.2	-0.2	-1.4	0.6	-3.9	-1.0	0.4	1.7	1.4	0.0
2wk	-1.6	-3.6	-1.1	-2.8	1.3	-1.0	-1.3	0.4	1.5	0.9	-0.6
6wk	-0.8	-5.2	-0.3	-0.8	0.6	-1.8	-1.0	0.1	1.4	0.2	-1.9
6M	*	-0.7	-1.4	-0.7	-1.2	-1.9	0.0	-2.3	-0.6	1.2	0.2
12M	0.7	0.0	-3.6	-1.9	-1.4	-1.7	-1.3	-0.7	1.0	-0.9	-1.1
IGFBP-3 (ng/ml)											
BL	2.6	2.6	5.0	4.1	2.5	1.7	4.3	4.3	3.8	5.9	3.5
2wk	3.6	2.4	3.9	3.3	2.7	2.8	4.2	3.7	3.7	3.3	3.3
6wk	4.4	2.1	3.8	2.8	2.9	3.0	4.5	3.5	3.6	3.3	3.2
6M	*	4.4	2.1	3.8	2.9	2.9	3.0	4.5	3.5	3.6	3.3
12M	4.1	2.8	2.5	3.4	3.2	2.8	5.8	3.5	3.3	3.4	3.1
IGFBP-3 Ht-age-matched SDS											
2wk	-0.8	0.1	1.5	0.7	-0.1	-2.8	1.3	0.9	0.0	2.4	0.1
6wk	0.6	-0.2	0.5	-0.2	0.3	-0.7	1.2	0.3	0.0	-0.1	-0.1
6M	1.5	-0.9	0.4	-0.9	0.6	-0.4	1.5	0.1	0.0	-0.1	-0.3
12M	*	2.6	-2.1	0.4	0.3	-0.6	-0.4	1.1	0.0	0.2	-0.1
	0.8	0.3	-1.3	0.0	0.6	-0.7	2.3	0.1	0.0	-0.1	-0.3
IGFBP-3 age-matched SDS											
BL	-1.1	-1.0	1.6	0.7	-0.9	-2.9	1.3	1.0	0.5	2.2	0.1

2wk	0.3	-1.4	0.6	-0.2	-0.6	-0.8	1.1	0.3	0.4	-0.2	-0.2
6wk	1.1	-1.9	0.5	-0.9	-0.3	-0.5	1.4	0.1	0.2	-0.2	-0.3
6M	*	1.1	-2.0	0.4	-0.4	-0.7	-0.4	1.1	0.1	0.2	-0.2
12M	0.7	-0.8	-1.2	0.0	0.0	-0.8	2.3	0.1	0.0	-0.1	-0.4
IGFBP-2(ng/ml)											
BL	965.8	1460.0	819.0	713.1	528.9	896.6	628.0	756.8	357.6	237.5	679.5
2wk	117.2	1306.8	825.3	832.0	461.8	696.5	786.1	509.4	374.7	152.9	764.4
6wk	483.6	533.3	728.9	842.5	428.4	767.0	674.8	561.8	299.3	242.4	864.1
6M	*	845.8	877.3	735.8	485.1	748.5	526.8	385.1	686.1	106.3	414.5
12M	374.0	504.6	1089.5	572.6	777.3	478.3	337.0	323.1	198.3	277.5	352.1
ALS(mU/ml)											
BL	1308.4	829.3	1986.1	1562.0	1056.0	612.2	1606.6	2212.9	4291.1	3251.7	2083.3
2wk	1633.2	822.6	2000.0	1476.8	1699.7	1365.4	1572.3	1841.6	723.9	2516.0	2177.2
6wk	1915.7	527.3	2480.7	1910.2	1270.4	1148.0	1426.2	2120.7	3366.4	2076.0	1465.7
6M	*	823.1	1874.9	1832.2	735.8	1257.8	1425.4	2324.1	2633.4	1712.4	1751.6
12M	2411.2	766.3	1010.1	2088.1	1100.8	1108.1	1432.1	1805.2	2530.0	1693.2	1848.4

Figure 5.4.31: Individual change in Insulin like growth factor-I (IGF-1 (ng/ml), Insulin like growth factor-I height-age and chronological-age-matched standard deviation scores (IGF-ISDS ht-age-matched and IGF-1SDS age-matched) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD





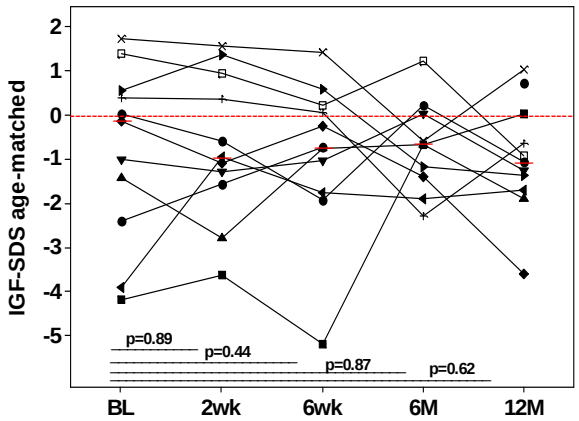


Figure 5.4.32: Association between height standard deviation score (HtSDS) and (insulin like growth factor-1) IGF-1(ng/ml)

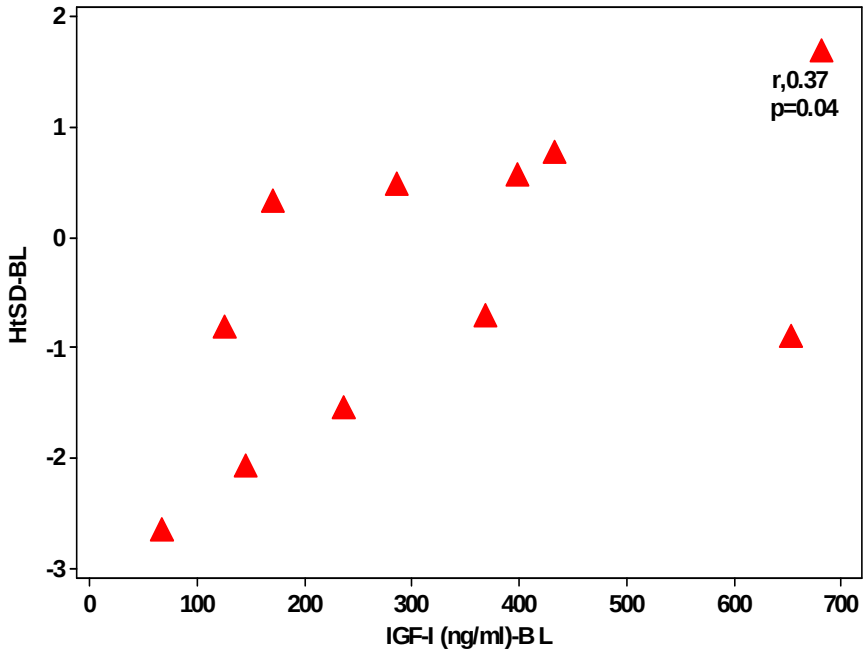


Figure 5.4.33: Individual change in Insulin like growth factor binding protein-3 (IGFBP-3(ng/ml),, Insulin like growth factor binding protein-3 ht-age-matched and chronological-age-matched standard deviation scores (IGFBP-3SDS ht-age-matched IGFBP-3SDS age-matched) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

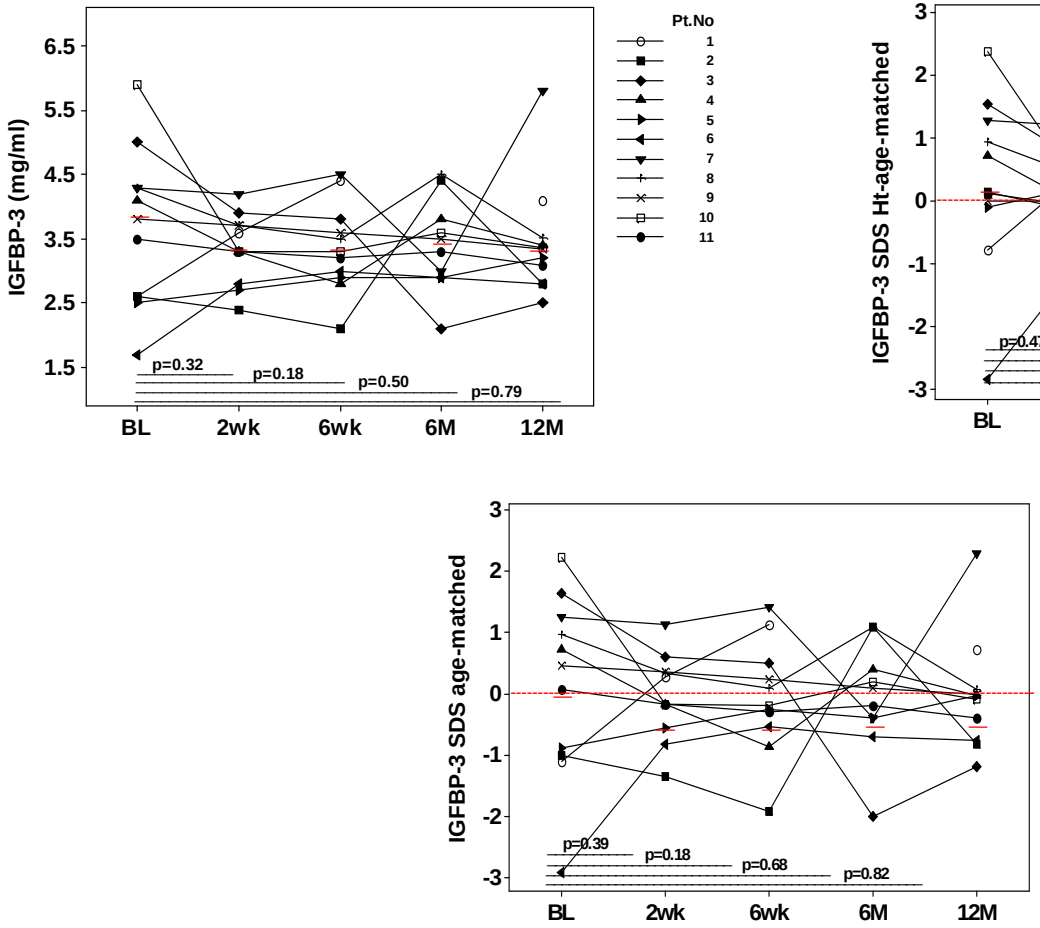


Figure 5.4.34: Individual change in insulin like growth factor binding protein-2 (IGFBP-2 (ng/ml), and acid labile subunits (ALS (ng/ml) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD

