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Predicting Response to Anti-TNF Therapy in Crohn's Disease – Clinical and Metabolic Studies

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**Submitted in total fulfilment of the requirement of the degree of
Doctor of Philosophy**

Department of Medicine

St Vincent's Hospital

The University of Melbourne

Abstract

Crohn's disease is a chronic, disabling inflammatory condition that affects the gastrointestinal tract that is associated with significant morbidity. Up to 80 percent of patients require surgery at some point in their lives. A third of patients require surgery within five year of diagnosis even in the era of biologic therapy. Anti-Tumour Necrosis Factor alpha (Anti-TNF) therapies form the backbone of drug therapy in patients with moderate to severe Crohn's disease and remain the most effective biologic drugs available.

There are currently three classes of biological drugs available on the Pharmaceutical Benefits Scheme (PBS) for Australians with many more under development. Up to thirty percent of patients fail to have an adequate response to anti-TNF therapy and loss of response occurs at a rate of ten percent per year. There are at present no highly sensitive biomarkers of response to anti-TNF therapies. Without biomarkers to assist with selecting the most effective biologic for a particular patient, there is a risk of exposing patients to ineffective drug therapies with unnecessary side-effects and costs.

This thesis comprises a range of clinical and scientific studies that seek to identify predictors of loss of response to anti-TNF therapies in Crohn's disease. Two types of loss of response have been identified: Primary nonresponse (PNR), is defined as a lack of response to the initial drug therapy as determined at 12 weeks post induction, and Secondary Loss of Response (SLOR) is defined as the loss of response after the patient has had an initial response to the drug.

Based on the clinical observation that patients with altered body composition and those with strictures have a variable to response to anti-TNF therapy, we sought to identify whether there were specific clinical, endoscopic, histologic and biochemical parameters that correlated with response to anti-TNF therapy amongst patients from whom longitudinal body composition and endoscopic data had been collected.

A prospective cohort study of patients who had been newly started on anti-TNF therapies was also undertaken. In this cohort samples of urine, blood and faeces were taken prior to anti-TNF therapy delivery (baseline) and then at 3 monthly

intervals thereafter. A combination of bioinformatic analyses and novel techniques evaluating the metabolome were applied to the cohort, in order to find clinical factors and biomarkers that might correlate with therapeutic response to anti-TNF therapy.

This series of studies presented in this thesis on clinical and metabolic predictors of outcomes in patients receiving anti-TNF therapy for Crohn's disease reveals new insights into the pathophysiology of Crohn's disease and has identified biomarkers for the prediction of therapeutic response – thereby helping to come a step closer towards the ultimate goal of precision medicine.

Declaration

This is to certify that this thesis:

- i) Contains no material that has been accepted for the award of any other degree or diploma in any university or other institution,
- ii) Comprises only my original work except where indicated in the Preface,
- iii) Due acknowledgement has been made in the text to all other material used,
- iv) Is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices

Preface

This thesis forms the culmination of years of work which have been primarily under the supervision of Professor Ailsa Hart, Imperial College, London. It has been co-supervised by Dr Peter De Cruz, Director of IBD, Austin Hospital and Prof Michael Kamm, Department of Gastroenterology, St Vincent's Hospital.

The laboratory work was co-supervised by Professor Elaine Holmes, Director of Metabonomics, Imperial College, London. Bioinformatic analysis was co-supervised by Dr Magali Sarafian, Imperial College, London.

Dr Ryan Choi taught me techniques relating to bioinformatics and analysis of the IBD cohort at St Mark's Hospital.

These studies were partly funded by the European Crohn's and Colitis Organisation grant, ForCrohn's grant, St Mark's Foundation grant and Post-graduate Scholarship awarded by Melbourne University.

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Generous funding support from the European Crohn's and Colitis Organisation grant, ForCrohn's grant, St Mark's Foundation grant and Post-graduate Scholarship awarded by Melbourne University are gratefully acknowledged.

I would like to thank my parents for their love, instilling the confidence to know that I can achieve what I set my mind to and inspiring me to always learn, improve and give my all. Thank you to my parents-in-law for their support and love throughout the years.

To my wife Natalie, you are my rock and so much of what I have achieved is because of your love, words and grace. You always pick me up when I am down, show me love when I least deserve it and bring out the best in all of us. I hope to be that same rock to you.

My daughters, Emily and Grace, I love your spirit, determination and strength. I will always learn more from the both of you than you will ever know. Thank you for teaching me the power of humility and to think with child-like imagination.

Published Articles

Ding, N.S., Hart, A. & De Cruz, P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease – algorithm for practical management. *Aliment Pharmacol Ther* **43**, 30–51 (2016).

Ding, N.S. et al. The body composition profile is associated with response to anti-TNF therapy in Crohn's disease and may offer an alternative dosing paradigm. *Aliment Pharmacol Ther* **46**, 883–891, doi:10.1111/apt.14293 (2017).

Ding, N.S. et al. Endoscopic dilatation of Crohn's anastomotic strictures is effective in the long-term and escalation of medical therapy improves outcomes in the biologic era. *J Crohns Colitis* **10**, 1172–1178 (2016).

Articles Submitted for Publication

Under review:

Metabonomics and the Gut Microbiome Predict Response to Anti-TNF Therapy in Inflammatory Bowel Disease

The impact and importance of Body Composition in Inflammatory Bowel Disease

Conference Abstracts

ECCO 2017

Oral presentation: Metabonomic and microbiota profile of Crohn's patients is a predictor of primary nonresponse to anti-TNF therapy N. Ding, M. Sarafian, A. Perdonés-Montero, R. Misra, P. Hendy, L. Penez, E. Holmes, A. Hart.

UEGW 2017

Ding, N.S., *et al.* Metabonomic and microbiota profile of Crohn's patients is a predictor of primary nonresponse to anti-TNF therapy. *United European Gastroenterol J* **5**, (2017).

UEGW 2016

Oral presentation: Metabolic profiling of urine and faeces differentiates inflammatory bowel disease phenotype and has implications on therapeutic response. Basic science award prize.

Poster champion award: Body composition profile: a predictor of therapeutic outcome in patients with moderate to severe Crohn's disease. Best submitted abstract.

ECCO 2015

Oral presentation: Escalation of medical therapy decreases need for repeat dilatation in Crohn's anastomatic strictures. N.S. Ding, W. Yip, R. Choi, B. Saunders, S. Thomas-Gibson, N. Arebi, A. Humphries, A. Hart.

Lipid biomarkers to diagnose Crohn's disease using metabonomic profiling approach in serum and faeces. (A-1239). N. S. Ding, M. Sarafian, E. Holmes, A. Hart.

Body composition: a biomarker of therapeutic outcome in patients with Crohn's disease. Ding, N., Malietzis, G., Lung, P., Yip, W., Penez, L., Ganeshanathan, T., Gabe, S., Jenkins, J., Hart, A.

DDW (Digestive Disease Week) Conference 2016

Oral presentation: Body composition profile: a predictor of therapeutic outcome in patients with moderate to severe Crohn's disease. Nik S. Ding, George Malietzis, Philip F. Lung, Wai M. Yip, Lawrence Penez, Thenugan Ganeshanathan, Simon M. Gabe, John T. Jenkins, Ailsa Hart.

Selected for Young Investigator award for an important investigator led study.

Prizes

Basic Science award: UEGW 2017

Poster champion award: UEGW 2017, 2016

Young AGA investigator award: DDW 2016

ECCO best poster award: ECCO 2016

Table of Contents

Abstract.....	ii
Declaration.....	iv
Preface	v
Acknowledgements.....	vi
Published Articles	vii
Articles Submitted for Publication	vii
Conference Abstracts	vii
Prizes.....	viii
List of Tables	xv
List of Figures	xvii
Chapter 1: Introduction, Background and Overall Research Design	1
1.1 General Introduction.....	1
1.2 The Clinical Problem	1
1.2.1 Anti-TNF α Drug Therapy	3
1.2.2 Predictors of Response to Therapy	4
1.3 Systematic Review: Predicting and Optimizing Response to Anti-TNF Therapy in Crohn's Disease – Algorithm for Practical Management	5
1.3.1 Introduction	5
1.3.2 Review Criteria and Methodology	6
1.3.2.1 Selection Criteria.....	6
1.3.2.2 Search Strategy	6
1.3.3 Definitions of Response and Remission	7
1.3.3.1 CDAI.....	7
1.3.3.2 HBI	8
1.3.4 Primary Nonresponse	8
1.3.4.1 Definition	8
1.3.4.2 Incidence.....	8
1.3.4.3 Mechanisms Underlying Primary Nonresponse	9
1.3.4.4 Drug Factors	10
1.3.4.5 Treatment Factors.....	12
1.3.4.6 Clinical.....	13
1.3.5 Therapeutic Drug Monitoring as a Predictor of Primary Nonresponse.....	15
1.3.6 Endoscopic Factors	16
1.3.7 Histologic Factors	16

1.3.8	Faecal Inflammatory Markers	17
1.3.9	Biochemical Markers.....	17
1.3.10	Serological Antibodies	17
1.3.11	Radiology	18
1.3.11.1	Stricturing Phenotype	18
1.3.12	Experimental Techniques	18
1.3.12.1	Pharmacogenetics	19
1.3.13	Failure at Induction of Second Anti-TNF	21
1.3.14	Secondary Loss of Response	22
1.3.14.1	Definition	22
1.3.14.2	Incidence	22
1.3.14.3	Mechanisms Underlying Secondary Loss of Response	23
1.3.14.4	Drug Factors	23
1.3.14.5	Predictors of Secondary Loss of Response to Anti-TNF Therapy	25
1.3.14.6	Clinical.....	26
1.3.15	Therapeutic Drug Monitoring as a Predictor of Secondary Loss of Response	28
1.3.15.1	High Trough Levels Versus Development of Undetectable Trough Levels	28
1.3.15.2	Effect of Anti-Drug Antibody Status on Trough Levels	28
1.3.15.3	Low Trough Levels with Absent Anti-Drug antibodies.....	28
1.3.15.4	Clinically-Guided Dose Escalation	29
1.3.15.5	High Trough Levels and Possible De-Escalation	30
1.3.16	Endoscopic	30
1.3.17	Faecal Inflammatory Markers	31
1.3.18	Biochemical.....	31
1.3.18.1	CRP, ANA, Anti-dsDNA	31
1.3.19	Radiologic	31
1.3.20	Pharmacogenetic	32
1.3.21	Metabonomic	32
1.3.22	Strategies to Recapture Response	33
1.3.22.1	Dose Escalation	33
1.3.22.2	Addition of an Immunomodulator	33
1.3.22.3	Switching Anti-TNF	34
1.3.23	Systematic Approach to the Management of Primary Nonresponse and Secondary Loss of Response.....	34
1.3.23.1	Approach to Patient with Symptom Deterioration Despite Anti-TNF therapy	34

1.3.24	Prevention of Primary Nonresponse and Secondary Loss of Response.....	36
1.3.25	Conclusion	39
1.4	Review Article: Body Composition Analysis in Inflammatory Bowel Disease – Implications for Clinical Practice and Research	40
1.4.1	Introduction	40
1.4.2	Definitions	41
1.4.3	Pathogenesis	42
1.4.3.1	Chronic Inflammatory Activity.....	42
1.4.3.2	Malabsorption.....	43
1.4.3.3	Malnutrition.....	43
1.4.3.4	The Role of Fat (Adipose Tissue) in Inflammatory Regulation	43
1.4.3.5	Physical Activity and Body Composition	44
1.4.4	Assessment and Monitoring.....	45
1.4.5	Computed Tomography and Magnetic Resonance Imaging.....	47
1.4.6	Techniques for Assessment and monitoring of body composition in IBD	47
1.4.6.1	Active Disease Versus Clinical Remission and Association with Body Composition.....	52
1.4.6.2	Impact of Body Composition Changes on Muscle Strength in IBD Patients	52
1.4.6.3	Relationship Between Medical Therapy and Body Composition	53
1.4.6.4	Incidence of Muscle Depletion in IBD	54
1.4.7	Management.....	54
1.4.7.1	Nutrition	54
1.4.7.2	Physical Activity and Exercise.....	55
1.4.7.3	Rehabilitation	55
1.4.7.4	Medical Therapy.....	56
1.4.8	Discussion.....	56
1.5	Supplementary Text	58
1.5.1	Methods	58
1.5.2	Selection Criteria.....	58
1.5.3	Search Strategy	59
1.5.4	Data Extraction	59
1.6	Metabolic Profiling in IBD	60
1.6.1	Metabolic Profiling Methods and Workflow	61
1.6.2	Application of Metabolic Phenotyping in Gastrointestinal Diseases	63

1.6.3	Application of Metabolic Phenotyping in IBD	64
1.7	Overall Thesis Hypothesis.....	67
1.8	Overall Thesis Aims	67
1.9	Details of the Studies in this Thesis	68
Chapter 2: Body Composition Profile is Associated with Response to Anti-TNF Therapy in Crohn's Disease and may offer an Alternative Dosing Paradigm		69
2.1	Introduction.....	69
2.2	Methods.....	70
2.2.1	Patient Population.....	70
2.3	Computed Tomography Image Analysis	71
2.4	Statistical Analysis.....	72
2.5	Results	72
2.5.1	Primary Nonresponse	75
2.5.2	Secondary Loss of Response.....	78
2.5.3	Obesity.....	81
2.5.4	Pharmacokinetic Sub-study	81
2.6	Discussion	82
2.7	Conclusion.....	85
Chapter 3: Crohn's Anastomotic Dilatation and Anti-TNF Therapy Response.....		86
3.1	Introduction.....	86
3.2	Methods.....	87
3.2.1	Management Protocol.....	87
3.2.2	Outcomes Measures.....	88
3.2.3	Statistics.....	89
3.3	Results	89
3.3.1	Patient Characteristics	89
3.3.2	Disease Characteristics	90
3.3.3	Efficacy of Endoscopic Dilatation.....	91
3.3.4	Effect of Clinical and Endoscopic Factors on Repeat Dilatation....	91
3.3.5	Effect of Medical Therapy on Repeat Dilatation.....	92
3.3.6	Effect of Clinical, Radiologic, Endoscopic Factors and Medical Therapy on Surgery	94
3.3.7	Radiology and Correlation with Endoscopic Disease Activity	95
3.3.8	Complication	96
3.4	Discussion	96
3.5	Conclusion.....	99

Chapter 4: Endoscopic and Histologic Predictors of Response to Anti-TNF Therapy in Crohn's Disease	100
4.1 Introduction.....	100
4.2 Assessment of Severity and Healing.....	100
4.3 Medical Management	101
4.3.1.1 Evidence for Biological Therapy.....	101
4.3.1.2 Histologic or Endoscopic Predictors of Primary Nonresponse or Loss of Response	101
4.4 Aim	102
4.5 Method	102
4.5.1 Statistics.....	104
4.6 Results	104
4.6.1 Endoscopic and Histologic Predictors.....	104
4.6.1.1 Endoscopy and Histologic Activity Affecting Response to Anti-TNF Therapy.....	105
4.7 Discussion	106
Chapter 5: Metabonomics and the Gut Microbiome Predict Response to Anti-TNF Therapy in Inflammatory Bowel Diseases	109
5.1 Introduction.....	109
5.2 Methods.....	111
5.2.1 Sample Collection	111
5.2.2 Baseline Disease Activity Assessment	112
5.2.3 Response Index to Anti-TNF Treatment Using Objective Markers of Inflammation	113
5.3 Results	118
5.3.1 Demographics.....	118
5.3.2 Classification of Patient Response to Anti-TNF Therapy According to an Objective Response Index.....	118
5.3.3 Differences in Metabolic Profiles Observed Between Healthy Controls, CD Patients and UC Patients using UPLC-MS Metabonomic Approaches	119
5.4 Metabolic Predictors of Anti-TNF Response Using UPLC-MS Profiling in CD Patients	121
5.4.1 Exploration of 16S rRNA Gene Sequencing of Longitudinal Samples with Respect to Response to Anti-TNF Therapy.....	126
5.5 Discussion	127
5.5.1 IBD Biofluid Metabolite and Metataxonomic Profiles Exhibit a Distinct Metabolic Phenotype from those of Healthy Controls	127
5.5.2 Prognostic Metabolic Phenotypes are Associated with Response to Anti-TNF Therapy	129
5.5.3 Metataxonomic.....	131

5.6	Limitations, Current and Future Work	132
5.7	Conclusion.....	133
5.8	Supplementary Information	134
Chapter 6: Summary and Future Directions		148
6.1	Summary of Findings	148
6.1.1	Body Composition.....	148
6.1.2	Anastomotic Crohn's Disease.....	148
6.1.3	Endoscopy and Histology as Predictors of Response to Anti-TNF Therapy.....	149
6.1.4	Metabolic Profiling	149
6.2	Future Directions	150
Appendix I: Abbreviations and Definitions		152
Appendix II: Ethics Approvals		154
References		164

List of Tables

Table 1. Rates of response and primary nonresponse to induction of anti-TNF therapy in clinical trials.	9
Table 2. Noninflammatory causes of anti-TNF failure.	35
Table 3. Studies of body composition in Crohn's disease.	49
Table 4. Studies of body composition in ulcerative colitis.	51
Table 5. Background and demographics of study cohort.	74
Table 6. Univariate and multivariate analysis of patient demographics, medical therapy, biochemistry and body composition factors on outcome of primary nonresponse (PNR) to anti-TNF therapy.	77
Table 7. Primary nonresponse rates to anti-TNF therapy with each quartile of muscle mass with correlation between lean skeletal muscle index and primary nonresponse.	78
Table 8. Univariate and multivariate analysis of patient demographics, medical therapy, biochemistry and body composition factors on outcome of secondary loss of response to anti-TNF therapy.	79
Table 9. Demographics of patients in cohort.	90
Table 10. Univariate analysis of clinical, radiologic, endoscopic and medical therapy on outcome of repeat dilatation.	92
Table 11. Multivariate analysis – repeat dilatation outcomes.	93
Table 12. Univariate analysis of clinical, radiologic, endoscopic and medical therapy on outcome of surgical resection.	95
Table 13. Multivariate analysis – surgical outcomes.	95
Table 14. Basic demographic information for the 237 patients.	104
Table 15. Endoscopic and histologic parameters identified at baseline colonoscopy.	105
Table 16. Table showing multivariate analysis for PNR.	106
Table 17. Summary of clinical and biochemical characteristics of IBD patients.	113
Table 18. Scoring system of response index using objective markers of mucosal healing and inflammation on CD and UC patients. Seven criteria were selected to classify response of CD patients to anti-TNF therapy (Table 18). Each criteria is given a score of 1 if the patient meets the criteria and 0 if not with the sum totalling the response index. CD patients are classified as 0–1 non-responders, 2–4 partial responders and 5–7 responders according to the response index.	114
Table S1. Pairwise OPLS-DA models of the three classes (HC, CD and UC) for the three UPLC-MS profiling methods and the three biofluids. OPLS-DA models are built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples. OPLS-DA parameters (R^2X , R^2Y , Q^2Y and p-value from CV ANOVA) demonstrate the robustness of each model. Data analysed at day 0 (before treatment).	142
Table S2. Pairwise OPLS-DA models between visits of CD patients for each of the three UPLC-MS profiling methods and the three biofluids. OPLS-DA models are built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples from CD. OPLS-DA parameters (R^2X , R^2Y , Q^2Y and p-value from CV ANOVA) demonstrate the robustness of each model. Data	

were analysed comparing visit 1 (V1) to visit 2 (V2), V1 to visit 3 (V3) and V1 to visit 4-5-6 (V4-5-6). 143

Table S3. Pairwise OPLS-DA models of CD anti-TNF treatment between responders and non-responders. OPLS-DA models were built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples from CD. OPLS-DA parameters (R^2X , R^2Y , Q^2Y and p-value from CV ANOVA) demonstrate the robustness of each model. Data were analysed on all visits, visit 1 (V1, before anti-TNF treatment) and V1 with only one point per patients. 144

Table S4. BA metabolites correlating with the longitudinal response of CD patients to anti-TNF treatment. List of significant BA markers identified to vary between patient that respond and do not respond to the anti-TNF treatment in serum, urine and faeces samples. Two-tailed Student's *t*-test * $p < 0.05$ *, ** $p < 0.01$ and *** $p < 0.001$ 145

Table S5. Summary metabolites and pathways variations observed between HC vs. IBD and CD vs. UC. Two-tailed Student's *t*-test. 146

List of Figures

Figure 1. Flowchart demonstrating study design with PRISMA.....	7
Figure 2. Proposed management algorithm for primary nonresponse to anti-TNF therapy in Chron's disease.	35
Figure 3. Proposed management algorithm for secondary loss of response to anti-TNF therapy in Crohn's disease.	38
Figure 4. Diagram representing the attenuated muscle hypertrophy pathway as reflected by signalling factor levels in CD.	45
Figure 5. PRISMA flow chart detailing study design.....	58
Figure 6. Flowchart of study protocol.	71
Figure 7. Kaplan-Meier plots showing overall rates of primary nonresponse to anti-TNF therapy by myopenia status.....	76
Figure 8. Kaplan-Meier plots showing overall rates of secondary loss of response to anti-TNF therapy depending on immunomodulator therapy	80
Figure 9. (A) Association between lean body mass and BMI in patients with anti-TNF therapy, (B) Estimated anti-TNF per kg fat-free mass in male and female patients based on 5 mg/kg anti-TNF dosing; all patients ranked from lowest to highest anti-TNF dose per kg fat-free mass.....	81
Figure 10. Flowchart with study design.	88
Figure 11. Impact of combination therapy on dilatation-free survival.	94
Figure 12. Flowchart detailing study design	103
Figure 13. Survival curve demonstrating that lymphoid aggregates are protective against SLOR.	106
Figure 14. Longitudinal study on IBD patients' response to anti-TNF treatment (A). Urine (n=181), serum (n=214) and faecal (n=147) samples were collected from CD and UC with patient samples over 16 months (B).	112
Figure 15. Significant variations in faecal bacterial composition of healthy control (HC) and Crohn's disease (CD) patients observed at all taxonomic levels. Faecal samples were analysed by 16S rRNA gene sequencing, and data were evaluated using White's non-parametric t-test with Benjamini-Hochberg FDR.	120
Figure 16. BA metabolites in urine, serum and faeces correlate with the longitudinal response of CD patients to anti-TNF treatment. BA markers vary between responders and non-responders to anti-TNF therapy in faeces (A), serum (B) and urine samples (C). Two-tailed Student's <i>t</i> -test * $p < 0.05$ *, ** $p < 0.01$ and *** $p < 0.001$. Supplementary information is available in Table S4.....	123
Figure 17. Predictive model demonstrating area under curve for classifying non-responders to anti-TNF therapy from lipid, bile acid and Hilic profiling biomarkers. (A) Serum lipid, (B) Faecal lipid, (C) Faecal bile acid, (D) Serum bile acid, (E) Urine bile acid, (F) Faecal histidine, (G) Serum histidine and (H) Urinary cysteine biomarkers for predicting nonresponse to anti-TNF therapy. Confusion matrix parameters are true positive (TP), true negative (TN), false negative (FN) and false positive (FP) with calculated accuracy, specificity and sensitivity.	124
Figure 18. Polar metabolites (Hilic) correlating with the longitudinal response of CD patient's anti-TNF treatment. Histidine and cysteine vary between patient that did and	

did not respond to anti-TNF treatment in faeces (A), serum (B) and urine samples (C). Two-tailed Student's <i>t</i> -test * $p < 0.05$ *, ** $p < 0.01$ and *** $p < 0.001$	125
Figure 19. Lipid classes correlating with the longitudinal response of CD patients to anti-TNF therapy. Mean levels of lipid markers according to classes (PC, Cer, SM and TG) which vary between patients responders and non-responders to anti-TNF treatment in faeces (A) and serum samples (B). Two-tailed Student's <i>t</i> -test * $p < 0.05$ *, ** $p < 0.01$ and *** $p < 0.001$	126
Figure 20. Differences in bacterial composition of IBD patients before and after receiving anti-TNF therapy. IBD patients included responders, non-responders, and intermediate responders, and post samples were taken from one time point 5–7 months after treatment.	127
Figure S1. OPLS-DA scores plots of faecal UPLC-MS data. Healthy participants were compared to CD (A, D and G) and to UC patients (B, E and H) at day0. Three assays were implemented, lipid profiling (A, B and C), BA profiling (D, E and F) and polar metabolite profiling (G, H and I). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 2168; 405 and 2346 features detected by respectively lipid; BA and Hilic profiling (C, F and I).	135
Figure S2. OPLS-DA scores plots of serum UPLC-MS data. Healthy participants were compared to CD (A, D and G) and to UC patients (B, E and H) at day0 (before treatment). Three assays were implemented, lipid profiling (A, B and C), BA profiling (D, E and F) and polar metabolite profiling (G, H and I). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 1278; 387 and 2288 features detected by respectively lipid; BA and Hilic profiling (C, F and I).	136
Figure S3. OPLS-DA scores plots of urine UPLC MS data. Healthy participants were compared to CD (A and D) and to UC patients (B and E) at day0 (before treatment). Two assays were implemented, BA (A, B and C) and polar metabolite profiling (D, E and F). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 518 and 2790 features detected by respectively BA and Hilic profiling (C and F).	137
Figure S4. OPLS-DA scores plots of faecal UPLC-MS data comparing CD vs. UC on BA profiling. Data analysed at day 0 (before treatment).	138
Figure S5. OPLS-DA scores plots of urine UPLC-MS data comparing CD vs. UC. BA profiling (A) and polar metabolite profiling (Hilic, B) of urine. Data analysed at day 0 (before treatment).	138
Figure S6. OPLS-DA scores plots of faecal samples for the prediction of the response of CD patients to anti-TNF therapy using the three UPLC-MS profiling methods. Three assays were implemented, lipid profiling (A and B), BA profiling (C and D) and polar metabolite profiling (Hilic, E and F). Models were built with all visits and visit 1 (baseline prior to anti-TNF therapy delivery).	139
Figure S7. OPLS-DA scores plots of serum samples for the prediction of the response of CD patients to anti-TNF therapy using the three UPLC-MS profiling methods. Three assays were implemented, lipid profiling (A and B), BA profiling (C and D) and polar metabolite profiling (Hilic, E and F). Models were built with all visits and visit 1 (before anti-TNF treatment).	140
Figure S8. OPLS-DA scores plots of urine samples for the prediction of the response of CD patients to anti-TNF therapy using the two UPLC-MS profiling methods. Two	

assays were implemented, BA profiling (A and B) and polar metabolite profiling (Hilic, C and D). Models were built with all visits and visit 1 (before anti-TNF treatment). ... 141

Figure S9. Levels of primary BAs in serum of responder and non-responder patients.

Cholic acid and chenodeoxycholic acid UPLC- MS data were analysed comparing visit 1 (V1) and all visits (Vall). Two-tailed Student's *t*-test * $p < 0.05$ *, ** $p < 0.01$ and

*** $p < 0.001$ 142

Chapter 1: Introduction, Background and Overall Research Design

1.1 General Introduction

Crohn's disease is a chronic disabling inflammatory disorder of the bowel which can affect any part of the gastrointestinal tract from mouth to anus which may result in complications such as strictures and abscess formation due to disease progression if left untreated.¹ The underlying cause is unknown but it is hypothesized that Crohn's occurs due to an exaggerated immune response to the gut microbiota in immunosuppressed patients.² Crohn's disease is heterogeneous in its presentation in relation to disease location, extent, activity and behaviour. Response to treatment is similarly heterogeneous thereby raising the need for biomarkers of both prognostic and predictive value to determine disease course and anticipate treatment outcome.

1.2 The Clinical Problem

Australia has one of the highest incidence rates of inflammatory bowel disease worldwide with 29.6 cases per 100,000 people.³ The cost to the healthcare system is estimated to be approximately \$2.7 billion a year.⁴ The main driver of expense is the cost of biologic therapy, with further increases in cost anticipated due to the increasing number of biologics available via the Pharmaceutical Benefits Scheme.⁵ Real world data suggest that there is a law of diminishing returns in relation to the efficacy of each successive biologic that is introduced to a given patient. This highlights the importance of the quality use of biologics if there is to be any chance of altering the natural history of disease.

Primary Non-Response (PNR) rates to biologics are in the order of 30%. However, specific predictors of PNR are unclear and seldom applied to choices regarding therapeutic decision making. Recent data suggest that pharmacokinetic and pharmacodynamic monitoring is of benefit in guiding the assessment and monitoring of treatment response and adjustment of biologic dose.^{6,7} Whilst most of the focus of pharmacokinetic monitoring has been on Therapeutic Drug Monitoring with Trough Level and Anti-Drug Antibody testing there has been less emphasis on the impact of body composition analysis and its impact on pharmacokinetics. This may be a particularly important issue within Western nations such as Australia which is estimated to have at least 63% of its

population within the overweight or obese range.⁶ Moreover the incidence of obesity has also been found to be increasing amongst IBD patients.⁴ Although raised Body Mass Index has been found to be associated with non-response to biologic therapy little is known about which specific body composition parameters can be used to predict response to biologic therapy.⁷

The implications of altered body composition appear to have consequences beyond non-response to biologics alone. Disease progression of Crohn's disease has been associated with fat-wrapping at the time of surgical resection which is associated with fibrosis and stricture formation. Up to 80% of patients with Crohn's require surgery at some point in their lives and up to a third of patients require surgery within five years of their diagnosis even in this era of biologic therapy. Surgical resection is not a cure. Disease recurs, at, and above the anastomosis. The development of anastomotic strictures following resectional surgery for Crohn's disease is a clinical scenario that is associated with PNR or secondary loss of response. However, little is known about the impact of anti-TNF therapy on patients with known anastomotic strictures in relation to the need for repeat balloon dilatation or re-operation.