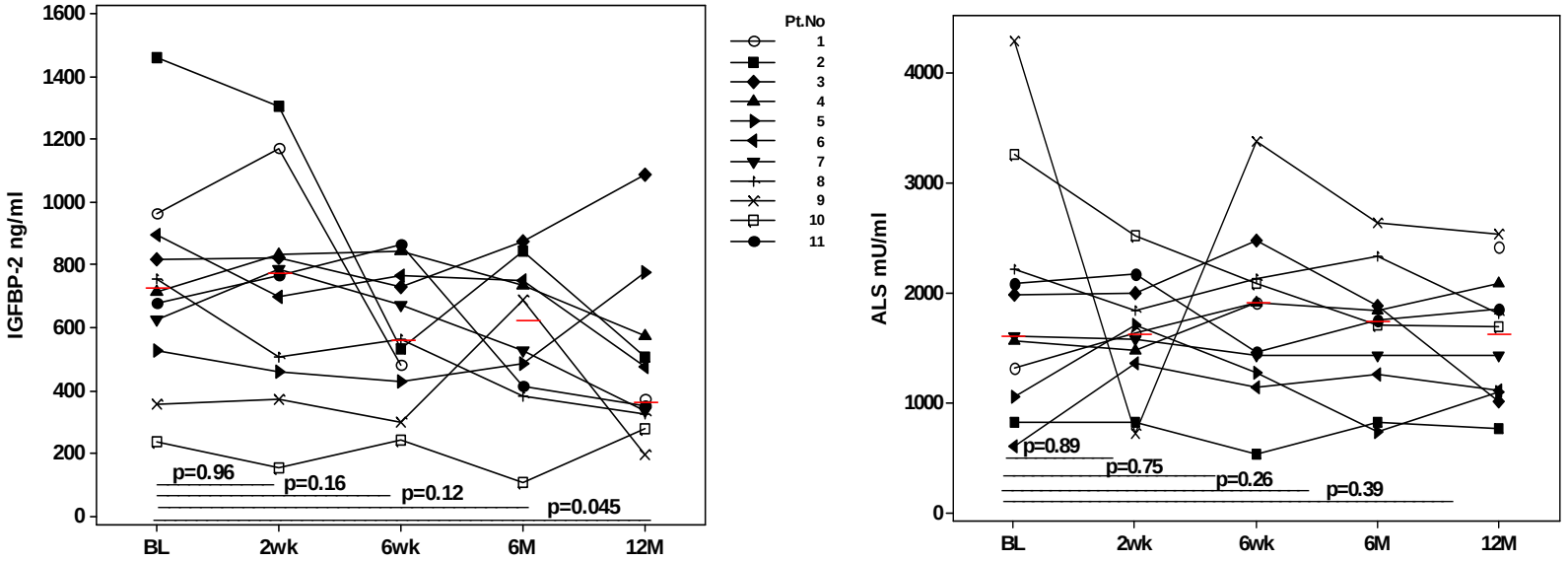


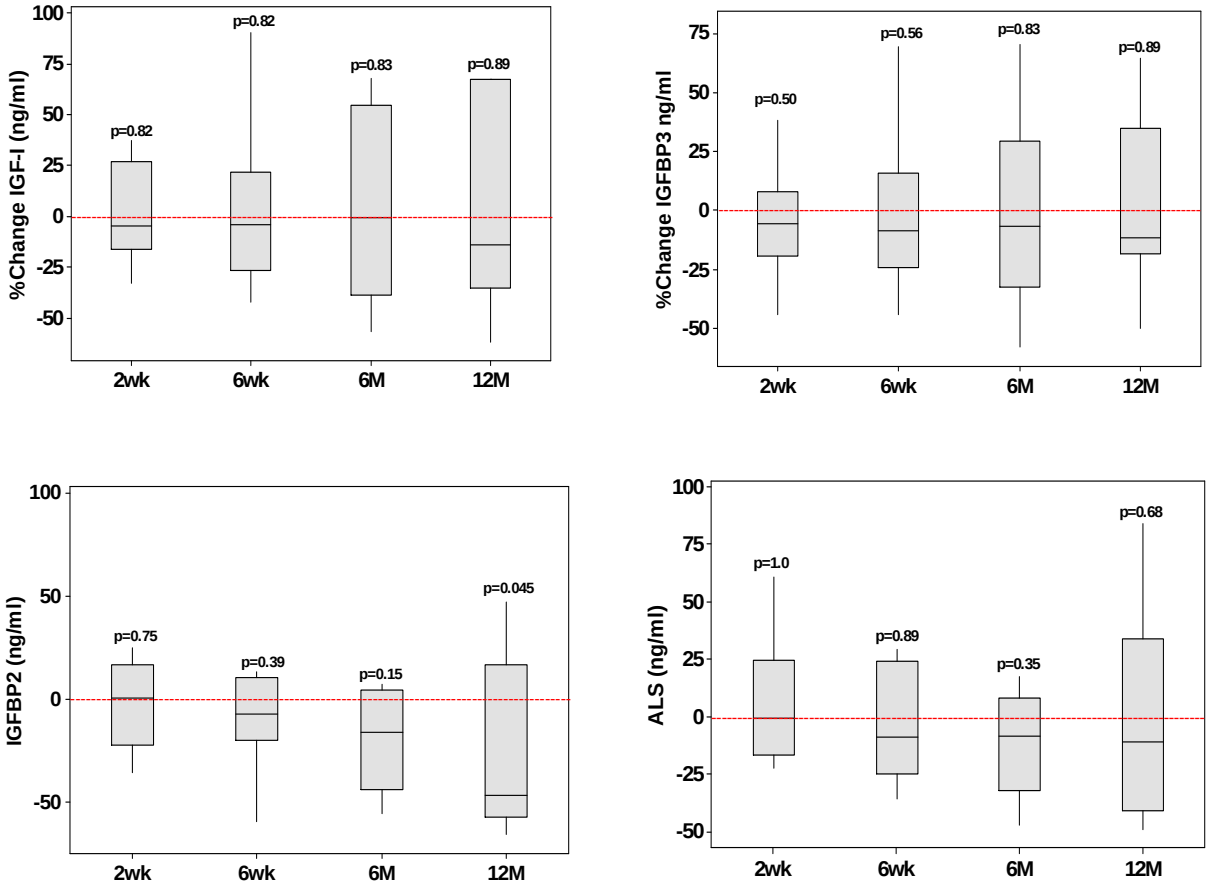
Table 5.4.34:Changes in Insulin like growth factor-I (IGF-1 (ng/ml), Insulin like growth factor-I height-age and chronological-age-matched standard deviation scores (IGF-ISDS ht-age-matched and IGF-1SDS age-matched), Insulin like growth factor binding protein-3 (IGFBP-3(ng/ml), Insulin like growth factor binding protein-3 ht-age-matched and chronological-age-matched standard deviation scores (IGFBP-3SDS ht-age-matched IGFBP-3SDS age matched), Insulin like growth factor binding protein-2 (IGFBP-2 (ng/ml), and acid labile subunits (ALS (ng/ml) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD (median-range)

						p-Value			
Value	**BL N,11**	**2wk N,11**	**6wk N,11**	**6M N,11**	**12M N,11**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
IGF-1(ng/ml)	286 (68,682)	300 (82,649)	258 (49.6,623)	259 (151,624)	303 (141,524)	0.56	0.35	0.68	0.56
IGF-1 Ht-age-matched SDS	-0.3 (-2.4,2.3)	-0.4 (-2.8,1.9)	-0.3 (-3.2,1.2)	-0.2 (-2.6,1.8)	-0.5 (-3.3,2.1)	0.91	0.48	0.90	0.68
IGF-1S age-matched SDS	-0.2 (-4.2,1.7)	-1.0 (-3.6,1.5)	-0.8 (-5.2,1.4)	-0.7 (-2.3,1.2)	-1.1 (-3.6,1.0)	0.89	0.44	0.87	0.62
IGFBP-3(ng/ml)	3.8 (1.7,5.9)	3.3 (2.4,4.2)	3.3 (2.1,4.5)	3.4 (2.1,4.5)	3.3 (2.5,5.8)	0.32	0.18	0.50	0.79
IGFBP-3 Ht-age-matched SDS	0.1 (-2.8,2.4)	0.0 (-0.7,1.2)	0.0 (-0.9,1.5)	0.1 (-2.1,2.6)	0.0 (-1.3,2.0)	0.47	0.23	0.81	0.83
IGFBP-3 age-matched SDS	0.5 (-2.9,2.2)	-0.2 (-1.4,1.1)	-0.2 (-1.9,1.4)	0.0 (-2.0,1.1)	0.0 (-1.2,2.3)	0.39	0.18	0.68	0.82
IGFBP-2(ng/ml)	713.1 (237.5,1460)	764.1 (153,1307)	561.8 (242.4,864.1)	606.5 (106.3,877.3)	374.1 (198.3,1089)	0.96	0.16	0.12	**0.04**
ALS(ng/ml)	1607 (612,4291)	1633 (724,8516)	1910 (527,3366)	1732 (736,2633)	1693 (766,2530)	0.89	0.75	0.26	0.39

Table 5.4.35:Percentage (%) change in insulin like growth factor-I (IGF-1 (ng/ml), Insulin like growth factor binding protein-3 (IGFBP-3 (ng/ml), Insulin like growth factor binding protein-2 (IGFBP-2 (ng/ml), and acid labile subunits (ALS (ng/ml) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD (median-range)

	%Change				p-Value			
Value	**BL-2wk**	**BL-6wk**	**BL-6M**	**BL-12M**	**BL -2wk**	**BL- 6wk**	**BL -6M**	**BL-12M**
IGF-1(ng/ml)	-7.8 (-32.7,122.6)	-3.9 (-42.2,90.6)	-0.5 (-56.5,251.9)	-13.7 (-61.8,413.1)	0.82	0.82	0.83	0.89
IGFBP-3(ng/ml)	-5.7 (-44.1,64.7)	-8.6 (-44.1,76.5)	-6.5 (-58.0,70.6)	-11.7 (-50.0,64.7)	0.50	0.56	0.83	0.89
IGFBP-2(ng/ml)	0.7 (-35.6,25.1)	-7.2 (-59.2,58.5)	-16.3 (-55.2,91.9)	-46.3 (-65.4,47.1)	0.75	0.39	0.15	**0.045**
ALS(ng/ml)	-0.8 (-83.1,123)	-9.3 (-235.9,365)	-8.4 (-47.3,105.4)	-10.9 (-49.1,84.3)	1.00	0.89	0.35	0.68

Figure 5.4.35: Percentage (%) change in Insulin like growth factor-I (IGF-1), Insulin like growth factor binding protein-3 (IGFBP-3(ng/ml), Insulin like growth factor binding protein-2 (IGFBP-2(ng/ml), and acid labile subunits (ALS (ng/ml) at BL, 2wk, 6wk, 6M & 12M following treatment with biologic therapy in paediatric patients with CD (median range)



5.4.14 : Infliximab pharmacokinetics

Changes in Infliximab pharmacokinetics were observed at 2wk, 6wk, 6M and 12M and are shown in Table 5.4.36. The results show wide variation in pharmacokinetic levels. Some of them are high presuming that these are not trough but peak levels. As the trough levels were not reliable therefore it was difficult to observe association between Infliximab pharmacokinetic levels and disease activity.

Table 5.4.36 : Pharmacokinetic levels of Infliximab in paediatric patients with CD

	Infliximab (IFX) conc. (µg/mL)			
Pt.No	**2wk**	**6wk**	**6M**	**12M**
1	37.2	28.2	*	10.6
2	0.3	0.2	0.4	Stopped IFX/ started ADA
3	↑	7.6	0.1	7.9
4	ADA	ADA	ADA	ADA
5	20.5	4.8	0.6	0.4
6	18.4	11.1	15.5	2.8
7	↑	↑	↑	↑
8	↑	↑	14.1	↑
9	↑	↑	10.2	↑
10	↑	27.7	0.4	Stopped IFX/ started ADA
11	↑	27.6	0.2	1.2
Median(range)	**19.5(0.3,37.2)**	**11.1(0.2,28.8)**	**0.5(0.1,15.5)**	**9.3(0.5,40.3)**

5.5 Discussion

In the present study, although with a small sample, treatment with biologic therapy over twelve months seems to induce beneficial effects on bone status, muscle function and body composition in the majority of children with CD who responded to and tolerated infliximab: it is possible that controlling disease activity with biologic therapy may positively outweigh the effects of CD on growth, body composition, muscle function and bone health.

5.5.1 Effect on growth

This study has shown improvement in both ΔHtSDS and HVcms/yr at 12M, but increase in median values did not reach significance as compared to BL. This observation is comparable to the results of the previously published studies by our own group (367)and others which reported improvement in growth following biologic therapy (156;207;247;279;310-312).

5.5.2 Effects on bone

The data demonstrated a significant change and in biomarker of bone formation (BALP) and a non-significant change in a biomarker of bone resorption (CTX-1) from BL to 12M. The median percentage(%) change of BALP was -1.5%(p=0.62),14.8%(p=0.02), 68.1%(p=0.01) and 128.7%(p=0.01) and CTX-1 was -7.9%(p=0.96), 13.6%(p=0.14),62.1%(p=0.03) and 19.9%(p=0.26) from BL to 2wk 6wk, 6M and 12M, respectively. Moreover, data demonstrated that the bone formation marker was lower in patients with greater disease activity at baseline as depicted by a significant negative association of BALP and PCDAI score at BL and was found to be greater in patients who had a reduction in PCDAI score at 12M following biologic therapy. Furthermore, a significant positive association was observed between BALP and HV cm/yr at 6M and 12M. However, no association was observed for CTX-1 with PCDAI, inflammatory markers and HV. This finding is comparable to the results of previously published REACH study (207) which observed changes in the bone biomarkers at week 10 after Infliximab induction therapy in paediatric patients with CD. In this study Thayu et al (207) reported improvement in markers of bone formation, BSAP and P1NP during the 10-week interval with the median increase of 87% and 103% respectively (both p<.001). Moreover, increase in BSAP at week-10 was significantly associated with the reductions in PCDAI at week 10 but no association was observed between changes in PINP and PCDAI. The bone resorption markers CTX-1 and DPD also increased significantly during the induction period (both p<.001); however, the magnitude of the changes (18% and 23%) were markedly less

pronounced than the changes in markers of bone formation. This study also reported association of changes in the bone biomarkers with subsequent changes in disease activity and linear growth during the 54-week interval after initiation of Infliximab therapy.

Tumor necrosis factor- α (TNF-α) has direct effects on bone formation by inhibiting osteoblast differentiation and osteoblast collagen secretion, causing increased resorption by inducing osteoblasts secretion of IL-6, and inducing osteoblast apoptosis (203;204). In addition TNF-α promotes accelerated bone resorption by inducing osteoblast secretion of interleukin (IL)-6 and the expression of the receptor activator of nuclear factor kappa B ligand (RANKL). RANKL stimulates osteoclast differentiation and activation and inhibit osteoclast apoptosis thereby dramatically increasing osteoclast survival and increasing bone resorption (198;199). Adult studies that examined bone formation biomarkers after a single dose of Infliximab reported a mean increase in BALP of 15%-18% (206;319;321). The study by Franchimont et al (319) demonstrated low formation markers and elevation resorption markers at baseline, with a significant further decrease in resorption marker after Infliximab therapy. One study reported an increase in resorption markers at baseline and with a significant increase during Infliximab induction therapy (322). For conclusive explanation of these changes, it would have been useful to have a control group in the study. The results presented here suggest the beneficial effect of Infliximab on bone formation might be due to the inhibition of TNF-α or through the indirect improvement in disease activity.

5.5.3 Effect on muscle function

This is the first study that charts the effect of biologic therapy on changes in lower limb muscle function using jumping mechanography in paediatric patients with CD. In our study we observed a non significant increase in jump height (m), V-max (m/s), EFI (%), efficiency % from BL to 12M following biologic therapy and a significant increase in both F-max (kN), and P-max (kW) at 12M. Despite the fact that the increase in efficiency % of the movement was not significant but however, the increase was likely to be through improvements in jump height and velocity thereby indicating higher muscular flexibility. These data are suggestive of an effect of biologic therapy on lower limb muscle function through improvements in the mechanical efficiency of the muscle. Thus, it is plausible that the better muscle function that we have observed is mediated through the effect of biologic therapy on muscle mass.

Moreover, we also observed a significant negative association of jump height (m), V-max (m/s, EFI (%), efficiency % with PCDAI at BL which shows the impact of disease activity on muscle function in patients with CD. We also observed significant improvement in dominant and non dominant MIGF and in age and height adjusted MIGFSDS.

5.5.4 Effect on body composition

This study is the first longitudinal study to show the effect of biologic therapy on changes in body composition in paediatric patients with CD. The study showed a significant increase in FM in paediatric patients with CD following biologic therapy an effect that has not been reported extensively in previously published studies. Thayu et al (365) did report a significant association between increased FM-ht z-score with cumulative glucocorticoid exposure, methotrexate and Infliximab at the end of any given interval but when all three medications were included in the same model the effect of Infliximab on FM-ht z-score was found to be attenuated. This observation is however, in concordance with the results of adult studies which reported a specific effect of anti-TNF treatment on body composition with an increase of FM after short term treatment in a group of patients with rheumatoid arthritis (407) and spondylarthropathy (408), a study in which patients gained FM during 2 years of treatment with TNF antagonists. As no significant correlation was observed between FM and PCDAI score and markers of inflammation therefore increase in FM seems to be an anti-TNF-α therapy specific effect. We also observed significant improvement in both FFM and FFMI which shows that the treatment with anti-TNF-α therapy also had a significant impact on FFM. Our result is consistent with the previously published study in paediatric CD patients (365). Thayu et al (365) have shown that the concurrent Infliximab therapy was associated with improvement in LM ht-z-scores.