There has been a change in the management paradigm for Crohn's in that treatment based on symptoms alone has not changed the natural history of disease.⁸ Emerging evidence suggests that therapeutic strategies should adopt a treat-to-target approach aimed at healing the mucosa.^{9,10} Mucosal healing has been associated with better clinical outcomes such as lower relapse rates and risk of surgery.¹¹ Histologic remission represents a potentially even greater depth of remission than mucosal healing.¹² However, whether histologic disease activity has any predictive value in relation to response to anti-TNF therapy is unknown.

Although endoscopic and histologic parameters are the gold-standard by which Crohn's is diagnosed and monitored ileo-colonoscopy is an invasive test which is associated with potential risks and cost. Non-invasive techniques to diagnose, monitor, predict and prognosticate represent an attractive alternative. Fecal calprotectin and C-Reactive Protein are two biomarkers that have been found to correlate with disease activity in IBD that can be used to non-invasively monitor patients as part of a treat-to-target strategy and predict treatment response.

Although FC and CRP have entered mainstream clinical practice there remains widespread inter-individual variability in relation to both biomarkers and their correlation with disease activity and treatment response. There is a consequently a need for other non-invasive biomarkers.

Whilst previous investigation has focused on biomarkers that are linked to the immune response it has more recently become apparent that the gut microbiota plays a key role in disease pathogenesis. Although serologic markers evaluating antibodies to microbial antigens such as ASCA and ANCA have been identified as being of predictive and prognostic value in IBD they still represent biomarkers that have focussed on the immune response rather than antigenic stimulus for IBD. In contrast metabolic profiling represents functional characterisation of the microbiome and its composition.¹³ The majority of previous studies using metabolic profiling have focussed on distinguishing Crohn's disease from Ulcerative Colitis.¹⁴ More recent studies have demonstrated that metabolic profiling is able to distinguish active disease from disease remission. However, whether metabolic profiling is able to predict therapeutic response is unknown.

Although the recent STRIDE guidelines suggest a treat-to-target approach using non-invasive biomarkers such as FC and CRP, IBD remains a heterogeneous group of diseases with heterogeneous treatment responses and FC and CRP do not correlate with disease activity and treatment response in all patients. Further non-invasive biomarkers are therefore required. Predictive biomarkers may be identified by exploring whether or not there is an association between body composition, metabolic profiling and treatment response.

This thesis describes a series of clinical and translational studies that seek to explore potential pharmacokinetic and pharmacodynamic mechanisms behind anti-TNF therapy primary non-response in pursuit of potential predictive biomarkers of response to anti-TNF therapy.

1.2.1 Anti-TNF α Drug Therapy

Although anti-TNF agents are highly effective, they are associated with significant adverse effects and costs. Primary Non-Response (PNR) rates are approximately 30% and secondary loss of response (SLOR) occurs at a rate of 10% per year. Identifying predictors of response is therefore important to identify

which patients are most likely to benefit from therapy. Moreover, it is important to identify patients who are less likely to respond in order to minimise exposure to futile drug therapies and reduce associated costs. Identifying predictors of response and non-response to drug therapy is therefore likely to help tailor drug therapy to the individual patient, facilitating a personalised or precision-medicine approach to drug therapy.

Central to personalized or precision medicine is the need for accurate and accessible biomarkers. Biomarkers are classically defined as measurable indicators of biological processes which can be used to quantify drug metabolism, disease activity or adverse drug effect.¹⁵ They can be of predictive value in relation to measuring and monitor disease activity or of prognostic value in relation to determining disease course and treatment response. Biomarkers which are useful in clinical practice need to be easily accessible, cost-effective and validated. Their application should facilitate a nuanced assessment of risk versus benefit for any patient when considering treatment selection or dose escalation.

1.2.2 Predictors of Response to Therapy

This first chapter will focus on (1) The introduction to clinical predictors of outcome to anti-TNF therapy (2) Endoscopic and histologic parameters as predictors of outcome with anti-TNF therapy in Crohn's disease (3) Body composition parameters as predictors of outcome and (4) Metabonomic approach to discovery of biomarkers for prediction of response to anti-TNF therapy.

This thesis involves the retrospective evaluation of a cohort of IBD patients with moderate to severe luminal Crohn's disease to identify clinical predictors of response to anti-TNF therapy. My initial study on an area of complex Crohn's disease with post-operative complications of anastomotic recurrence with stricturing disease, is an area that continues to lack data. Currently, patients may receive balloon dilatation if the strictures are amenable and accessible. However, little is known about whether subsequent escalation in medical therapy can either reduce the need for balloon dilatation or repeat surgery. Through the undertaking of these studies it became evident that there are also factors other than drug

related; body composition, endoscopy and histology, which are important to determining outcomes of response to anti-TNF therapy. A final prospective study was designed to collect data at various time-points to facilitate metabolic profiling in order to find predictive biomarkers of response to anti-TNF therapy using various biofluids.

1.3 Systematic Review: Predicting and Optimizing Response to Anti-TNF Therapy in Crohn's Disease – Algorithm for Practical Management

1.3.1 Introduction

Early intensive therapy with anti-tumour necrosis factor alpha (anti-TNF) monoclonal antibody drugs (infliximab, adalimumab and certolizumab) in moderate to severe Crohn's disease (CD) has been shown to achieve mucosal healing.¹⁶ Such an approach may alter the progression of CD by reducing disease-related complications. However, anti-TNF drugs are expensive and associated with unpredictable side-effects including infusion reactions, infections and lymphoma.¹⁷

Of the patients with CD treated with anti-TNF drugs, approximately 70–85% have an initial response to therapy.^{17,18} Treatment failure may occur as a result of: (1) adverse drug reactions at the time of infusion; (2) lack of primary response to anti-TNF therapy (primary nonresponse), or; (3) diminished response or loss of response to anti-TNF therapy (secondary loss of response). Adverse drug reactions occur in up to 5% of patients and range in severity from rashes to anaphylactic reactions. Primary nonresponse occurs in approximately 13–40% of patients.^{19,20} Secondary loss of response is estimated to occur at a rate of 13% per year in infliximab treated patients and at a similar rate in adalimumab-treated patients.^{21,22} The ability to predict and differentiate which patients are unlikely to benefit from drug therapy (primary nonresponse), from those likely to lose response (secondary loss of response) or develop adverse drug reactions would allow anti-TNF therapies to be tailored to the patient's needs, thereby maximising efficacy and minimizing toxicity and unnecessary costs.

The purpose of this systematic review which focuses on CD, is to collate information gathered from clinical trials, cohort studies and translational evidence regarding the incidence, mechanisms and predictors of primary nonresponse and

secondary loss of response. Although it is acknowledged that de-escalation or cessation of anti-TNF is an important aspect of anti-TNF management, it has not been covered in this review as it has been comprehensively explored in a review by Pariente *et al.*²³ In this review, clinical, endoscopic, radiologic, biochemical, serologic, pharmacogenetic/genomic and metabonomic predictors of nonresponse will be explored with the intention of formulating practical clinical algorithms to direct the optimal management of primary nonresponse and secondary loss of response respectively.

1.3.2 Review Criteria and Methodology

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

1.3.2.1 Selection Criteria

A search of the medical literature was conducted using MEDLINE (1970 to January 2018, EMBASE (1984 to June 2015), the Cochrane central register of controlled trials and the Cochrane IBD Group Specialized Trials Register and conference abstracts (ECCO, UEGW, DDW). The following key words were included alone or in combination: 'loss of response', 'infliximab', 'adalimumab', 'certolizumab', 'anti TNF- α ', 'biologics', 'Crohn's disease', 'lose', 'loss', 'dose escalation', 'intensification', 'radiology', 'MRI', 'Computer Tomography', 'ultrasound', 'clinical', 'biochemical', 'endoscopy', 'histology', 'metabolomics', 'proteomics', and 'genomics'.

1.3.2.2 Search Strategy

All relevant articles in English published until June 2015 were reviewed by two independent authors (NSD, PD). Where it was appropriate, a methodological 'filter' was applied to identify RCTs, citations of relevant studies, research registries of ongoing trials including Current Controlled Trials and hand search of conference abstracts from 2010 to 2015: British Society of Gastroenterology, Digestive Disease Week, United European Gastroenterology Meeting, European Crohn's and Colitis Organisation. Five hundred and eighty-one articles were found and screened, of which eighty-six were excluded. Six relevant abstracts were identified from conferences and included (Figure 1).

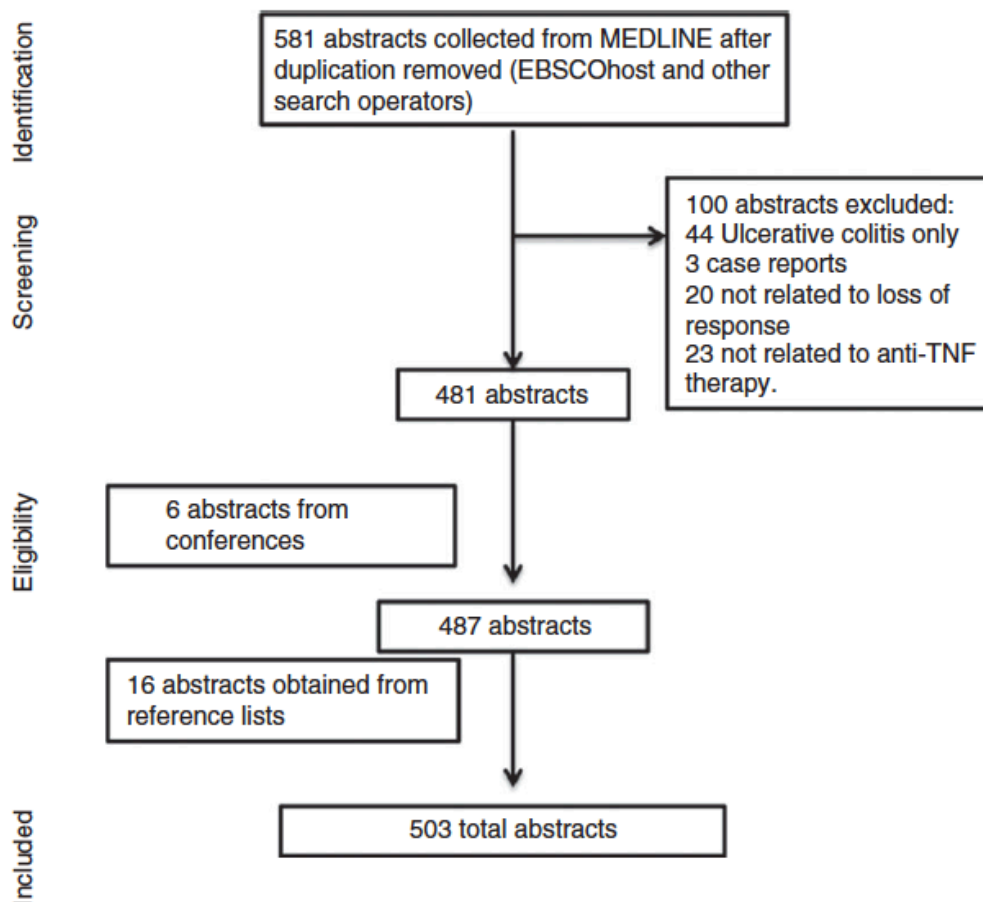


Figure 1. Flowchart demonstrating study design with PRISMA.

1.3.3 Definitions of Response and Remission

Determining the impact of a therapeutic intervention in relation to response or remission relies on adequate clinical assessment to quantify and monitor the symptoms of patients with CD. Response and remission following the administration of drug therapy have traditionally been defined based on the clinical indices that have been used in clinical trials for CD. Two validated scoring systems that combine patients' symptoms and laboratory parameters have been utilized for CD, the Crohn's disease activity Index (CDAI) and the Harvey-Bradshaw Index (HBI).

1.3.3.1 CDAI

In clinical trial settings, the definition of initial *clinical response* to anti-TNF therapy has varied and has been identified as: 1) either an initial CDAI reduction of more than 70 points^{12,24,25} or 100 points²⁶ from baseline; 2) a 25% or more reduction in the total CDAI score,²⁴ or; a combination of these criteria. According to the CDAI,

clinical remission of CD has been defined as a score below 150, which is regarded as an asymptomatic state.

1.3.3.2 HBI

Clinical trials utilizing HBI define initial response as a decrease in score of ≥ 2 during or after induction therapy. Clinical remission is defined as a score of < 5 . In clinical practice response and remission is simply defined by improvement in symptoms, blood test parameters and global assessment by the treating clinician.

1.3.4 Primary Nonresponse

1.3.4.1 Definition

There is currently no consensus around the definition of primary nonresponse. Nonetheless, primary nonresponse is recognised as a failure to achieve the previously described decrease in CDAI or HBI scores from baseline following anti-TNF induction therapy.²⁷ The time frame within which primary response or nonresponse is determined has varied between trials^{24,28} and clinical practice. However, currently in clinical trials, there is agreement that primary nonresponse to anti-TNF drugs should not be assessed prior to 14 weeks following initial induction infusions with use of infliximab, or prior to 12 weeks for adalimumab injections²⁹ and after an induction phase of 8 weeks for certolizumab. However, in some of the earlier clinical trials, assessment for primary nonresponse was not undertaken until week 26. In clinical practice, anti-TNF therapy is often continued for a period of 6 months before patients are considered as primary non-responders.

1.3.4.2 Incidence

Response rates for the TNF-alpha monoclonal antibodies used in Crohn's disease; infliximab, adalimumab³⁰ and certolizumab pegol, are summarized in Table 1. The incidence of primary nonresponse ranges from 36–40% in clinical trials^{24,27} to 13–33% in clinical practice.³¹

Table 1. Rates of response and primary nonresponse to induction of anti-TNF therapy in clinical trials.

	Response at weeks 2 to 6	Of initial responders	All treated patients	Initial responders	All treated patients	Primary nonresponse
Infliximab (ACCENT I)	59%	39%	23%	29%	23%	40%
Adalimumab (CHARM)	58%	40%	24%	36%	21%	40%
Certolizumab (PRECISE 2&3)	64%	48%	31%	42%	27%	36%

1.3.4.3 Mechanisms Underlying Primary Nonresponse

Recent evidence suggests that the mechanisms underlying primary nonresponse are multifactorial and include disease characteristics (phenotype, location, severity); drug (pharmacokinetics, pharmacodynamics, immunogenicity) and treatment strategy (dosing regimen) related factors.