5.5.5 Effect of GH/IGF-1 system

In this study no significant change was observed in IGF-1, height-age and chronological-age-matched IGF-1 SDS, IGFBP-3 and height-age and chronological-age-matched IGFBP-3 SDS following biologic therapy. Moreover height-age and chronological-age-matched IGF-1SDS and IGFBP-3 SDS remained below zero during the study interval. Our data demonstrated that IGF-1 and IGFBP-3 were lower in patients with greater disease activity at BL as depicted by a significant negative association of IGF-1 and IGFBP-3 with PCDAI score. Furthermore, a

significant positive association was observed between IGF-1 and HtSDS at BL and IGFBP-3 and HVcm/yr at 12M. A significant negative association was also observed between age and height matched IGF-1SDS with PCDAI, ESR, CRP and ALB during induction therapy. Our finding is also similar to the previously published data by Vespasiani et al (328) who reported a negative association between IGF-1 and IGFBP-3 with CRP in adult CD patients.

The effect of Infliximab on the GH/IGF-I axis has been assessed in a series of 14 adult IBD patients with low levels of IGF-I and IGFBP-3 treated with three induction doses (weeks 0, 2 and 6) plus two additional infusions every eight weeks (328). Peripheral resistance to GH is apparently reversed after the second infusion of Infliximab, as judged by the significant increase in the serum levels of IGF-I and IGFBP-3 as compared to baseline. However, this effect is not sustained since they return to baseline values between the first and second maintenance dose(328). TNF blockade by Infliximab could account for the initial improvement of GH sensitivity as increased TNF activity suppresses the GH/IGF-I axis in the liver. Another study conducted by Eivindson et al (329) assessed changes in the IGF system in patients with active CD before and during Infliximab treatment and studied 13 patients with therapy refractory CD, treated with Infliximab at baseline and after 2 weeks. The IGF system and markers of inflammation were examined at baseline, on days 2-5 and after 1, 4, and 8 weeks. Ten healthy age- and gender-matched persons served as controls. This study has shown treatment with Infliximab normalized circulating levels of total IGF-I and IGFBP-3, and partially normalized IGFBP-2, whereas free IGF-I remained suppressed and suggest that the changes in the IGF system may be part of the catabolic state in active CD and may have an association with metabolic bone disease and muscle wasting (329).

Children with CD have normal stimulated and spontaneous GH secretion but have reduced plasma IGF-1 concentrations and IGFBP concentrations. The reduction in IGF-1 concentration is mediated by multiple factors including increased cytokines expressed in active inflammation and malnutrition (409). The presence of lower levels of inflammatory cytokines in paediatric CD has been correlated with increased height velocity. A study by Thayu et al (365), which evaluated 78 paediatric patients with CD, showed significantly greater IL-6 and TNF-a levels in the CD patients as compared with normal population controls. Significantly lower IGF-1 levels and higher IGFBP-3 levels were also present in patients with CD compared with controls. The authors correlated increased height velocity in this cohort to increased serum IGF-1 and decreased serum levels of TNF-α and IL-6.

We observed significant reduction in IGFBP-2 at 12M as compared to BL. Moreover, a significant negative association was observed between IGFBP-2 and HVcms/yr at 6M. High IGFBP-2 levels have previously been described in IBD (410). IGFBP-2 is known as an inhibitor of IGF-1 activities. The elevated circulating IGFBP-2 levels may be caused by a certain degree of catabolism induced by inflammation itself. No significant change was observed in ALS following biologic therapy. However, we observed a significant positive association between ALS and HtSDS at BL, 6M and 12M and with ALB at BL and 12M. As the effect of biologic therapy on ALS have not been reported previously in patients with IBD therefore it is not possible to compare these results with those of other observations.

5.5.6 Effect on bone mineral density

In this study we did not observe any significant change in total body, LS (L2-L4) and proximal femur BMD following biologic therapy. Moreover, the bone area adjusted TB-BMCSDS and LS-BMCSDS remained below zero throughout the study interval. No significant change was observed in LS(L2-L4) and femoral neck BMAD. LS(L2-L4) and femoral neck BMADSDS also remained below zero during the study interval. The results of our study are not consistent with the previously published studies (324-327). Pazianas et al (325), conducted a retrospective study on 61 CD patients who had low BMD. In this study a number of confounding factors which could affect study outcome were analyzed including sex, number of postmenopausal women, number of women on hormone replacement therapy, glucocorticoid use and Infliximab infusions. In this study 23 patients were on Infliximab therapy and 36 patients were on bisphosphonates. After controlling for corticosteroid use, patients with concurrent Infliximab and bisphosphonate treatment had a greater increase in BMD as compared to those who had bisphosphonate alone; corticosteroids inhibited this effect. However, Infliximab alone had no effect on BMD(325). Mauro et al(326), conducted a retrospective study of 15 CD patients who had Infliximab therapy for the first time and who underwent DXA before and during Infliximab therapy. These patients were then compared with 30 CD patients who were naïve to Infliximab therapy and who had DXA done at least one year apart. Age, gender, age at diagnosis, disease duration, final weight, change in weight, disease activity and the use of bisphosphonate and corticosteroids were used as confounding factors in this study. Patients in this study had Infliximab therapy at intervals of 4-8 weeks for a mean period of 18 months. Those patients who had Infliximab therapy had a significant increase in lumbar bone area,

BMC and BMD as compared to the control group but these data were not corrected for size. The increase in BMC in patients who had Infliximab therapy was significant when compared with those who had received glucocorticoid therapy or who had evidence of disease activity (326). Bernstein et al(327), also found that the maintenance treatment with Infliximab (5mg/kg) at 6-8 weeks interval for 1 year in 46 CD patients resulted in the improvement in BMD after 1 year in lumbar spin, at the femoral trochanter and at the femoral neck and this effect was independent of the glucocorticoids, calcium supplementation or changes in C-reactive protein (CRP)(327). Paganelli et al (324) reported increase in bone mineral apparent density BMAD in Infliximab treated group than those never treated with Infliximab. This is the only study which reported the effect of Infliximab on BMD in children with IBD. It is speculated that significant differences in BMD may have been observed if the cohort was larger or had been followed up for longer than one year.

5.5.7 Effect on bone and muscle parameters

The current prospective study is the first longitudinal study which evaluated bone health using pQCT technique for radius and tibia both in children with CD following biologic therapy. We observed significant change in TotBMD and percentage change TotBMD of tibia at 6M. SSI and percentage change SSI for tibia was also changed significantly at 12M. Mus-CSA and percentage change Mus-CSA changed significantly at 6M and 12M. Fat-CSA and percentage change Fat-CSA significantly changed at 6M only. Only Mus-CSA and percentage change Mus-CSA of radius changed significantly and rest of the parameters did not show any significant change. Individual changes in height-adjusted z-scores for radius TotBMD, TrbBMD, CrtBMD-z-scores, SSI, Mus-CSA, fat Fat-CSA, Crt-BA and Mus-Bone CSA remained below zero throughout the study interval. Tibia bone and muscle parameters with did not show any association with PCDAI score. However a positive significant association was observed between Tibia TrbBMD and ALB at BL and tibia Fat-CSA with ALB at 6M. A significant association was observed between greater PCDAI score and radius TotBMD and TrbBMD at BL and with radius CrtBMD at 12M. The improvement in muscle and fat cross sectional area reflects the positive impact of biologic therapy on muscle and fat mass.

5.5.8 Infliximab pharmacokinetics

This study could not provide much detail about Infliximab pharmacokinetic levels in children with CD due to unreliable “trough” levels therefore future studies are needed looking at Infliximab pharmacokinetic levels in children.

5.6 Conclusion

In conclusion, these prospective studies of growth, body composition and muscle function suggests that biologic therapy in children with CD has a beneficial effect on muscle mass and muscle function and which can be observed over the first year of therapy but for comprehensive conclusion this data needs to be adjusted for body size. These positive changes are also associated with an increase in bone turnover where the change in bone formation is much greater than bone resorption. These favourable effects on bone health were not accompanied by marked changes in BMD as assessed by DXA but did show some beneficial effects on pQCT assessed SSI, a surrogate marker of bone strength. The results of this preliminary study need to be confirmed in a larger group of children.

CHAPTER 6

Conclusion and Future Directions

6.1 General discussion and conclusions

Crohn's disease affects increasing numbers of children worldwide. Growth retardation associated with delayed puberty, is a frequent feature in paediatric patients with IBD especially CD. Control of disease activity and mucosal healing are paramount to promote growth and adequate pubertal onset. Current therapeutic strategies for maintenance in IBD include anti-inflammatory drugs, immunosuppressives and more recently, biologics. Although these treatments are efficient in minimising inflammation and inducing prolonged remission, their long-term effects on growth and final height remain controversial. Biologic drugs present remarkable advantages in terms of disease control for children, especially in those whose disease cannot be controlled with conventional therapies. Data regarding biologic use in children is limited but results have been promising, both in terms of controlling disease activity and improving growth parameters.

Four different studies have been conducted in an attempt to explore the effects of the biologic therapy on changes in physical growth, puberty, GH/IGF-1 system, bone health, body composition and muscle function in paediatric patients with CD.

In summary the hypothesis explored in the studies that comprised this PhD were:

1. To summarize and evaluate effects of biologic therapy on growth and skeletal development in children with chronic inflammatory conditions
2. To assess the frequency of short stature and poor growth and their relationship to disease course and therapy in children with CD
3. To assess the effect of Infliximab therapy on growth, puberty and markers of disease in children with CD
4. To assess the effect of Adalimumab therapy on growth in children with CD
5. To investigate the effect of biologic therapy prospectively on
    a) Physical growth and puberty
    b) Bone mineral density, bone geometry and bone strength
    c) Bone metabolism
    d) GH/ IGF-1 system
    e) Body composition
    f) Muscle function in children with CD

Effect of therapeutic interventions on growth in children with CD

Three different retrospectives studies were conducted to explore the long-term and short-term impact of contemporary disease specific therapies, Infliximab therapy and Adalimumab therapy on growth and puberty in children with CD.

The study about the impact of contemporary disease specific therapies in children with CD (Chapter 2) provides clear evidence that despite advances in therapy, short stature and slow growth continue to be encountered in a sub-group of children with CD. This study examined the relationship of a number of factors including therapeutic modalities to two commonly used anthropometric markers of growth - HVSDS and ΔHtSDS. The mean HtSDS of the study population as well as the percentage of children with HtSDS of less than -2 were similar to that reported in other contemporary studies (310;335). HtSDS did not show an improvement despite a significant improvement in HVSDS this observation suggest that the reduction in growth deceleration, as reflected by an improving HV is not sufficient to improve overall height but simply prevents any further deterioration in height. This study recommended that the change in HtSDS may be a more valid method of assessing and reporting longitudinal growth in children with chronic disease, particularly when there is a high prevalence of children of a peripubertal age.

Several studies have reported the effect of Infliximab therapy on growth in children with CD (Section 1.9). The study about the effect of Infliximab therapy on growth in children with CD (Chapter 3) supports these findings (233;273;311). This study has shown an average improvement of approximately 50% in HV in the 6 months after the initiation of Infliximab therapy which was further sustained for a further 6 months. It was also observed that initiation of Infliximab was associated with an improvement in growth in those children who had not been exposed to exogenous GC at all, is noteworthy, and suggests that the growth-promoting effects of Infliximab are not solely due to its 'GC-sparing effect'. The finding, that growth was more likely to improve in those children who were judged to be 'responders' also suggests that growth improves as a result of improved disease control. Improvement of the disease status would be expected to be associated to pubertal progression and improved growth as improvement in growth was also observed in children who remained pre-pubertal. Previous report of growth following Infliximab therapy have suggested that improvement is more likely in those, which are in early puberty (310), and this may be due to the possibility of pubertal progress following initiating Infliximab therapy.

At present there are no published paediatric studies that adequately assess the effect of Adalimumab on linear growth in children with CD. The study about the effect of Adalimumab therapy on growth in children with CD (Chapter 4) provides evidence that Adalimumab is associated with improvement in short term linear growth in children with CD who enter remission but not in those who do not. It is also more likely to happen in children who are on immunosuppression and those in early puberty but seems to be relatively independent of steroid use. These findings suggest that growth improves as a result of several interrelated factors, including improved disease control. Use of co-immunosuppression therapy with biologics remains controversial (369). It is also interesting to note that the growth response to Adalimumab varied dependent on the reason for discontinuing Infliximab; those who had an allergic reaction to Infliximab fared best paralleling preliminary Adalimumab clinical trial data suggesting clinical response and remission are higher in patient who are anti-TNF naïve rather than those who have had no/lost response to Infliximab previously (376).

The above mentioned studies provided evidence that short term biologic therapy improves growth. Although these studies are reporting short term improvement in growth, future prospective studies need to look at final height as an outcome rather than the short term changes in height.

The effects of biologic therapy on bone metabolism

To date, few published studies have examined the effect of anti-TNF-α therapy on bone metabolism in patients with IBD and most of the data originate from studies performed in adult patients (Section 1.10). This longitudinal study demonstrated a significant increase in biomarker of bone formation (BALP) and a non-significant increase in a biomarker of bone resorption (CTX-1) from BL to 12M. Moreover, this study depicted that the bone formation marker was lower in patients with greater disease activity at baseline as depicted by a significant negative association of BALP and PCDAI score at BL and was found to be greater in patients who had a reduction in PCDAI score at 12M following biologic therapy. Furthermore, a significant positive association was observed between BALP and HV cm/yr at 6M and 12M. However, no association was observed for CTX-1 with PCDAI, inflammatory markers and HV cms/yr. This finding is comparable to the results of previously published REACH study (207). Suggesting the beneficial impact of biologic therapy on bone formation may be due to the inhibition of proinflammatory cytokine TNF-α or through the indirect

improvement in disease activity. A limitation of this study is lack of controls therefore, in order to have conclusive explanation of these changes; it would have been useful to have a control group in the study.

The effect of biologic therapy on bone geometry and bone mineral density

This is the first longitudinal observational study which explores the impact of biologic therapy on bone geometry and muscle function in children with CD using a novel imaging device, pQCT. In this study, pQCT scans for distal radius and tibia did show increase in parameters of muscle function and bone strength following biologic therapy in paediatric patients with IBD. It is difficult to compare the results of this study with previous reports and to assess sequential changes in bone and muscle parameters following biologic therapy due to the lack in currently available data in paediatric population with IBD.

Moreover, no clear conclusions can be made due to the number of issues and limitations related to the use of pQCT in paediatric population. Firstly, the results are not comparable to any other study results due to the lack in currently available data regarding the impact of biologic therapy on bone geometry and muscle function. Secondly, pQCT reference data for children and adolescents are not yet available. Therefore, it is impossible to compare data obtained from clinical populations to normative datasets. Large population-based studies of healthy children across the growing years are essential to generate a reference data set and for determining the clinical application of pQCT technique, in children with chronic inflammatory conditions.

This study did not show significant changes in BMD following biologic therapy in paediatric patients with CD. This result is not consistent with the previously published studies in adult and paediatric patients both (324-327). This may be due to small size of the cohort moreover; changes in BMD following biologic therapy were only assessed at three time points (BL, 6M and 12M). A more frequent, and possibly longer, follow-up of patients would provide a more comprehensive understanding of the long-term effects of biologic therapy on BMD of the total body, lumbar spine, proximal femur and femoral neck.

The effect of biologic therapy on body composition

This study is the first longitudinal prospective study to show the effect of biologic therapy on changes in body composition in paediatric patients with CD. The study showed a significant increase in FM in paediatric patients with CD following biologic therapy an effect that has not been reported extensively in previously published studies. This observation is however, in concordance with the results of adult studies which reported a specific effect of anti-TNF treatment on body composition with an increase of FM after short term treatment in a group of patients with rheumatoid arthritis (407) and spondylarthropathy (408), a study in which patients gained FM during 2 years of treatment with TNF antagonists. As no significant correlation was observed between FM and PCDAI score and markers of inflammation therefore increase in FM seems to be an anti-TNF-α therapy specific effect. We also observed significant improvement in both FFM and FFMI which shows that the treatment with anti-TNF-α therapy also had a significant impact on FFM. This result is consistent with the previously published study in paediatric CD patients (365).

Although this result is an interesting finding of this study and provide evidence for potential benefits of biologic therapy on FM and FFM in paediatric patients with CD. This result needs to be interpreted with care as the body composition measurements were not adjusted for body size. In growing subjects, body composition measurements must be adjusted to body size, specifically height, in addition to age and sex. Future studies should report the impact of biologic therapy on body size adjusted measurements of body composition for a better conclusion.

The effect of biologic therapy on GH/IGF-1 system

In this study no significant change was observed in height and age adjusted IGF-1SDS and IGFBP-3 SDS following biologic therapy and remained below zero during the study interval. IGF-1 and IGFBP-3 levels were lower in patients with greater disease activity at BL as depicted by a significant negative association of IGF-1 and IGFBP-3 with PCDAI score. Furthermore, a significant positive association was observed between IGF-1 and HtSDS at BL and IGFBP-3 and HV cm/yr at 12M. A significant negative association was also observed between age and height matched IGF-1SDS with PCDAI, ESR, CRP and ALB during induction therapy. Our finding is also similar to the previously published data by Vespasiani et

al (328) who reported a negative association between IGF-1 and IGFBP-3 with CRP in adult CD patients.

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inhibitor of IGF-1 activities. The elevated circulating IGFBP-2 levels may be caused by a certain degree of catabolism induced by inflammation itself. No significant change was observed in ALS following biologic therapy. As the effect of biologic therapy on GH/IGF-1 system have not been reported previously in paediatric patients with IBD therefore it is not possible to compare these results with other reports. The exact interaction between anti-TNFα therapy and GH/IGF-1 system could be explored in group of paediatric patients with IBD in whom there is a continuous supporting evidence of impaired GH/IGF-1 system prior to starting biologic therapy.

Infliximab pharmacokinetic levels

This study could not provide much detail about Infliximab pharmacokinetic levels in paediatric patients with CD due to unreliable “trough” levels therefore future studies are needed looking at Infliximab pharmacokinetic levels in paediatric patients.

Conclusion

In conclusion, these prospective studies of growth, body composition and muscle function has clearly shown that biologic therapy in children with CD has a beneficial effect on muscle mass and muscle function and which can be observed over the first year of therapy but these observation needs to be interpreted with care as the measurements were not adjusted for body size. These positive changes are also associated with an increase in bone turnover where the change in bone formation is much greater than bone resorption. These favourable effects on bone health were not accompanied by marked changes in BMD as assessed by DXA but did show some beneficial effects on pQCT assessed SSI, a surrogate marker of bone strength. The results of this preliminary study need to be confirmed in a larger group of children.

Clinical implications

1. Markers of bone formation and bone resorption can be useful to determine the bone metabolism in children with IBD and may be used to explore the mechanism of bone loss in these children. It is easy to measure them, and the results are easy to construe. At present, these markers are only used in research. It is advisable to use

them in routine clinical practice in order to evaluate the impact of treatment particularly with glucocorticoids and anti-TNF-α on bone turnover.