Disease Phenotype

Disease phenotype and behaviour may contribute to primary nonresponse. Fibrostenotic disease may have lower response rates and may be more amenable to surgical resection or endoscopic dilatation therapy as shown in a retrospective cohort study of 425 patients amongst whom a stricturing phenotype was significantly associated with surgery despite anti-TNF therapy, (adjusted HR 6.17, 95% CI, 2.81–13.54).³²

Disease Location

Localized ileal stricturing disease may be associated with primary nonresponse but data have been conflicting. Whilst one previous study suggested ileal resection as the primary treatment for localized ileal stricturing disease,³³ a separate study showed that patients with isolated ileal stricturing disease treated with anti-TNF therapy progressed to surgery at the same rate as other patients with stricturing disease of an unspecified location.³² The results of the Laparoscopic Ileocolic Resection versus infliximab treatment of recurrent distal ileitis in Crohn's disease: A randomized multicenter trial (LIR!C-C) comparing anti-TNF versus ileal resection as first line treatment for localized ileal disease demonstrated comparable outcomes in quality of life as measured by the Inflammatory Bowel Disease Questionnaire (IBDQ) at 12 months.³⁴

Disease Severity

Anti-TNF therapies have been demonstrated to have lower efficacy in highly active inflamed tissue due to non-immune clearance of the drug.^{35,36} Therefore, severely active disease may not be sufficiently controlled by lower induction doses. Faecal loss of anti-TNF into the stool via the ulcerated denuded mucosa has been hypothesized as the mechanism for primary nonresponse in patients with very high inflammatory disease burden in Crohn's disease similar to that described by Brandse *et al.* in a cohort of patients with moderate to severe ulcerative colitis.³⁷ In a randomized control trial of certolizumab pegol, response rates were increased in those with less severe CD as determined by CDAI which coincided with better response rates in patients with higher levels of drug at week 6 compared with those with lower drug concentrations.³⁸

1.3.4.4 Drug Factors

There are pharmacokinetic, pharmacodynamic and immunogenicity factors that contribute to primary nonresponse to anti-TNF therapy.

Pharmacokinetic Failure

Pharmacokinetic failure results in decreased drug levels with typically absent or low anti-TNF antibodies. Pharmacokinetic failure arises from accelerated non-immune clearance of drug through tissue or systemic circulation. The three main mechanisms underlying pharmacokinetic failure are: proteolytic catabolism within the reticuloendothelial system; binding of the monoclonal antibody to Fc gamma receptors; and degradation in lysosomes by binding to membrane-bound TNF.^{39,40,41} Non-immune clearance may be caused by leakage of drug across the mucosal membrane^{37,42} due to ulcerated mucosa which may result in significant loss of protein and electrolytes including anti-TNF.³⁷

Pharmacodynamic Failure

Pharmacodynamic failure is characterized by a lack of improvement of Crohn's disease symptoms in the setting of normal drug levels without anti-drug antibodies. Pharmacodynamic failure occurs due to a shift in disease course to a non-TNF driven pathway in the setting of adequate serum drug levels.⁴³ In a study by Ainsworth *et al.*⁴³ primary nonresponse was observed in CD patients despite the presence of adequate TNF binding capacity of infliximab and the absence of

antibodies to infliximab, suggesting a non-TNF-driven inflammatory process as a cause of primary nonresponse in such patients.

Immunogenicity Failure

Immunogenicity failure is defined by the absence of improvement in patient symptoms in the setting of low anti-TNF levels with high levels of anti-drug antibodies and is a further mechanism that may contribute to primary nonresponse. Although initially thought to be an issue isolated to secondary loss of response, neutralizing antibodies can be detected after the first infusion of anti-TNF therapy. In a prospective study of 32 CD patients (15 treated with infliximab, 17 with adalimumab) after induction, those who were in clinical remission had higher anti-TNF trough levels (IFX: 5.60 µg/mL and adalimumab: 9.07 µg/mL) compared with those with active disease (IFX 0.032 µg/mL; adalimumab: 2.62 µg/mL ($p < 0.01$)).⁴⁴ Furthermore, 26% of infliximab-treated patients developed sustained antibodies (defined by two consecutive detected levels) which was associated with low trough drug levels and a greater chance of infliximab infusion reactions and primary nonresponse. Taken together these data indicate that a low concentration of drug and the presence of neutralizing and sustained (non-neutralizing) antibodies (which are associated with an increased rate of infusion reactions) contribute to primary nonresponse in 83% of patients.⁴⁴

There have been two studies that have reviewed the use of a second anti-TNF in the case of primary nonresponse in small cohorts.^{45,46} Ho *et al.* showed that three out of the initial six patients who had primary nonresponse to infliximab achieved remission with adalimumab. A retrospective review of adalimumab-treated primary non-responders to IFX demonstrated that three out of six showed clinical improvement on initiation of adalimumab. However, data was only collected to 6 weeks.⁴⁵ These preliminary data suggest that the likelihood of capturing response with a second anti-TNF in the setting of primary nonresponse is in the order of approximately 50% which was supported by a recent meta-analysis which demonstrated a response rate of 53% in patients with primary failure following their first anti-TNF.⁴⁷ Adding weight to the premise that primary nonresponse is associated with inferior response to second-line biologics, a recent meta-analysis showed that patients with prior PNR were 27% less likely to achieve remission with induction therapy with second-line biologics.⁴⁸

1.3.4.5 Treatment Factors

Emerging evidence suggest that primary nonresponse may be minimized via optimization of dosing regimen and use of combination therapy.

Dosing Regimen

In the Clinical assessment of Adalimumab Safety and efficacy Studied as Induction therapy in Crohn's disease) CLASSIC 1 dose-finding induction study, remission at 4 weeks was achieved in more patients receiving the higher dose adalimumab (160mg followed by 80mg 2 weekly thereafter (36%)) than those on lower doses (80mg followed by 40mg 2 weekly (24%)).⁴⁹ Similarly, in the Pegylated Antibody Fragment Evaluation in Crohn's Disease: Safety and Efficacy (PRECISE)-2 trial⁵⁰ with certolizumab and A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen (ACCENT-1) trial with infliximab, higher doses of anti-TNF during the induction phase resulted in lower primary nonresponse rates.²⁴

The CALM study showed that escalation of therapy according to objective measures of inflammation; CRP and faecal calprotectin, was superior to clinical management alone with 46% achieving the primary endpoint of mucosal healing in the tight control arm than 30% in the clinical management group.^{9,51} These therapeutic strategy trials can help to demonstrate improved efficacy and decreased primary nonresponse rates for anti-TNF therapy when used in combination with thiopurines.

Combination Therapy

The SONIC (Study of biologic and Immunomodulator Naïve Patients in Crohn's disease) study indicated that infliximab and azathioprine have an additive effect on mucosal healing. Early immunosuppressive therapy with introduction of infliximab and azathioprine in patients who were treatment naïve showed that a higher proportion of patients on combination therapy achieved mucosal healing compared with those on monotherapy at week 26 (43.9% vs 30.1%; $p=0.06$).⁵²

Predictors of Primary Nonresponse

Clinical, endoscopic, biochemical, serologic, and radiologic predictors of primary nonresponse have been identified. Novel methods of detecting primary

nonresponse using pharmacogenomics, proteomics and metabonomics are currently under investigation.

1.3.4.6 Clinical

Patient Factors

Patient factors that have been found to predict response to anti-TNF therapy include: age, duration of disease, CD phenotype and smoking status.^{53,54} Of these clinical factors a study of anti-TNF naïve CD patients on infliximab recently established a matrix-based prediction tool with BMI, prior surgery and age identified as three factors for predicting primary nonresponse with an Area Under Curve (AUC) of 0.79.⁵⁵ However, its utility as a predictive tool is yet to be validated. Data from PRECISE-3 have recently demonstrated in 377 patients treated with certolizumab with a follow-up time of 7 years that older age, haematocrit, prior IBD surgery and entry HBI are all factors that are predictive of primary nonresponse in certolizimab-treated patients.⁵⁶

Age

Early age at diagnosis (<17 years) is associated with a worse prognosis.⁵⁷ However, younger patients are more likely to respond to anti-TNF drugs than older patients.⁵⁸ In certolizumab-treated patients, a multivariate analysis of the PRECISE-3 study showed that for every year of age, the probability of achieving primary response reduced [HR 0.989].⁵⁶ In a systematic review by Torres *et al.* disabling CD course, need for multiple surgeries and increased risk of intestinal failure were associated with young age at onset.⁵⁹

Disease Duration

The data pertaining to short-term (4 week) induction of remission, and the earliest post-induction results at week 20 to 30 were evaluated in a systematic review and meta-analysis on the efficacy of biological therapies.³¹ According to results of the Crohn's Trial of the Fully Human Antibody Adalimumab for Remission Maintenance (CHARM) trial which evaluated the maintenance of response and remission to adalimumab 40mg fortnightly, the week-26 rate of maintenance of remission was 56% in patients with disease duration less than 2 years, 35% in those with disease duration of 2 to 5 years, and 37% in those with disease longer than 5 years.²⁵ Similar findings were demonstrated in the PRECISE 2 study

evaluating response and remission with certolizumab pegol in CD, where 62% of patients were primary responders at week 26. However, in those with elevated CRP and a disease duration of less than 2 years, rates of response were 90% ($p=0.02$), suggesting that early use of an anti-TNF agent is a more successful strategy in such patients.⁵³ A subanalysis of the Crohn's patients treated with Adalimumab: Results of a safety and Efficacy (CARE) study which evaluated adalimumab in treatment-naïve patients showed a trend toward higher remission rates in CD patients with disease duration less than 2 years compared with more than 5 years.⁶⁰ A disease duration of less than 2 years therefore appears predictive of higher medium and longer-term remission rates than those with disease duration greater than 2 years. In the "A Randomized, multicenter, open-label study to Evaluate the safety and efficacy of Anti-TNF- α Chimeric monoclonal antibody in pediatric subjects with moderate to severe Crohn's disease (REACH)⁶¹ and D'Haens *et al.* "top-up versus step down"⁶² study, response rates were also improved in patients with shorter disease duration. The impact of a short disease duration on anti-TNF outcome was also observed in a post-hoc analysis of the SONIC trial which found that those with a disease duration ≤ 18 months after diagnosis who did not have fistulas and were anti-TNF naïve were more likely to achieve clinical remission, mucosal healing and CRP normalization with combination therapy compared with those with > 18 months of disease (64.7% vs 44.4%) as assessed at week 26.⁶³ Similar findings which associated short disease duration with improved outcome were observed in the EXTend the Safety and Efficacy of Adalimumab Through ENDoscopic Healing (EXTEND) study which found higher deep remission rates in patients with Crohn's disease for < 2 years compared with those > 2 years (33% vs 24%) as assessed at week 12.⁶⁴

Phenotype

Disease phenotype as defined by the Montreal classification may be associated with anti-TNF treatment response. A study of 201 patients on adalimumab with Crohn's disease demonstrated that patients with only luminal disease were likely to be primary responders and have sustained clinical remission to week 52 (OR, 3.89 95% CI, 1.43–10.6; $p=0.008$).⁶⁵ In the same study remission rates in patients with luminal and fistulising phenotype were numerically lower at each time-point

assessed compared to those with a luminal phenotype alone (week 12: 42.5% vs. 56.3%, $p=0.06$).⁶⁵

In addition, one of the strongest predictors of disabling course of CD is perianal disease.⁵⁹ The typical course for patients with perianal CD includes frequent relapses and long periods of active disease with draining fistulas. The cumulative risk of developing a perianal fistula is ~10% at 1 year and 20% at 10 years.⁶⁶ For the purposes of this review, the focus will be on luminal Crohn's disease.

Smoking

Smoking is the only environmental factor that has been associated with primary nonresponse. Smokers have been found to be 30% less likely to respond to infliximab at week 4.⁶⁷ Smoking was also found to be a predictor of nonresponse in adalimumab-treated patients in a study of 221 patients of whom 21.2% were smoking at induction (OR 0.52, $p=0.049$).⁶⁵

1.3.5 Therapeutic Drug Monitoring as a Predictor of Primary Nonresponse

Recent published guidelines by the American Gastroenterology Association (AGA) have advocated for the use of TDM in clinical practice with a reactive approach whereby patients not responding to anti-TNF should have TDM performed to determine the possible underlying cause. The measurement of anti-TNF and anti-drug antibodies level at week 12–14 may help determine adequacy of drug levels in the treatment of CD following induction therapy.^{68,69} Karmiris *et al.* recently demonstrated in a study of 168 CD patients on adalimumab that there is a trough level which correlates with mucosal healing which may be used to predict clinical response.⁷⁰ The median (interquartile range, IQR) adalimumab serum concentrations were 8.6 (IQR, 6.5–10.8) mcg/mL and 5.3 (IQR, 2.8–10.9) mcg/mL at weeks 2 and 4, respectively. In comparison with patients who received 80/40 mg, those who received 160/80 mg as a loading dose had higher adalimumab serum concentrations at week 4 (3.6 versus 11.6 mcg/mL; $p < 0.0001$) and had a lower incidence of primary nonresponse (odds ratio [OR] 0.02; 95% confidence interval (CI), 0.003–0.2; $p < 0.0001$). A recent follow-up exploratory study of 144/168 of the original patients, in the study by Karmiris *et al.*⁷⁰ with measurement of adalimumab concentrations and antibodies at week 4

showed that those with concomitant immunomodulator therapy at induction were less likely to develop antibody formation (HR 0.23; 95% CI 0.06–0.86; $p=0.029$).⁷¹ Furthermore, using a cut-off of 5mcg/ml, it was demonstrated that those with < 5mcg/ml at week 4 had an increased future risk of antibodies to adalimumab (ATA) formation (HR: 25.12; 95% CI 5.64–111.91; $p=0.0002$).

A separate prospective study of 32 CD patients (15 treated with infliximab, 17 with adalimumab) after induction showed that the median trough concentration of anti-TNF drug was observed to be higher in responders (infliximab 5.60 mcg/mL and adalimumab 9.07 mcg/mL) compared with non-responders as determined by the Harvey–Bradshaw Index, CRP or faecal calprotectin concentration (infliximab 0.032 mcg/mL and adalimumab 2.62 mcg/mL; $p=0.01$) at week 14.⁴⁴ The positive predictive value of high trough concentration (infliximab >3 mcg/mL and adalimumab > 4.5 mcg/mL) at week 6 for predicting response and remission after anti-TNF induction was >90%. Sustained anti-drug antibody concentrations were detected in 26% of infliximab-treated patients and 0% of adalimumab-treated patients, respectively at week 6.