2. Body composition measurements and specifically FM and FFM measurements would be useful to provide appropriate treatment to paediatric patients with bone mass deficits. Moreover, body composition measurements should be adjusted for body size particularly in growing children these measurements should be adjusted for height along with sex and age.
3. It is also recommended that clinical studies should report growth changes in terms of change in HtSDS. As change in HtSDS may be a more valid method of assessing and reporting longitudinal growth in children with chronic disease, particularly when there is a high prevalence of children of a peripubertal age. This will also allow comparison with studies that report results of endocrine growth promoting therapy.
4. BMD should be monitored in all children with IBD. It is therefore important for paediatric gastroenterologist to focus on the prevention of generalized bone loss, particularly in those patients with long-term disease, recurrent flare-ups and glucocorticoid users.

Future directions

The studies in this thesis are cohort studies without appropriate control groups, which makes it tough to draw clear-cut conclusions from them. However they raise questions for further research to expand and explain the results. Multicentre prospective studies are essential to achieve better recruitment and acquire adequate sample sizes. Further, well-designed prospective studies on effect of biologics on growth and skeletal development are required. An improved understanding of the effect of biologic therapy may improve future therapy directed at promoting growth and skeletal development in a diverse group of children. Further studies are required to understand the duration of the window of opportunity during which linear growth, bone and muscle mass in children with CD can be optimised. It will also be useful to follow-up patient cohort for longer than one year in order to observe significant changes in BMD following biologic therapy. There is a need to explore biomarkers that identify children who are at risk for growth retardation, bone and muscle mass deficits.

Future studies may also look at the impact of biologic therapy on changes in calprotectin levels as a marker for the presence and severity of gastrointestinal inflammation in IBD.

Moreover, future studies should be conducted to observe relationship between Infliximab pharmacokinetic levels and disease activity in patients with paediatric CD. Determination of antibodies against Infliximab would also be helpful to decide about continuation of biologic therapy and will further clarify the clinical use of these antibodies. It will also be worthy to determine the predictors of response for long-term anti-TNF-α therapy.

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Appendix A

Inclusion/exclusion criteria

Biologics in Crohn's Disease (BCD Study)

Exclusion Inclusion criteria

Screening date:----------
Patient ID: ----------
Patient Initials:

DOB:

Inclusion/exclusion

Confirmed IBD diagnosis	□No □ Yes
Children on the same biologic over Prior six months	□No □ Yes
Age 6-18 years at start of biologic therapy	□No □ Yes
Adults more than 18 years at start of Biologic therapy	□No □ Yes

Comments:

Appendix B

Study information sheet for the child under 8yrs

Yorkhill Division

NHS Greater Glasgow and Clyde

STUDY INFORMATION SHEET FOR THE CHILD UNDER 8 YRS

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

We would like to ask you to take part in a study. This sheet will tell you more about the study. Do read this sheet or ask your Mum and Dad to read it to you.

1. **What happens to growth and bones in children with Crohn's disease?**

   Crohn's Disease can sometimes affect your growth or your bones.

2. **What is this study about?**

   Some medicines for Crohn's Disease may help your growth and bones. We want to find out if this really happens.

3. **Why have you been chosen?**

   We know that your doctor has decided to start you on one of these medicines.

4. **What will happen to me if I take part?**

   We would like to measure your size. Also we would like to take a sample of your poo and wee. We will also take a sample from the blood that your doctors take when you come to the clinic. To check your bones, we will also take some special X-rays and scans of your body. There are no extra jags.

5. **What do I have to do?**

   If you want to take part, let your Mum or Dad know.

6. **Do I have to take part?**

   YOU DO NOT HAVE TO SAY 'YES'. You can also change your mind later and decide to stop taking part.

7. **What happens if you are not happy with anything?**

   Ask your Mum or Dad to talk to us.

8. **Who is organizing the study?**

   The study is being organized by Doctor Faisal Ahmed and the doctors you meet in your clinic. You can talk to them as well as your parents.

You can keep this sheet with you.

Appendix C

Study information sheet for the child under 8-14yrs

Yorkhill Division

NHS Greater Glasgow and Clyde

STUDY INFORMATION SHEET FOR THE CHILD 8-14 YRS

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

We would like to ask you to take part in a study. This study is done to see how a group of new medicines (biologics) affect growth and bones in children with Crohn's disease. Before you decide you need to find out more about this study. Please read this sheet carefully and discuss it with your parents. Take your time and ask us if anything is not clear.

1. What happens to growth and bones in children with Crohn's disease?

Children with Crohn's disease often do not grow as well as healthy children and their bones might be weaker. This is due to the effects of some substances (called cytokines) that are increased in children with Crohn's disease. Steroid medicines that are sometimes used can also slow growth and affect the bones.

2. What is this study about?

We want to find out how some medicines, called biologics, which are used to treat Crohn's disease can help your growth and your bones.

3. Why have you been chosen?

We know that your doctor has decided to start you on a biologic medicine as part of your clinical care.

4. What will happen to me if I take part?

If you agree to take part, you will have measurements of your growth, height and weight, at 5 time points i.e. at the time of first infusion, after 2 weeks, 6 weeks, 6 months and 12 months. On these occasions, we will also take some stool and urine samples and takes some blood (2 teaspoons) samples too. All the blood samples will be collected when you are having blood collected for other reasons. You will not notice anything different and will not need any extra jags. At the start and 6 months, and 12 months after your first biologic treatment, we will also check an x ray of your left hand to check the amount of growth there is. On these occasions, you will also

have a special scans, called a DXA and pQCT scan which will check the strength of your bones.

5. What do I have to do?

If you would like to help us with the study we will ask your Mum or Dad to sign a piece of paper called a 'consent form'. All you have to do is come for your biologic treatment. You can also sign an assent form.

6. What are the possible problems of taking part?

Taking the extra small amount of blood from you and having the special scans will not cause any problems. Sometimes getting the scans may prolong your visit. We will try our best to be as quick as we can.

7. What are the possible benefits of taking part?

We can tell you how your growth and bones are doing. By taking part in the study you will provide us with information that will help us help other children with Crohn's disease with problems with their growth.

8. Who can I contact for further information?

If you want to discuss the study further, please contact: Dr Faisal Ahmed telephone number 0141 201 0767 or Dr R Russell telephone number at the Royal Hospital For Sick Children, Yorkhill, Glasgow G3 8SJ.

9. Do I have to take part?

YOU DO NOT HAVE TO SAY 'YES' to taking part in this study if you do not want to.

10. What happens at the end of the study?

When the results of the study are published, your name will not be shown, so there is no way of anyone knowing that you have taken part unless you tell them.

11. What if something goes wrong?

We are not expecting any problems. However, if you are not happy about anything about this study, talk to your parents or your family doctor and ask them to speak to us. You can also contact Kate Colquhoun, Patient Contact Office at Yorkhill on 0141 201 9278.

12. Who is organizing the study?

The study is being organized by Dr Faisal Ahmed who looks after children with bone and growth problems at Yorkhill.

13. Who is helping these doctors with the study?

Most of the work in this study will be performed by a senior student, Ms Salma Malik, who is doing a PhD at the University of Glasgow. Other people who will be helping are members of the Bone & Endocrine Research Group and members of the GI team.

14. Who has looked at this study?

The study has also been looked at by a group of people who belong to the Research Ethics Committee and the Research & Development Department.

A copy of this information sheet and consent form will be given to you to keep. If you have any further questions about the study, please feel free to ask the doctors above.

Appendix D

Study information sheet for the young person over 14yrs

NHS Greater Glasgow and Clyde

Yorkhill Division

STUDY INFORMATION SHEET FOR THE YOUNG PERSON OVER 14 YEARS

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

You are being invited to take part in a research study. The study is being performed to see how biologic medicines may improve growth and bone health in Crohn's disease. Before you decide, you need to find out more about this study. Please read this sheet carefully and discuss it with your parents. Take your time and ask us if anything is not clear.

1. **What happens to growth and bone health in children with Crohn's disease?**
   Children and young people with Crohn's disease may not grow as well as healthy people and their bones may also be affected This may be due to the effects of some substances called cytokines that are increased in Crohn's disease. Cytokines cause the symptoms of Crohn's disease but may also affect growth. Steroid medicines (e.g. Prednisolone) that are sometimes used to treat Crohn's disease can also slow growth and affect the bones.

2. **What is this study about?**

   Biologics are special medicines that block the cytokines that may be increased in Crohn's disease. We want to find out how these new medicines, affect growth and bones in Crohn's disease. We are also interested in finding out how these new medicines can improve the hormones and substances that control growth and bone health.

3. **Why has your child been chosen?**

   Your gastroenterologist has indicated that you will be started on biologic therapy to treat Crohn's disease. We would like to add this study to your clinical treatment. The study will be fitted around your visits to receive the medicine or when you attend the clinic.

4. **What will happen to your child on participation?**

   If you agree to take part, we will take some extra blood from your cannula (2 teaspoons) at the time of the biologic therapy. Routine blood tests are required before the infusion anyway and we will just take a little bit more. You will not notice anything different and there will not be any extra jags. We will also ask for a urine and stool sample each time you child attends for the study visits. We will also check your height and weight at each visit. If you do not continue on biologics after the first 3 doses, we will see you and organize for the tests at the clinic. At baseline and 6 months and 12 months after the first dose of biologic, we will ask for an x-ray of your left hand to check the amount of bone growth there is. In addition, at baseline and 6 months and 12 months, we will also ask for

special scans, called DXA and pQCT scans that check how strong your bones are. Some of these scans involve a very small amount of x-rays that are not harmful. The amount of radiation is about the same as you would get from playing outside in the sun on one average day.

5. What do I have to do?

If you would like to help us with the study we would like to ask you to sign a piece of paper called a 'consent form'. All you have to do is to come for the biologic treatment or to the clinic visit as usual.

6. What are the possible problems of taking part?

Taking the extra small amount of blood from you and the special scans to measure how strong your bones will not cause any harm. Occasionally, your visit may be prolonged when waiting for the scans. We will try our best to shorten the visit as much as possible.

7. What are the possible benefits of taking part?

We are not introducing any treatment we would not have used to treat your condition or manage you any differently. The study may allow close monitoring of your growth and bone health. By taking part in the study you will provide us with information that will help us with addressing problems in other children with Crohn's disease.

8. Who can I contact for further information?

If you want to discuss the study further, please contact: Dr Faisal Ahmed telephone number 0141 201 0571 or Dr R Russell telephone number at the Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ.

9. Do I have to take part?

YOU DO NOT HAVE TO SAY 'YES' to taking part in this study if you do not want to. You will still have your biologic treatment.

10. What if something goes wrong?

We are not expecting any problems. However, if you are not happy about anything about this study, talk to your relatives, other hospital staff or your family doctor and ask them to speak to us. You can also contact Kate Colquhoun, Patient Contact Office at Yorkhill on 0141 201 9278.

11. Who is organizing the study?

The study is being organized by Dr Faisal Ahmed who looks after children and young people with bone and growth problems at Yorkhill.

12. Who is helping these doctors with the study?

Most of the work in this study will be performed by a senior student. Ms Salma Malik who is doing a PhD at the University of Glasgow. Other people who will be helping are members of the Bone & Endocrine Research Group and members of the GI team.

13. Who has looked at this study?

The study has also been looked at by a group of people who belong to the Research Ethics Committee and the Research & Development Department.

Appendix E

Study information sheet for the parent

Yorkhill Division

STUDY INFORMATION SHEET FOR THE PARENT

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

You child is being invited to take part in a research study. The study is being performed to see how biological medicines may improve growth and bone health in your child with Crohn's disease. Before you decide, you need to find out more about this study. Please read this sheet carefully and discuss it with your child. Take your time and ask us if anything is not clear.

1. **What happens to growth and bone health in children with Crohn's disease?**
   Children with Crohn's disease often do not grow as well as healthy children and their bones may also be affected This may be due to the effects of some substances called cytokines that are increased in children with Crohn's disease. Cytokines causes the symptoms of Crohn's disease but may also slow down the growth of your child. Steroid medicines (e.g. Prednisolone) that are sometimes used to treat Crohn's disease can also slow growth and affect the bones.

2. **What is this study about?**

   Biologics are special medicines that block the cytokines that may be increased in Crohn's disease. We want to find out how these new medicines, affect growth and bones in children with Crohn's disease. We are also interested in finding out how these new medicines can improve the hormones and substances that control growth and bone health.

3. **Why has your child been chosen?**

   We know that your child will be started on biologic therapy to treat their condition. We would like to add this study to your child's clinical treatment. The study will be fitted around the visits your child has to receive the medicine or when your child attends the clinic.

4. **What will happen to your child on participation?**

   If you agree to take part, we will take some extra blood from your child's cannula (2 teaspoons) at the time of the biologic therapy. Routine blood tests are required before

the infusion anyway and we will just take a little bit more. Your child will not notice anything different and there will not be any extra jags. We will also ask for a urine and stool sample each time your child attends for the study visits. We will also check your child's height and weight at each visit. If your child is not to continue on biologics after the first 3 doses, we will see your child and organize for the tests at the clinic. At baseline and 6 months and 12 months after the first dose of biologic, we will ask for an x-ray of your child's left hand to check the amount of bone growth there is. In addition, at baseline and 6 months and 12 months, we will also ask for special scans, called DXA scans that check how strong your child's bones are. Some of these scans involve a very small amount of x-rays that are not harmful. The amount of radiation is about the same as your child would get from playing outside in the sun on one average day.

5. What do I have to do?

If you would like to help us with the study we would like to ask you to sign a piece of paper called a 'consent form'. All you have to do is to bring your child for their biologic treatment or to the clinic visit as usual.

6. What are the possible problems of taking part?

Taking the extra volume of blood from your child and the special scans to measure how strong your child's bones are will not cause any harm. Occasionally your visit may be prolonged waiting for scans.

7. What are the possible benefits of taking part?

We are not introducing any treatment we would not have used to treat your child's condition or manage your child any differently. The study may allow close monitoring of your child's growth and bone health. By taking part in the study you will provide us with information that will help us with addressing problems in other children with Crohn's disease.

8. Who can I contact for further information?

If you want to discuss the study further, please contact: Dr Faisal Ahmed telephone number 0141 201 0571 or Dr R Russell telephone number at the Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ.

9. Do I have to take part?

YOU DO NOT HAVE TO SAY 'YES' to taking part in this study if you do not want to. Your child will still have his/her biological treatment.

10. What if something goes wrong?

We are not expecting any problems. However, if you are not happy about anything about this study, talk to your relatives, other hospital staff or your family doctor and ask them to speak to us. You can also contact Kate Colquhoun, Patient Contact Office at Yorkhill on 0141 201 9278

11. Who is organizing the study?

The study is being organized by Dr Faisal Ahmed who looks after children with bone and growth problems at Yorkhill.

12. Who is helping these doctors with the study?

Most of the work in this study will be performed by a senior student. Ms Salma Malik who is doing a PhD at the University of Glasgow. Other people who will be helping are members of the Bone & Endocrine Research Group and members of the GI team.

14. Who has looked at this study?

The study has also been looked at by a group of people who belong to the Research Ethics Committee and the Research & Development Department.

Appendix F

Assent form for the child 8-14 years

Yorkhill Division

NHS Greater Glasgow and Clyde

ASSENT FORM FOR THE CHILD 8-14YEARS

Patient Identification Number:

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

The study has been explained to me and I have read the information sheet and understand what I have to do.	☐
I know my child can come out of the study at any time.	☐
I understand that all medical information about my child is STRICTLY CONFIDENTIAL	☐
I would like my child to take part in this study.	☐
I am happy for my family doctor to know that my child is taking part in this study.	☐

Signatures:

Parent(s) name (block letters) ----------

Signature (s) ----------

Date ----------

Patient name (block letters) ----------

Patient signature (if applicable) ----------

Date ----------

Name of person taking consent ----------

(If not the researcher)

Signature ----------

Date ----------

Researcher's name (block letters) ----------

Signature ----------

Date ----------

THANK YOU FOR YOUR HELP

1 for patient/ parent; 1 for researcher; 1 to be kept with hospital notes

Appendix G

consent form for the young person over 14 years

NHS Greater Glasgow and Clyde

Yorkhill Division

CONSENT FORM FOR THE YOUNG PERSON OVER 14 YEARS OLD

Patient Identification Number:

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

The study has been explained to me and I have read the information Sheet and understand what I have to do. ☐

I know I can come out of the study at any time. ☐

I understand that all medical information about myself is STRICTLY CONFIDENTIAL. ☐

I would like to take part in this study. ☐

I am happy for my family doctor to know that I am taking part in this study. ☐

Signatures:

Parent(s) name (block letters) ----------

Signature (s) ----------

Date ----------

Patient name (block letters) ----------

Patient signature (if applicable) ----------

Date ----------

Name of person taking consent ----------

(If not the researcher)

Signature ----------

Date ----------

Researcher's name (block letters) ----------

Signature ----------

Date ----------

THANK YOU FOR YOUR HELP

1 for patient/ parent; 1 for researcher; 1 to be kept with hospital notes

Appendix H

Parent consent form

Yorkhill Division

NHS Greater Glasgow and Clyde

PARENT CONSENT FORM

Patient Identification Number:

The effects of biologic therapy on linear growth, puberty and bone health in children with Crohn's disease (CD)

The study has been explained to me and I have read the information Sheet and understand what I have to do.	☐
I know my child can come out of the study at any time.	☐
I understand that all medical information about my child is STRICTLY CONFIDENTIAL	☐
I would like my child to take part in this study.	☐
I am happy for my family doctor to know that my child is taking part in this study.	☐

Signatures:

Parent(s) name (block letters) ----------

Signature (s) ----------

Date ----------

Patient name (block letters) ----------

Patient signature (if applicable) ----------

Date ----------

Name of person taking consent ----------

(If not the researcher)

Signature ----------

Date ----------

Researcher's name (block letters) ----------

Signature ----------

Date ----------

THANK YOU FOR YOUR HELP

1 for patient/ parent; 1 for researcher; 1 to be kept with hospital notes

Appendix I

Data collection form baseline

Biologics in Crohn's Disease (BCD Study)			
VISIT: BASELINE		**Date:**	
Patient ID: **BCD……..** Patient Initials:	**Sex:** ☐ Male ☐ Female	**Date of Birth:** **Age in years:**	**Parents height** Mother: _____ father: _____
Baseline	Height	Weight	Sitting height
Date:	cms:_____	Kgs: _____	cms:_____
Diagnosis	**Height**	**Weight**	**Decimal age**
Date:	cms:_____	Kgs: _____	Years:_____

PARENT'S ETHINIC GROUP

	Mother	Father		Mother	Father
White	☐	☐	Jewish	☐	☐
Black –Caribbean	☐	☐	Indian	☐	☐
Black –African	☐	☐	Pakistani	☐	☐
Black –other	☐	☐	Bangladeshi	☐	☐
Others			Chinese	☐	☐
			Others		

MONTREAL DISEASE CLASSSIFICATION

Age at diagnosis	**Location**	**Behaviour**
A1□ Below 16 years	L1□ Ileal	B1 □non stricturing, non penetrating
A2□ Between 17&14 years	L2□ Colonic	B2□ stricturing
A3□ Above 40 years	L3□ Ileocolonic	B3□ penetrating
	L4□ Isolated upper disease	