Taken together these data suggest that adequate anti-TNF at weeks 4 to 6 may predict response to anti-TNF therapy whereas low anti-TNF level with antibody formation predicts primary nonresponse at week 14.

1.3.6 Endoscopic Factors

Despite mucosal healing having become an important outcome measure in clinical trials there are no specific endoscopic features other than stricturing disease (as previously described) that are associated with primary nonresponse to anti-TNF therapy.⁷²

1.3.7 Histologic Factors

There is currently no validated scoring system for evaluating Crohn's disease other than the Crohn's disease: Colonic and Ileal Global Histological Activity Score (CGHAS)⁷³ which was developed in a post-operative group of patients with a histologic grading of severity. However, none of these histologic variables have been assessed for predicting primary nonresponse.⁷⁴

1.3.8 Faecal Inflammatory Markers

Faecal inflammatory markers faecal lactoferrin and faecal calprotectin are surrogate markers of luminal disease activity which have been found to predict clinical response to anti-TNF therapy. In a cohort of 5 patients with severe CD, Buderus *et al.* demonstrated that a falling faecal lactoferrin after one week of therapy was associated with clinical response to initial infliximab therapy.⁷⁵

1.3.9 Biochemical Markers

It is unclear whether pre-treatment CRP is predictive of response to anti-TNF therapy. In the ACCENT 1 study, high baseline CRP ($>0.7\text{mg/dl}$) predicted increased response to infliximab therapy at week 14 and maintained remission to 1 year.⁷⁶ A study conducted by Louis *et al.* found similarly that those with higher CRP before treatment were more likely to respond to infliximab therapy.⁷⁷ Of 73.2% responders and 26.8% non-responders, pre-treatment CRP level was significantly higher in responders than in non-responders (16.8 mg/l (5–160) versus 9.6 mg/l (5–143); $p=0.02$) at week 4. Furthermore, response rates were significantly higher in patients with elevated CRP ($>5\text{ mg/l}$) than in patients with a normal CRP value ($<5\text{ mg/l}$) before treatment [76% versus 46%; $p=0.004$; OR: 0.26 (0.11–0.63)].

However, not all patients are known to have elevated CRP as part of their disease course, consequently use of this measure is not reliable in all patients.^{78,79} Among patients in clinical remission (CDAI <150) but with endoscopically active disease a single retrospective study found that CRP remained normal in 60% of cases.⁸⁰ Further studies are therefore required to evaluate the role of pre-treatment CRP in predicting primary response.

1.3.10 Serological Antibodies

Antibodies to microbial antigens may be useful in predicting response to anti-TNF therapy. Early studies using pANCA (Anti-neutrophil cytoplasmic antibody) and ASCA (anti-saccharomyces cerevisiae antibody) demonstrated a non-significant trend toward lower response rates to infliximab with the pANCA-positive/ ASCA-negative combination in CD at week 14.⁸¹ In contrast speckled ANCA (sANCA) was associated with a greater response to anti-TNF compared with pANCA or cytoplasmic (cANCA).⁸² However, a study with 74 CD patients treated with

infliximab was unable to confirm the latter association between serotype and response to infliximab.⁶⁷

Despite data supporting their value in predicting response to anti-TNF therapy, serologic markers have not been used widely as they are not sufficiently predictive of response when viewed in isolation.⁸³ Serologic markers are likely to be of greater utility when applied as part of a predictive model with clinical and other predictive factors.

1.3.11 Radiology

There are no data to demonstrate that radiologic findings are predictive of primary nonresponse to anti-TNF therapy. However, in clinical practice the identification of strictures is known to be associated with a lack of response to anti-TNF therapy.^{32,84} Moreover, cross-sectional imaging is important to determine contraindications to anti-TNF therapy such as collections complicating penetrating disease.

1.3.11.1 Stricturing Phenotype

A recent published study by Panes *et al.*⁸⁵ demonstrated that the presence of stenosis on MRI doubled the risk of subsequent surgical resection, while the presence of intra-abdominal fistulae increased the risk of resectional surgery four-fold despite the use of anti-TNF therapy. Thus, in addition to monitoring treatment response to anti-TNF, MRI provides valuable prognostic information regarding the need for surgical resection in patients with CD.

1.3.12 Experimental Techniques

At present, pharmacogenomics, proteomics, transcriptomics and metabonomic predictors of primary nonresponse are in the investigational phase of development and cannot be applied to clinical practice as they require further large-scale longitudinal studies.⁸⁶ There has been recent impetus to develop a System Dynamics Analysis (SDA) to address the complex interactions between patient, disease, drug and treatment related factors in order to devise predictive models that will help individualise therapy in the future.⁸⁷

1.3.12.1 Pharmacogenetics

Pharmacogenomics provides a means to investigate the influence of genetic variation on drug efficacy or toxicity by assessing differences in gene expression. Using targeted studies for sites of genetic polymorphisms such as in the TNF-receptor and apoptosis genes, prediction of primary response may be possible.

TNF-receptor Polymorphisms

TNF alpha and TNF-receptor polymorphisms may predict response to infliximab.^{82,88} In a study by Taylor *et al.*⁸² individuals homozygous for the TNF-alpha/ lymphotoxin A (LTA) polymorphism were identified as nonresponders to infliximab. A subsequent study in a Belgian cohort demonstrated an association between TNFR1A36 G polymorphism and response to infliximab with a lower rate of response in those carrying the G allele.⁸⁸ Neither of these associations have been replicated in subsequent cohorts.^{77,89} Louis *et al.* initially identified an association between a polymorphism in the Fc receptor III a (FcYRIIIa),⁹⁰ a mediator of antibody-mediated cell cytotoxicity and nonresponse to infliximab, but a subsequent analysis in the ACCENT I trial failed to replicate this relationship.⁹¹

Several authors have investigated the potential role of NOD2 polymorphisms and response to infliximab and have failed to identify a correlation.^{92,93} In contrast, the homozygous variant of the IBD5 locus (5q31) was found to be associated with a 3-fold increase in likelihood of nonresponse to infliximab at week 4 in luminal disease and at week 12 for fistulising disease.⁹⁴

In a more recent study of 474 patients with IBD treated with anti-TNF therapy, that sought to identify and validate clinical and genetic factors that predict anti-TNF response, 2 loci (rs116724455 in TNFSF4/18, rs2228416 in PLIN2) were found to be associated with PNR. Using a combination of clinical and genetic predictors a preliminary anti-TNF refractory score was constructed to help differentiate anti-TNF nonresponders (mean [SD] score, 5.49 [0.99]) from responders (2.65 [0.39]; $p=4.33E-23$).⁹⁵ These preliminary data, which require prospective validation, suggest that the combination of clinical and genetic predictors may be useful in the future to help develop predictive models to differentiate anti-TNF responders from non-responders.

Apoptosis Genes

In a study by Hlavaty *et al.* 287 consecutive patients on infliximab with refractory luminal or fistulising CD were genotyped and it was found that Fas-ligand TT genotype and caspase-9 CC/CT genotypes were associated with lower rates of response to infliximab.⁹⁶ In a subsequent study, the same authors developed an apoptotic pharmacogenetic index (API) that ranged from 0 (low apoptotic response) to 3 (high apoptotic response). Response in both luminal and fistulising CD increased with increasing apoptosis scores; individuals with an API <1 had remission rates of 39.5% and 28.6% for luminal and fistulizing CD, respectively, compared with 56.1% and 44.9% in those with an API of 2.⁹⁷ Further genome-wide association studies will be important in better understanding these polymorphisms and their impact on anti-TNF response.⁹⁸

Transcriptomics

Several studies have investigated the utility of gene-expression profiling to predict response to infliximab in both RA and IBD^{86,99} but from these studies, there were no specific transcriptomic profiles distinguishing anti-TNF responders from non-responders in CD have been identified.

Recent work by Gaujoux *et al.* has demonstrated that cell-centred meta-analysis of immune pathways, can be used as a predictor of anti-TNF response in patients with IBD.¹⁰⁰ In this study, pre-treatment plasma was used with good accuracy with an AUC of 82%. There was upregulation of the triggering receptor expressed on myeloid cells 1 (TREM-1) and chemokine receptor type 2 (CCR2)-chemokine ligand 7 (CCL7).

Microbiome

There is growing interest in the use of microbial signatures to predict response to biologic therapy. The use of 16s rRNA and subsequently metagenomics on faecal microbiota has revealed microbial signatures which can be used to classify patients according to their likelihood to respond to specific drugs. Although data linking microbial signatures to anti-TNF response are lacking the newer biologics; anti-integrins and anti-p40 have both been found to be associated with microbial signatures that are predictive of response. A study of 85 patients with IBD (43 UC, 42 CD) with moderate to severe disease activity requiring vedolizumab (anti-

integrin) identified specific metagenomic pathways which were linked to therapeutic responsiveness in patients with Crohn's disease. There were higher abundance of butyrate producers at baseline and 13 microbial pathways at baseline were linked with response at week 14.¹⁰¹ During the phase 2 CERTIFI study for ustekinumab for Crohn's disease, stool biobanking allowed for 16s rRNA analysis at multiple timepoints. Patients who were anti-TNF refractory underwent separate analysis which determined that specific operational taxonomic units (OTUs) were identified which included faecalibacterium and Escherichia or Shigella. Two OTUs with faecalibacterium ($p=0.003$) and Bacteroides ($p=0.022$) in particular were more abundant at baseline in subjects who were in remission at 6 weeks after treatment than those with ongoing active CD. It was also noted that the microbial diversity of clinical responders increased over the 22 weeks of the study compared with those who were nonresponsive ($p=0.012$).¹⁰² These preliminary data suggest that the use of faecal microbial analysis may help to better classify patients into responders and non-responders and perhaps determine the various mechanisms by which patients may, or may not, respond to drug therapy.

Metabonomics

There are currently no published data on metabonomic predictors of response to anti-TNF therapy.

1.3.13 Failure at Induction of Second Anti-TNF

In the CHARM and PRECISE-2 trials, 42% and 36% of patients respectively had a primary nonresponse to open-label adalimumab and certolizumab induction therapy respectively.^{25,50} A factor contributing to the primary lack of response in the adalimumab and certolizumab studies relates to previous exposure and loss of response to infliximab, which was observed in approximately 50% of the patients – a clinical situation which has been referred to as primary secondary loss of response.

In the case of failure to respond to two previous anti-TNF agents, there is evidence to suggest that a third anti-TNF may be of benefit. A retrospective review of 57 patients who had failed two prior anti-TNF agents and were commenced on a third anti-TNF (certolizumab in 85%) demonstrated the ability

to recapture response with a decrease in HBI from 9.6 to 6.4 ($p < 0.005$) after initiation in 60% of patients. The same study found that the probability of remaining on the third anti-TNF agent decreased over time from 0.69, 0.55, 0.37 and 0.25 at 6, 12, 24 and 36 months respectively.¹⁰³ Similar rates of anti-TNF discontinuation were found in a separate group of 67 patients, of whom 61% had demonstrated an initial response at week 6. In the same group of patients the rate of response to a third anti-TNF decreased to 45% at 9 months.¹⁰⁴

1.3.14 Secondary Loss of Response

1.3.14.1 Definition

Loss of response refers to the clinical situation that arises when a patient has an initial response to a biological drug, which is followed by a diminished or less durable response over time. Secondary loss of response has variously been defined as: an increase in CDAI of more than 70 points^{25,26} from the pre-induction score combined with a total score of greater than 175; an increase in CDAI of $\geq 35\%$ from the baseline score, or alternatively the need to introduce a new treatment for active CD.⁶

Two key clinical features which are consistent in secondary loss of response are: 1) the re-emergence of symptoms in a patient whose disease was previously controlled by induction treatment, and; 2) that the cause of symptoms is due to an inflammatory process attributable to the underlying IBD.¹⁰⁵ In situations where the cause of symptoms is considered unrelated to IBD (e.g. irritable bowel syndrome and infection) or related to non-inflammatory attributes of IBD (e.g. fibrostenotic stricture and bile-salt diarrhea), patients are deemed to be worsening with the use of anti-TNF, rather than thought to be losing response to these drugs.²¹ The true magnitude of secondary loss of response has been difficult to establish.

1.3.14.2 Incidence

Infliximab

In the ACCENT 1 study the median duration of response to a single infusion of infliximab was 19 weeks (interquartile range 10–45 weeks). In patients who initially responded to pharmacotherapy, the median time to loss of response to maintenance infliximab therapy was 46 weeks (interquartile range, 17 to >54

weeks).²⁴ Approximately one-third of patients on maintenance treatment lost response to infliximab over the first year of treatment.^{24,26,61} The proportion of patients with loss of response in clinical practice has been lower than in clinical trials. In a large series of 614 infliximab-treated patients with long-term follow-up, about 60% of patients had sustained benefit, with 20% losing response to infliximab at a median follow-up of 5 years.¹⁰⁶

More recently the frequency of loss of response to infliximab (defined as the need for an increased infliximab dose or a decreased interval between doses) was reviewed across 16 studies in which the majority of patients had received induction followed by regular maintenance therapy. The mean percentage of patients who lost response to infliximab was 37%; however, the duration of follow-up varied between studies. When expressed as incidence per patient-year follow-up, the annual risk of loss of infliximab response has been estimated to be 13% per patient-year.¹⁰⁷

Adalimumab

A meta-analysis exploring the extent of secondary loss of response with adalimumab use has demonstrated an annual risk of 20.3% per patient/year. A minority (37%) of patients treated with adalimumab require dose intensification, with 71.4% of these patients regaining remission after one year follow-up.²²

1.3.14.3 Mechanisms Underlying Secondary Loss of Response

The mechanisms underlying secondary loss of response are also thought to be multifactorial and include disease characteristics (phenotype, location, severity); drug (pharmacokinetics, pharmacodynamic, immunogenicity) and treatment strategy (dosing regimen) related factors.

1.3.14.4 Drug Factors

Pharmacokinetic and Pharmacodynamic Failure

The mechanisms underlying secondary loss of response related to pharmacokinetic and pharmacodynamic failure are the same as for primary nonresponse.

Immunogenicity Failure

Immunogenicity is a common cause of loss of response in patients due to the formation of antibodies directed against the drug. The use of infliximab and adalimumab has been known to elicit the formation of antibodies, directed against the F(ab)₂ fragment of the molecule.¹⁰⁸ Anti-drug antibodies may directly neutralize the biologic activity of anti-TNF by interfering with its binding to the TNF molecule, or hastening the clearance of the drug via the formation of immune complexes that are eliminated by the reticuloendothelial system.¹⁰⁹ The formation of anti-drug antibodies is associated with lower serum drug levels,^{108,110} shortened response to anti-TNF and increased rates of loss of response.^{43,111,112}

Loss of response to TNF antagonists due to development of neutralising anti-drug antibodies and subtherapeutic drug concentrations, is a challenging problem in the management of patients with IBD and is seen commonly in secondary loss of response.

Infliximab

The incidence of immunogenicity has been very high in episodic treatment (36–61%) and significantly lower in scheduled maintenance regimens (5–18%).¹¹³

Adalimumab

The presence of antibodies to adalimumab has not been commonly reported. Karmiris *et al.* found that 9.2% of CD patients receiving adalimumab developed antibodies which were associated with lower trough levels and higher rates of loss of response (82%).⁷⁰

Certolizumab

The PRECISE 2 study using certolizumab showed that 9% of CD patients developed antibodies after the induction phase but no comment on clinical loss of response was made.⁵⁰

It has been shown that antibodies developed against one of the anti-TNF agents does not affect the bioavailability of another anti-TNF drug;¹¹⁴ attempts should therefore be made at recapturing response with dose escalation or switching to a different anti-TNF drug.¹¹²

Dosing Regimen

A higher adalimumab induction regimen of 160/80mg significantly reduces the risk of loss of response compared with lower doses (HR 0.48, $p < 0.0001$).¹¹⁵ This was similarly shown in the CLASSIC (Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in CD) study.⁴⁹ It is also well described that episodic treatment of infliximab resulted in higher rates of secondary loss of response due to development of antibodies to infliximab.¹¹³

Combination Therapy

CD patients with an inflammatory phenotype have been demonstrated to have a longer duration of response on combination therapy with azathioprine or 6-mercaptopurine than those with a non-inflammatory phenotype. In a study involving 27 patients with inflammatory CD and 35 patients with perianal fistulising CD a longer duration of response was observed in CD patients with an inflammatory phenotype on concurrent immunomodulators.¹¹⁶ The differences in the duration of response between the 2 groups did not reach statistical significance, most likely due to the small sample size. However, a trend towards significance was seen in a separate study of 52 patients with CD on infliximab amongst whom a mean duration of response of 18 weeks was observed in patients on concurrent immunomodulators compared with 9 weeks for those not on immunomodulators ($p < 0.05$).¹¹⁷ A further study of 104 out of 117 patients (89%) on concomitant immunosuppressive therapy with infliximab showed that those receiving combination therapy had a longer duration of clinical response (97 weeks vs 68.5 weeks, $p = 0.25$) with a lower loss of response rate (29.8% vs 38.5%, $p = 0.36$) although this was not statistically significant.¹¹⁸

1.3.14.5 Predictors of Secondary Loss of Response to Anti-TNF Therapy

Factors influencing patient's duration of response to anti-TNF include patient factors (gender, smoking status, weight); disease characteristics (type, location, severity) and drug (pharmacokinetics, pharmacodynamics, immunogenicity) factors.

1.3.14.6 Clinical

Patient Factors

Gender

Data regarding the impact of gender on secondary loss of response are conflicting. Male gender was shown to be a risk factor for the need for dose escalation in 31 patients treated with adalimumab for CD with the time to dose escalation being shorter at 12.1 weeks versus 36.4 weeks for females ($p=0.0012$).¹¹⁹ In contrast gender was not a significant factor in clinical loss of response in a separate single-centre study of 210 patients with luminal or fistulising disease.²⁷

Smoking Status

Smoking reduces the efficacy of anti-TNF drugs and increases the risk of loss of response.^{119,120,121} Smokers exposed to adalimumab were more likely to lose response to therapy compared with non-smokers and had a shorter duration of response of 15.8 versus 36.3 weeks ($p=0.013$).¹¹⁹ In a recent study of 30 patients treated with adalimumab, smokers were significantly less likely to respond to treatment with a strong correlation between the number of cigarettes/day and rates of loss of response.¹²⁰ Smokers also had a shorter duration of response (1.8 months) compared with non-smokers (4.5 months).¹²² Similarly data from PRECISE-3 have indicated that smoking status was associated with a greater likelihood of active disease at any time point compared to former smokers or nonsmokers ($HR=1.404$; 95% CI 1.09–1.77 $p=0.007$).⁵⁶

Weight

Raised BMI has been found to significantly increase loss of response rates to anti-TNF therapy.^{123,124} A retrospective single-centre study which stratified patients with CD according to BMI showed that patients classified as obese ($BMI > 30 \text{ kg/m}^2$) were more likely to have an IBD flare than non-obese patients on infliximab.¹²¹ Higher BMI has also been identified as a predictor for loss of response in a separate study of 199 Crohn's patients of whom 38% treated with adalimumab required dose escalation within a median time of 21 weeks.¹²⁴

Whilst a mechanism for increased BMI is not completely understood, in a study of 73 patients of varying disease severity, a higher infliximab clearance was found amongst patients who had a higher BMI.¹²⁵

Disease Characteristics

Longer Duration of Disease and Higher CDAI

In a sub analysis of the CHARM trial, significant predictors of loss of response were longer disease duration and a greater baseline Crohn's Disease Activity Index in week 12 responders.¹²⁶ In a univariate analysis of PRECISE-3 higher baseline HBI scores were associated with a shorter time to loss of response [HR;1.08;95% CI 1.04–1.11, $p<0.0001$].⁵⁶

Disease Location and Activity

Cohen *et al.* found that isolated colonic CD was associated with a shorter time to dose escalation.¹¹⁹ Other studies have not shown any relationship between disease location and loss of response or dose escalation.¹²⁷

Phenotype

Disease phenotype plays a role in predicting loss of response. An inflammatory phenotype has been associated with better rates of response and sustained clinical benefit with a HR 0.55 ($p=0.03$) at 24 months.^{9,27} There was a significantly lower likelihood of failure to achieve sustained clinical benefit to infliximab in patients with an inflammatory phenotype ($p=0.04$).

Few studies have evaluated the efficacy of anti-TNF in patients with symptomatic strictures. A study by Naik *et al.* of 199 infliximab and 136 adalimumab patients found that a stricturing phenotype was associated with lower dose escalation (OR) = 0.18; 95% CI, 0.05–0.62) in a combined cohort of anti-TNF therapy.¹²⁸ In a retrospective study of 18 symptomatic patients treated with infliximab for symptomatic small bowel strictures after failure of conventional treatment, complete response, partial response, and failure were observed in 10, 7, and 1 patients respectively, at 8 weeks. At a median follow-up of 18 months, there were five patients with complete response, ten with partial response, and three patients failed therapy.¹²⁹ Together these studies suggest that the use of infliximab may be attempted in patients with symptomatic Crohn's related strictures. The risk of

secondary loss of response in such patients is likely to depend on the extent to which the stricture is inflammatory versus fibrostenotic.

1.3.15 Therapeutic Drug Monitoring as a Predictor of Secondary Loss of Response

1.3.15.1 High Trough Levels Versus Development of Undetectable Trough Levels

Several studies have demonstrated superior outcomes in patients with higher trough drug concentrations.^{130,131} In a study of 105 infliximab treated CD patients, the rates of clinical remission were higher for patients with detectable trough serum infliximab compared with patients in whom serum infliximab was undetectable, including those without antibodies (82% vs 6%; $p<.001$).¹³¹ In support of these data a post-hoc analysis of ACCENT-1 published recently identified that an infliximab trough level at week 14 $\geq 3.5\text{mcg/ml}$ and a $\geq 60\%$ CRP decrease in patients with a baseline CRP $> 8.0\text{mg/l}$, were predictive of sustained remission at week 54 with an OR of 3.5 and 7.3 respectively.⁶⁹ Therapeutic drug monitoring may possibly be used as a predictor of secondary loss of response in patients who develop undetectable trough levels.

1.3.15.2 Effect of Anti-Drug Antibody Status on Trough Levels

The effect of antibodies to infliximab status on serum IFX levels has been reported in one study of patients with CD ($n=58$), and two studies that pooled patients with UC and CD together ($n=185$).^{108,132,133} Significantly lower trough serum IFX levels were present in patients with detectable antibodies to infliximab compared with patients with undetectable antibodies to infliximab as confirmed in a subsequent meta-analysis (HR -0.8, 95% CI: -1.2, -0.4, $p<0.0001$).¹³⁴

Serum adalimumab levels and antibodies have also been found to correlate with clinical response; a level of $> 5.85\text{ mcg/L}$ was associated with response with AUC of 0.748 $p<0.001$ in 71 CD patients treated with adalimumab.¹³⁵ Antibodies were also correlated with lower adalimumab levels and positively correlated with disease activity $p<0.001$.

1.3.15.3 Low Trough Levels with Absent Anti-Drug antibodies

The scenario of low infliximab levels with absent antibodies to infliximab is seen in 10–60% of patients with loss of response to infliximab.^{108,130,131,136} Low IFX with

absent antibodies to infliximab may occur due to: non-immune clearance of anti-TNF; consumption of anti-TNF in the setting of highly active disease; false negative results from ELISA assays; incorrect timing of measurements, and; missed anti-TNF or sampling when all antidrug antibodies are cleared.^{108,130,131,136} The inability to detect antibodies to infliximab in patients with otherwise unexplained low infliximab level in serum may be mostly due to sampling within a window period during which all antibodies to infliximab are bound to the drug and are cleared from the circulation.^{130,131} As such, if the next dose of anti-TNF is not administered, a rising titre of antibodies to infliximab will become measurable. Non-adherence or delayed drug dosing of anti-TNF causing low drug level in the absence of antidrug antibodies, should also be considered in patients, especially those who receive their medication at home or at a location separate to where clinical follow-up takes place.¹¹³

1.3.15.4 Clinically-Guided Dose Escalation

Therapeutic drug monitoring has been shown to be useful in directing treatment strategy toward anti-TNF therapy. However, a clinically guided approach to loss of response with dose-intensification may be sufficient to recapture response. In a cohort of seventy-six patients, 39 had dose intensification of infliximab with 27 (69%) able to recapture response.¹³² There was no significant difference between the infliximab trough levels of the responders versus non-responders (3.3 +/- 4.1 mcg/mL vs 2.3 +/- 2.2 mcg/mL, $p=0.85$). Six out of ten (60%) patients with antibodies to infliximab recaptured response with dose intensification. The Trough Concentration Adapted Infliximab Treatment (TAXIT) randomized controlled trial comparing clinically based dosing versus trough concentration monitoring with primary end points defined as patients in clinical (HBI ≤ 4 for CD) and biological (CRP ≤ 5 mg/L) remission after 1 year post-optimisation.¹³⁷ Secondary end-points were durable remission, relapse (need for infliximab dose escalation, steroid use or switch to other agent), antibodies to infliximab positivity, cost-analysis and quality of life. TAXIT found that targeting therapeutic trough concentrations of 3–7 mcg/ml and a strategy of dose intensification in the case of low antibodies to infliximab and undetectable (<0.3 mcg/ml) trough concentration, showed no significant difference in remission rates.¹³⁷ Furthermore, dose escalation resulted in a higher proportion of CD patients in remission than prior

to dose escalation (88% vs 65%; $p=0.020$) and a decrease in median CRP compared with before dose increase (3.2 vs 4.3 mg/L; $p<0.001$). However, more flares were detected in those with clinically based dosing (17%) versus 7% in those with therapeutic drug monitoring ($p=0.018$). Taken together, these studies suggest that clinical parameters of HBI and biochemistry may be adequate in determining the need for dose escalation and that this may recapture response in the majority of patients irrespective of their anti-drug antibody status.

1.3.15.5 High Trough Levels and Possible De-Escalation

The only data on de-escalation in the case of supra-therapeutic trough levels from TAXIT, suggest that de-escalating did not result in negative clinical outcomes at one year follow-up, which was shown when 26.2% of patients at baseline, having infliximab TLs $> 7 \mu\text{g/ml}$ underwent dose reduction (to minimum 5 mg/kg) or increase of interval between infusions (by 2 weeks to maximum 12 weeks).¹³⁷ De-escalation resulted in 28% reduction in drug cost as a result of drug dose reduction ($p<0.001$).

1.3.16 Endoscopic

Mucosal healing in response to anti-TNF therapy has been associated with improved outcomes of CD and is predictive of long-term remission rates.¹³⁸ In the long term follow-up of 49/133 patients in the 'step up/top down' study,⁶² patients who achieved complete mucosal healing (identified as the absence of ulcers, SES-CD=0) at 2 years with anti-TNF therapy, had a higher rate of sustained steroid-free remission, over the course of years 3 and 4 compared with those with lesions detected on endoscopy (71.0% vs 27.3%, $p=0.036$).¹³⁹ A separate study found that a SES-CD <2 was associated with a cumulative non-relapse rate of 88% as assessed by a follow-up colonoscopy 12 months later.¹⁴⁰ Similarly, a study by Bjorkesten *et al.* of 71 patients demonstrated that mucosal healing found at colonoscopy in 45% of patients, 3 months after the induction therapy with infliximab, and was predictive of ongoing response at 12 months in 90% of patients.¹⁴¹ Taken together, these data suggest that depth of remission determined endoscopically can be used to predict which patients will maintain remission in the longer term and conversely which patients may be at risk of loss of response.

1.3.17 Faecal Inflammatory Markers

Faecal inflammatory markers, faecal lactoferrin and faecal calprotectin are biomarkers of luminal inflammatory disease activity which reflect clinical response to anti-TNF therapy and may predict sustained clinical remission/secondary loss of response.^{142,143,107} Molander *et al.*¹⁴⁴ found that faecal calprotectin of < 100 mg/g after induction therapy with anti-TNF in 34 patients with active CD predicted sustained clinical remission (defined as HBI < 4) in most patients at week 52. These results conflict with the findings of Laharie *et al.*¹⁴⁵ who could not demonstrate a relationship between faecal calprotectin 14 weeks after commencing infliximab and clinical relapse defined as CDAI >150 in 65 patients with CD. It is likely that these inconsistencies relate to the subjective nature of these clinical indices (CDAI, HBI) and their poor ability to correlate with mucosal disease.