PCDAI **□No □ Yes** **Date …….** **score: ----------**

GRIP STRENGTH

Date: _____	Dominant Grip	Non dominant Grip Strength
Dominant side: □ Left □ Right	1 | 2 | 3	1 | 2 | 3

REASONS FOR BIOLOGIC THERAPY

Date:_____

Sever disease despite of Immunomodulator therapy	
Sever disease awaiting response despite standard therapy	
Fistulating disease despite standard therapy	
Metastatic disease	
Non responder to Infliximab therapy	
Intolerant to Infliximab therapy	

Dose of INFLIXIMAB/ADALIMUMAB

CONCOMINTANT THERAPIES

Date : _____ **Nutritional Support** Elemental feeds Polymeric feeds	 □No □ Yes □No □ Yes	
5-ASA Osalazine Mesalazine	 □No □ Yes □No □ Yes	
Steroids Prednisolone	 □No □ Yes	
Background immunosuppressants Methotrexate Azathioprine	 □No □ Yes □No □ Yes	
Antibiotics	□No □ Yes	

MECHANOGRAPH:SINGLE TWO LEG JUMP **Date**		
F.Max	Yes	No
P.Max	Yes	No
Efficiency	Yes	No
RADIOGRAPHY **Date**		
DEXA	Yes	No
pQCT	Yes	No
Bone Age (X-ray of right hand)	Yes	No
PUBERTAL ASESSMENT **Date : _____** □No □ Yes		

BIOLOGIC SAMPLES DATE:

Blood	Yes	No
Urine	Yes	No
Stool	Yes	No

ADDITIONAL INFORMATION

ANY REACTION TO INFLIXIMAB/ADALIMUMAB

Appendix J

Data collection form 2 weeks

Biologics in Crohn's Disease (BCD Study)	
VISIT: 2weeks	**Date:**

Patient ID: **BCD……..** **Patient Initials:** | | | |	**Date of Birth:** | | | | **Sex:** ☐ Male ☐ Female
2weeks	**Weight**
Date: | | | |	Kgs: _____

PCDAI **□No □ Yes** **Date …….** **score: ----------**

GRIP STRENGT

Date: _____ **Dominant side:** □ **Left** □ **Right**	Dominant Grip 1 2 3	Non dominant Grip Strength 1 2 3

CONCOMINTANT THERAPIES

Date : _____ Nutritional Support Elemental feeds Polymeric feeds	 □No □ Yes □No □ Yes	
5-ASA Osalazine Mesalazine	 □No □ Yes □No □ Yes	

Steroids Prednisolone	□No □ Yes	
Background immunosuppressants Methotrexate Azathioprine	 □No □ Yes □No □ Yes	
Antibiotics	□No □ Yes	

MECHANOGRAPH:SINGLE TWO LEG JUMP Date:			Comments
F.Max	No	Yes	
P.Max	No	Yes	
Efficiency	No	Yes	

BIOLOGIC SAMPLES

Date:			Comments
Blood	Yes	No	
Urine	Yes	No	
Stool	Yes	No	

ADDITIONAL INFORMATION

Appendix K

Data collection form 6weeks

Biologics in Crohn's Disease (BCD Study)	
VISIT: 6weeks	**Date:**

Patient ID: **BCD……..** **Patient Initials:** | | | |	**Date of Birth:** | | | | **Sex:** ☐ Male ☐ Female
2weeks	**Weight**
Date: | | | |	Kgs: _____

PCDAI **□No □ Yes** **Date …….** **score: ----------**

GRIP STRENGTH

Date: _____ **Dominant side:** □ **Left** □ **Right**	Dominant Grip 1 2 3	Non dominant Grip Strength 1 2 3

CONCOMINTANT THERAPIES

Date : _____ Nutritional Support Elemental feeds Polymeric feeds	 □No □ Yes □No □ Yes	
5-ASA Osalazine Mesalazine	 □No □ Yes □No □ Yes	

Steroids Prednisolone	 □No □ Yes	
Background immunosuppressants Methotrexate Azathioprine	 □No □ Yes □No □ Yes	
Antibiotics	□No □ Yes	

MECHANOGRAPH:SINGLE TWO LEG JUMP			
Date:			Comments
F.Max	No	Yes	
P.Max	No	Yes	
Efficiency	No	Yes	

BIOLOGIC SAMPLES

Date:			Comments
Blood	Yes	No	
Urine	Yes	No	
Stool	Yes	No	

ADDITIONAL INFORMATION

Appendix L

Data collection form 6 months

Biologics in Crohn's Disease (BCD Study)	
VISIT: 6 months	**Date:**

Patient ID: **BCD…….** **Patient Initials:**	**Sex:** ☐ Male ☐ Female **Date of Birth:**

6 months	**Height**	**Weight**	**Sitting height**
Date:	cms:_____	Kgs: _____	cms:_____

PCDAI **□No □ Yes** **Date …….** **score: ----------**

PUBERTAL STATUS **□No □ Yes** **Date …….** **Tanner stage: ----------**

GRIP STRENGTH:

Date: _____ **Dominant side:** □ **Left** □ **Right**	Dominant Grip 1 2 3	Non dominant Grip Strength 1 2 3

CONCOMINTANT THERAPIES

Date : _____		
Nutritional Support Elemental feeds Polymeric feeds	 □No □ Yes □No □ Yes	
5-ASA		

Osalazine Mesalazine	□No □ Yes □No □ Yes	
Steroids Prednisolone	□No □ Yes	
Background immunosuppressants Methotrexate Azathioprine	□No □ Yes □No □ Yes	
Antibiotics	□No □ Yes	

MECHANOGRAPH:SINGLE TWO LEG JUMP Date			
F.Max		Yes	No
P.Max		Yes	No
Efficiency		Yes	No
RADIOGRAPHY Date			
DEXA		Yes	No
pQCT		Yes	No

BIOLOGIC SAMPLES

Date

Blood		Yes	No
Urine		Yes	No
Stool		Yes	No

ADDITIONAL INFORMATION

Appendix M

Data collection form 12 months

Biologics in Crohn's Disease (BCD Study)	
VISIT: 12 months	**Date:**

Patient ID: **BCD……..** **Patient Initials:**	**Sex:** ☐ Male ☐ Female **Date of Birth:**		
12 months	**Height**	**Weight**	**Sitting height**
Date:	cms:_____	Kgs: _____	cms:_____

PCDAI □No □ Yes Date …….. score: ----------
PUBERTY □No □ Yes Date …….. Tanner stage: ----------

GRIP STRENGTH

Date: _____ Dominant side: □ Left □ Right	Dominant Grip	Non dominant Grip Strength
	1	1
	2	2
	3	3

CONCOMITANT THERAPIES

Date : _____ Nutritional Support Elemental feeds Polymeric feeds	 □No □ Yes □No □ Yes	
5-ASA Osalazine Mesalazine	 □No □ Yes □No □ Yes	

Steroids Prednisolone	□No □ Yes	
Background immunosuppressants Methotrexate Azathioprine	 □No □ Yes □No □ Yes	
Antibiotics	□No □ Yes	

MECHANOGRAPH: SINGLE TWO LEG JUMP Date			
F.Max		Yes	No
P.Max		Yes	No
Efficiency		Yes	No
RADIOGRAPHY Date			
DEXA		Yes	No
pQCT		Yes	No
Bone Age (X-ray of right hand)		Yes	No

BIOLOGICAL SAMPLES

Blood		Yes	No
Urine		Yes	No
Stool		Yes	No

ADDITIONAL INFORMATION

TOTAL NUMBER OF INJECTIONS/IFUSIONS IN 12 m

Appendix N

Paediatric Crohn's disease activity index score form

Biologics in Crohn's Disease (BCD-Study)

Visit: Patient ID: BCD…….. DOB:

Visit date: Patient Initial:

Paediatric Crohn's Disease Activity Index

1.Abdominal pain	
None	0
Mild: Brief, does not interfere with activities	5
Mod/severe - daily, longer lasting affects activities, nocturnal	10
2.Stools	
Formed stools or up to 1 liquid stool, no blood	0
Up to 2 semi-formed with small blood, or 2-5 liquid with or without small blood	5
Any gross bleeding, or ≥ 6 liquid, or nocturnal diarrhoea	10
3.Functioning, well being	
No Limitation of activities, well	0
Occasional difficulty in maintaining appropriate activities, below par	5
Frequent limitation of activity, very poor	10
4.Weight	
Weight gain or voluntary weight stable/loss	0
Involuntary weight stable, weight loss 1-9%	5
Weight loss ≥ 10%	10
5.Abdominal exam	
No tenderness, no mass	0
Tenderness, or mass without tenderness	5
Tenderness, involuntary guarding, definite mass	10
6. Peri-rectal disease	
None, asymptomatic tags	0
Inflamed tags or 1-2 indolent fistula(e) or fissure(s), scant	5

drainage, no tenderness	
Active fistula, drainage, tenderness, or abscess	10
7. Extra-intestinal Manifestations, (Fever≥38.5℃ for 3 days over past week, oral ulcers, definte arthritis, uveitis, erythema nodosum, pyoderma gangrenosum)	
None	0
One	5
Two or more	10
Height at diagnosis	
<1 channel decrease	0
≥1<2 channel decrease	5
≥ channel decrease	10
Height velocity at follow-up	
Height velocity -1SD	0
Height velocity < -1SD, >- 2SD	5
Height velocity ≤ - 2SD	10
Hct(%)	
ESR	
Albumin	