1.3.18 Biochemical

1.3.18.1 CRP, ANA, Anti-dsDNA

Normalization of CRP (< 10mg/L) at week 12 has been found to be predictive of improved endoscopic score and mucosal healing at week 52, suggesting that CRP at week 12 can be used as an independent predictor of endoscopic response to therapy at week 52 in patients on adalimumab.⁶⁵ A lack of CRP normalisation has consistently been identified as an indicator of likely loss of response as demonstrated in a follow-up study of ACCENT 1 in patients treated with infliximab.^{76,78} Patients exposed to anti-TNF induced products of monocyte apoptosis in the setting of a low CRP may subsequently form antibodies such as ANA and anti-dsDNA antibodies to these antigens, which when in abundance may be associated with loss of response.¹⁴⁶ An increase in CRP occurring at the time of a decrease in infliximab level has been found to be predictive of subsequent loss of response.⁷⁸

1.3.19 Radiologic

Anti-TNF has been demonstrated to accelerate healing and precipitate stricture development in some patients which may lead to secondary loss of response.¹⁴⁷ Clinical suspicion of sub-acute bowel obstruction following anti-TNF administration warrants cross-sectional imaging to exclude a stricture associated

with pre-stenotic dilatation, which is a known cause of secondary loss of response. Cross sectional imaging may also be used to monitor response to anti-TNF and evaluate the depth of remission. Persistence of mucosal inflammation detected radiographically despite prolonged anti-TNF therapy may signal secondary loss of response.¹⁴⁸

1.3.20 Pharmacogenetic

The most promising pharmacogenetic studies on infliximab in CD have adopted a candidate gene approach, focusing primarily on variants that have the potential to influence monocyte and T-cell apoptosis, or the expression, metabolism and signal transduction of TNF.^{149,150} Data on the association of the SNP *TNFRSF1A* 36A>G (Pro12Pro) with infliximab response have been conflicting. Pierik *et al.*¹⁵¹ found that CD patients who were heterozygous or homozygous for the *TNFRSF1A* 36G allele were less likely to have a biological response (decrease in C-reactive protein) to infliximab compared to patients without this allele (p=0.034, OR = 0.47, 95% CI: 0.23–0.95). However, a subsequent study by Mascheretti *et al.*⁸⁹ comprising two independent CD cohorts, did not show any evidence of association of *TNFRSF1A* 36AG genotype with clinical or biological response to infliximab. The influence of C-reactive protein (CRP),¹⁵² ATP-Binding Cassette Subfamily B Member 1 (ABCB1)¹⁵³ and Nucleotide-binding Oligomerization Domain Protein 2 (NOD2) polymorphisms⁹² on infliximab response, and influence of IgG1 heavy chain polymorphisms on development of antibodies to infliximab,¹⁵⁴ have also been investigated but no associations have been reported.

1.3.21 Metabonomic

Metabolomics has not yet identified biomarkers that are predictive of secondary loss of response. However, a recent study of urinary metabolomics from 16 RA and 20 psoriatic arthritis (PsA) patients treated with infliximab or etanercept, demonstrated with 88.9% sensitivity and 85.7% specificity that several metabolites (histamine, glutamine, xanthurenic acid and ethanolamine) at baseline could predict response at 12 months.⁹⁹

1.3.22 Strategies to Recapture Response

Depending on the reason for loss of response, tailored therapeutic strategies available to recapture response in patients with worsening inflammatory disease despite anti-TNF using therapeutic drug monitoring include dose escalation, addition of an immunomodulator or switching anti-TNF.

1.3.22.1 Dose Escalation

In CHARM²⁵ and CLASSIC II,¹⁵⁵ approximately 50–60% of patients requiring dose escalation to weekly adalimumab therapy were able to regain clinical response. In real-life cohort experiences, the response to adalimumab dose escalation varies significantly, ranging from 36% to 86%.^{45,156,157} In a cohort of 547 initial responders to infliximab from Leuven, Belgium, 50% required at least one intervention to maintain response.¹⁰⁶ Interventions included reduction in dose interval to 6 weekly, increased dose to 10 mg/kg and re-induction with infusions at 0, 2 and 6 weeks, and a combination of dose escalation and shortening of dose interval in a small number of patients (3.8%). Overall, 103 of the 144 patients (71.5%) with an increase in dose and/or a re-induction with infliximab were able to return to a standard dose of 5 mg/kg, and 61.9% of patients (13/21) with an increase of dose and a shortening of the interval were able to return to 5 mg/kg and treatment every 8 weeks. A total of 28.7% of patients with a shortened interval between the infusions (31/108) could again extend the interval to 8 weeks. In the total cohort of initial responders, only 21.6% (n=118) had to stop IFX because of loss of response, despite interventions. When considered together these data suggest that dose escalation or shortening of the dose interval is an effective strategy in a majority of patients.

1.3.22.2 Addition of an Immunomodulator

The addition of an immunomodulator to patients losing response to anti-TNF monotherapy has been demonstrated to successfully restore response in patients who have previously failed azathioprine therapy.^{158,159} A study by Ben-Horin *et al.* demonstrated that the addition of an immunomodulators to 5 patients who developed antibodies to infliximab restored response to the drug via elimination of the antibodies and increase in anti-TNF trough levels.¹⁶⁰

1.3.22.3 Switching Anti-TNF

The Gauging Adalimumab Efficacy in Infliximab Non-Responders (GAIN) trial¹⁶¹ found that adalimumab (160mg at week 0 followed by 80mg at week 2) induced remission at week 3 in 21% of infliximab non-responders with moderate-to-severe CD compared with 7% on placebo ($p < 0.001$). A recent systematic review by Gisbert *et al.* demonstrated pooled remission rates of 62% when a patient had secondary loss of response.⁴⁷ These data suggest that switching to an alternative anti-TNF agent in the setting of secondary response is a feasible strategy to recapture response.

1.3.23 Systematic Approach to the Management of Primary Nonresponse and Secondary Loss of Response

Current strategies in the management of primary nonresponse and secondary loss of response should be aimed at preventing the development of loss of response. If prevention is unsuccessful and loss of response emerges, recognition and management of the scenarios of pharmacokinetic, pharmacodynamic and immunogenic failure should be undertaken.

1.3.23.1 Approach to Patient with Symptom Deterioration Despite Anti-TNF therapy

Eliminating non-inflammatory causes or non-TNF processes as the driver of symptoms is the first step in the assessment of patients who are losing response to treatment (Table 2). Loss of response to anti-TNF can only be confirmed after diagnostic efforts have proven that ongoing symptoms stem from uncontrolled inflammatory activity despite anti-TNF treatment. In particular fibrostenotic disease, abscess, sporadic malignancy, small intestinal bacterial overgrowth, bile salt malabsorption, intercurrent infections such as *Clostridium difficile*, or opportunistic infections such as Cytomegalovirus (CMV) all need to be excluded and managed on their own merits (Figure 2).

Table 2. Noninflammatory causes of anti-TNF failure.

Non-inflammatory mechanisms
Irritable bowel syndrome
Fibrotic strictures
Cancer
Dietary
Miscellaneous (bacterial overgrowth, bile salt diarrhea)

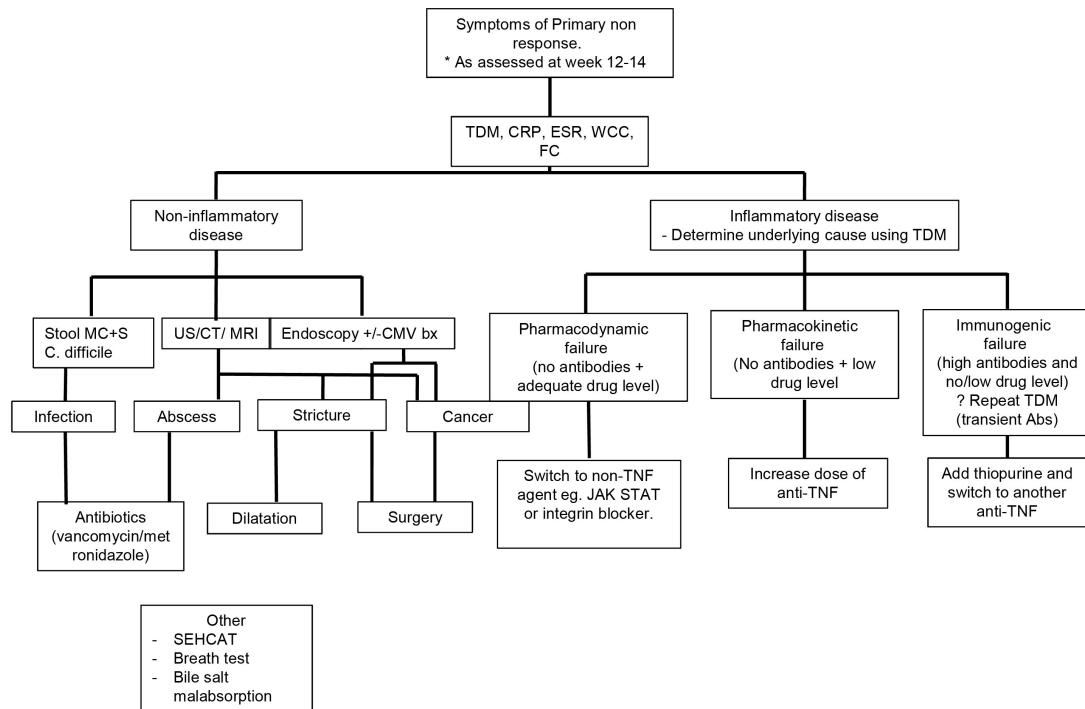


Figure 2. Proposed management algorithm for primary nonresponse to anti-TNF therapy in Crohn's disease.

Once non-inflammatory causes of a patient's symptoms are ruled out, unless precluded by the severity of symptoms, a 'watchful waiting' approach may be warranted. Such an approach is based on the premise that between 41% and 72% of trial patients designated as 'loss of response' while on blinded active-drug arm of anti-TNFs responded when transferred to open-label administration of the same dose,¹²⁶ suggesting that many patients with clinical worsening may improve in clinical practice without the need for intervention.

1.3.24 Prevention of Primary Nonresponse and Secondary Loss of Response

Preventive strategies to avoid primary and secondary loss of response include screening and counselling patients against smoking.

Treatment early in disease course (within 2 years of disease onset) when disease phenotype is inflammatory in nature using intensive combination therapy with an immunomodulator and anti-TNF may reduce the risk of primary nonresponse and secondary loss of response. In such patients, optimization of dosing regimen and use of combination therapy is important to ensure that those with a significant burden of TNF-driven disease have adequate levels of drug and are given the greatest opportunity to achieve mucosal healing.

Prior to commencement of anti-TNF, patients should be assessed endoscopically and with small bowel imaging such as small bowel MRI or abdominal ultrasonography. Among patients with fibrostenotic disease, if the disease is isolated to the terminal ileum (short stricture length), consideration should be given to endoscopic dilatation or earlier recourse to surgical induction of remission with ileal resection.

Once primary response has been established, preferably using combination anti-TNF therapy with immunomodulator, patients can be monitored endoscopically or with MRI or abdominal ultrasonography annually to establish depth of remission and consider de-escalation of therapy provided that complete mucosal healing has been achieved. Similarly, patients who develop obstructive symptoms in the setting of treatment with anti-TNF should be re-evaluated with endoscopy and MRI small bowel to confirm the presence of a fibrostenotic stricture and pre-stenotic dilatation to determine whether the developing stricture can be endoscopically dilated or managed with surgical resection.

Faecal biomarkers such as faecal calprotectin can be used to detect response to anti-TNF therapy,¹⁴⁵ predict primary nonresponse in patients monitored early in treatment course and predict sustained clinical remission in most patients out to one year (<100mcg/g post induction). As such faecal calprotectin may be used to monitoring disease activity in patients on anti-TNF, establish depth of

remission or confirm loss of response in patients developing symptoms despite anti-TNF therapy (>150mcg/g).

The role for early (week 4) therapeutic drug monitoring requires further investigation but recent studies have demonstrated that lower anti-TNF levels are associated with either presence or formation of antibody at the end of induction and primary nonresponse. If clinical response remains absent by week 12–14 of therapy and persistent inflammatory disease activity is confirmed clinically, endoscopically, biochemically and/or using imaging techniques, therapeutic drug monitoring at week 12–14 may help predict and guide ongoing treatment in such patients in the medium to longer term.^{135,162}

Emerging data suggest that the formation of sustained anti-TNF antibodies, prior to symptom development is predictive of loss of response. Furthermore, low anti-TNF levels may also be predictive of future antibody formation and/or development of loss of response; however, the role of therapeutic drug monitoring while in remission to predict the development of secondary loss of response is yet to be established.

Management of Primary Nonresponse

In patients with ongoing symptoms at weeks 12 to 14 with confirmed inflammatory activity despite anti-TNF therapy, anti-TNF level +/- anti-drug antibody monitoring should be obtained and a decision pathway followed based on the underlying cause for primary nonresponse; pharmacokinetic, pharmacodynamic or immunogenic treatment failure (Figure 2).

Pharmacodynamic treatment failure: If there are adequate levels and no anti-drug antibodies, then switching to a different class of medication is warranted using new therapeutic options targeting non-anti TNF biological pathways.

Pharmacokinetic treatment failure: If there are absent or low anti-TNF levels together with absent antibody levels it may indicate inadequate concentrations of drug necessary to control inflammation. In such cases a higher dose via doubling the dose or shortening the interval may be required to obtain response.

Immunogenic treatment failure: If patients have anti-drug antibodies and low anti-TNF level at week 12–14, then a switching to an alternative anti-TNF and /or

the addition of an immunomodulator may be indicated. Following the switch to an alternative immunomodulator and/or addition of an immunomodulator, therapeutic drug monitoring should be repeated to evaluate whether the antibodies were transient and dilutional and have reduced in titre.

Management of Secondary Loss of Response

In patients in whom inflammatory luminal disease activity has been confirmed following exclusion of non-inflammatory causes or non-TNF processes, therapeutic drug monitoring can provide information to help guide the therapeutic strategy that is most likely to re-capture response. A proposed therapeutic algorithm for assessment and management of secondary loss of response is presented in Figure 3.

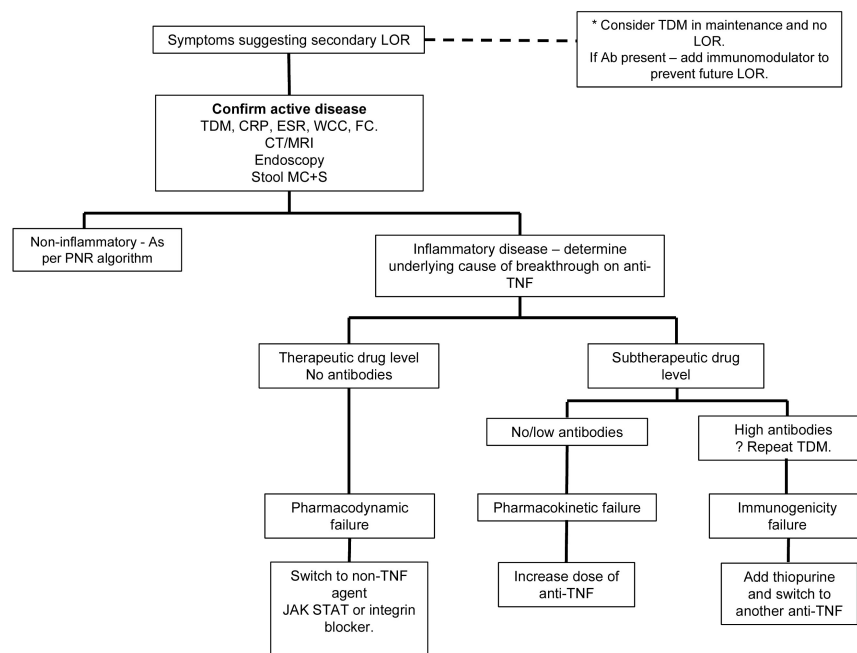


Figure 3. Proposed management algorithm for secondary loss of response to anti-TNF therapy in Crohn's disease.

Pharmacokinetic treatment failure: If there are low trough levels and absent or low antibodies it is important to check compliance with induction and maintenance drug therapy in order to ensure adequate dosing. If compliance is adequate consideration should be given to escalating the anti-TNF dose either via an increase in the dose in the case of infliximab or shortening of dose interval in the case of adalimumab. Dose escalation by scheduled re-induction of anti-TNF has been demonstrated to recapture response in 86% of patients who have

low drug levels and no antibodies causing secondary loss of response.^{110,114,138} However, the “window phenomenon”, where antibodies are maximally bound to anti-TNF and may therefore not be measurable, can be ruled out by measuring anti-TNF trough levels after delaying delivery of the next dose of anti-TNF.

Immunogenicity treatment failure is characterized by low trough levels together with high antibody titres. In such a situation either of two strategies can be used:

(1) Addition of immunomodulator – thiopurine/ methotrexate at adequate dose

In patients on anti-TNF monotherapy the addition of an immunomodulator may restore the response to the failing anti-TNF either via the elimination of anti-drug antibodies or resensitisation to anti-TNF.¹⁵⁸

(OR)

(2) Switch to alternative anti-TNF

Given that antibodies developed against one of the anti-TNF agents does not affect the bioavailability of another anti-TNF drug attempts should be made at recapturing response with a different anti-TNF.¹⁶³ The strategy of switching anti-TNF agents has been demonstrated to recapture response in 92% of patients with antibodies.¹¹⁴

Pharmacodynamic treatment failure: The finding of therapeutic drug levels and no antibodies suggest a non-TNF mediated pathway of disease. A switch out of anti-TNF class should be considered in such patients.

1.3.25 Conclusion

Anti-TNF therapy has revolutionized treatment of Crohn’s disease. However, not all patients respond to therapy and a significant proportion of patients lose response over time. Strategies to prevent loss of response should be employed to optimize drug therapy. Systematic exclusion and identification of non-inflammatory causes of nonresponse is the first step in the approach to a patient who has ongoing symptoms despite biologic therapy. Management of primary nonresponse subsequently involves dose optimisation or switching to an alternative TNF antagonist. In patients with secondary loss of response, there

may be a role for a period of expectant management as patient's symptoms often improve without alteration of drug therapy. If symptoms continue despite a defined period of expectant management and ongoing inflammatory disease is confirmed, therapeutic drug monitoring with drug levels and/or antidrug antibody measurement may help distinguish between non-adherence to therapy, immunogenicity and non-immune clearance of anti-TNF, or inflammatory activity despite adequate anti-TNF levels. The latter finding may be best addressed by a switch to another class of immunomodulator, whereas a low drug level may be best managed by dose intensification or a switch to another anti-TNF.

Given their cost and potential side-effects a tailored approach incorporating an individual's clinical, biochemical, radiological and endoscopic findings should be undertaken in order to optimize the use of Anti-TNF therapy. Future research should continue to explore the role of serology, pharmacogenomics and metabonomics, in order to enable clinicians and patients to make informed decisions about the likelihood of developing loss of response and strategies to overcome loss of response.

1.4 Review Article: Body Composition Analysis in Inflammatory Bowel Disease – Implications for Clinical Practice and Research

1.4.1 Introduction

Inflammatory bowel disease (IBD) results in significant changes to body composition which refers to the combination of both fat mass and fat-free mass, also known as lean body mass).^{164,165} The pathogenesis behind alterations in body composition is multifactorial and is thought result from changes in metabolism due to chronic inflammatory activity, malabsorption or malnutrition.¹⁶⁶

Alterations in body composition have been associated with complicated Crohn's disease (CD) course,¹⁶⁷ increased post-operative complications^{168,169} and decreased efficacy of medical therapy.¹⁷⁰ However, little is known about the extent to which altered body composition is associated with disease course and treatment outcomes in IBD.

This review brings together the definitions, pathogenesis and methods of assessment and monitoring of body composition and current treatment approaches to alterations in body composition in IBD. Using a systematic

approach this review also explores the impact/association of altered body composition on disease course, treatment response and outcomes in IBD.

1.4.2 Definitions

The existing literature has predominantly used Body Mass Index (BMI) as defined by weight divided by height in metres squared as a marker of nutritional outcomes and as benchmark for clinical outcomes including disease activity, need for surgery, hospitalisation and perioperative complications ^{171,172} However, BMI does not accurately assess fat and muscle mass which are the body's main tissue compartments.

Body composition analysis divides and measures the body's main tissue compartments of fat mass (FM) and fat-free mass (lean body mass).

Fat mass (FM) is comprised of the subcutaneous and visceral fat respectively which are the two main forms in which adipose tissue is stored. An increase in FM and obesity has been associated with elevated inflammatory markers and a more severe clinical course in CD patients.¹⁷³

Lean body mass (LBM) is anatomically defined as the sum of all non-adipose tissue of the body, whereas the term muscle mass usually refers to the sum of skeletal muscle tissue of the body. LBM appears to play a greater role than the equivalent weight of fat by applying stress to bones and thereby encouraging bone deposition thereby having an influence on bone mineral density (BMD).^{174,175,176} Bone mineral density is the content of the bone which is a marker of the strength of the bone as measured by various tools such as dual-energy X-ray absorptiometry (DXA). The morbidity associated with LBM deficits includes loss of muscle strength, altered energy metabolism and increased susceptibility to infection.¹⁷⁷

Skeletal muscle is considered to be one of the most plastic human tissues;¹⁷⁸ consequently alterations in skeletal muscle mass are particularly sensitive to fluctuations in physiological and metabolic conditions. Although skeletal muscle mass depletion is variably described as either myopenia or sarcopaenia,¹⁷⁹ sarcopenia specifically refers to clinically significant muscle wasting that is associated either with impaired functional capacity (disability as a result of

myopaenia), and/or with increased risk of morbidity or mortality. In contrast myopenia refers to the loss of muscle mass which may be a manifestation of disease or senescence.¹⁸⁰ The review methodology can be found in the supplementary text.

1.4.3 Pathogenesis

Although the pathogenesis behind alterations in body composition remains to be elucidated, key mechanisms include changes in metabolism due to chronic inflammatory activity, malabsorption and malnutrition.¹⁶⁶ Emerging data also support a role of fat (adipose tissue) in the regulation of inflammatory activity. These pathogenic changes associated with altered body composition are compounded by physical inactivity.

1.4.3.1 Chronic Inflammatory Activity

Chronic inflammation results in decreased muscle mass via catabolism. Increased metabolic demands associated with energy expenditure during active gastrointestinal inflammation have been associated with elevated whole-body protein synthesis and breakdown in IBD when using albumin as a surrogate marker of disease activity and protein synthesis.^{181,182} In animal models, it has been shown that disease activity diverts protein synthesis towards albumin production at the expense of muscle protein synthesis.¹⁸³ The systemic inflammatory response in IBD also involves stimulation of a number of mediators (mainly cortisol and proinflammatory cytokines), which can directly activate autophagy in skeletal muscle as well as inhibit synthesis of myofibrillar proteins.¹⁸⁴

The impact of chronic inflammatory activity on muscle depletion has been shown to correlate with self-reported fatigue, a symptom which is known to affect patients with IBD independent of anaemia¹⁸⁵ In a study by van Langenberg *et al.* subjects with muscle fatigue were found to have lower serum vitamin D, IGF-1 and magnesium together with higher IL-6 levels. In work from the same authors muscle size was 14% lower in patients with CD compared to healthy controls when measured at the quadriceps muscle. The reduction in muscle size was thought to be in part due to impaired activation of muscle protein synthesis pathways with IGF1-Akt pathway.¹⁸⁶ Figure 4 (with permission from Dr van

Langenberg) summarizes the signalling targets involved with active Crohn's disease resulting in reduced muscle hypertrophy.¹⁸⁶ Skeletal muscle preservation may also be protective against worsening IBD outcomes as cytokines released by contracting skeletal muscle may generate anti-inflammatory effects and inhibit proinflammatory mediators from visceral fat.¹⁸⁷

1.4.3.2 Malabsorption

Malabsorption due to Crohn's disease may occur due to either a reduction in the anatomical length of small bowel subsequent to resectional surgery or due to functionally reduced small bowel surface area due to disease involving the small bowel. Both clinical states are associated with altered absorption of micro and macronutrients.¹⁸⁸ Malabsorption can be measured via measurement of macronutrients such as fat and protein in the faeces which indicates poor absorption. Increased levels of fat, protein and caloric content have been found in the stool of patients with Crohn's disease indicating the propensity that patients with Crohn's disease have for malabsorption.¹⁸⁹

1.4.3.3 Malnutrition

Malnutrition according to BMI classification has been found in 14% of CD patients and 6% of UC patients.¹⁹⁰ Malnutrition also results in decreased muscle mass via catabolism. Dietary intake is frequently compromised in IBD due to cytokine-induced anorexia and food avoidance due to gastrointestinal symptoms.¹⁹¹ Therefore, metabolic demands of the inflammatory response, including the synthesis of acute phase proteins, are complicated by inadequate dietary intake during gastrointestinal inflammation.

1.4.3.4 The Role of Fat (Adipose Tissue) in Inflammatory Regulation

Mesenteric adipose tissue is thought to secrete hormones and molecules which have a role in regulation of inflammation¹⁹². A regulatory role of mesenteric adipose on inflammation is underpinned by the observation of fat-wrapping in CD patients on imaging and operative specimens.¹⁹³ Fat wrapping has been found to be an early event in the disease course of CD patients and has therefore led to interest in its role in disease pathogenesis.^{193,194} It has been found that pre-adipocytes have direct phagocytic function and can differentiate into macrophages.¹⁹⁵ The IL-17a inflammatory pathway is activated via interaction

with cytokines released from pre-adipocytes of IBD patients. In a study by Sideri *et al.* it was found that in patients with IBD there were higher levels of Substance P (SP) which resulted in upregulation of its respective receptor (NK-1R) along with differential inflammatory cytokine production between UC and CD patients, with lower expression of mRNA and protein level of IL-8 and IL-10 in response to SP treatment of adipocytes.¹⁹⁶ The study by Sideri *et al.* also reveals important alterations due to substance P and points to the possible role of adipocytes having inflammatory modulating effects which in an IBD setting may result in phenotypic variation as well as its role as a therapeutic target in the future.¹⁹⁶

1.4.3.5 Physical Activity and Body Composition

Reduced physical activity is thought to compound the pathogenic effect of chronic inflammatory activity, malabsorption, malnutrition and fat metabolism on body composition in IBD patients.¹⁹⁷ IBD is associated with decreased physical activity. Although bed rest alone can result in approximately 5% loss of LBM in healthy volunteers, the effects of severe stress combined with muscle unloading can be 2-to 3-fold greater than the impact of immobility alone.¹⁹⁸

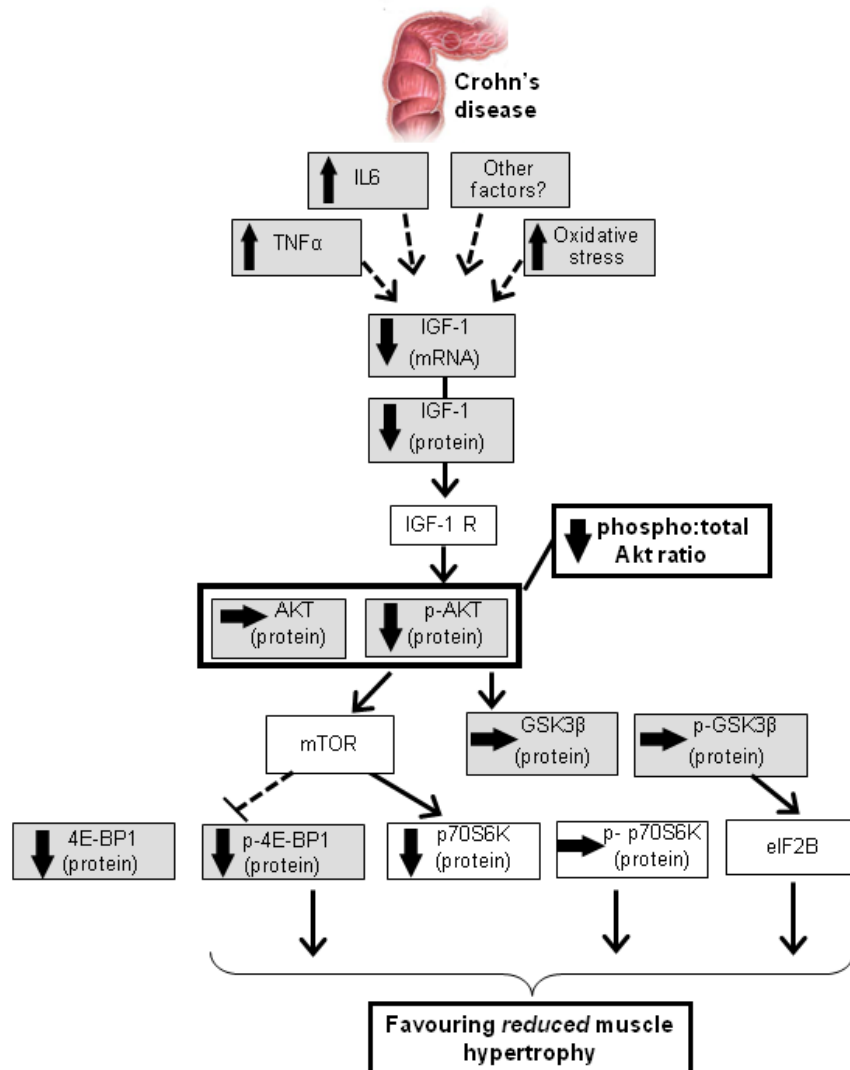


Figure 4. Diagram representing the attenuated muscle hypertrophy pathway as reflected by signalling factor levels in CD.

1.4.4 Assessment and Monitoring

A variety of methods can be employed to measure body composition, but none have been standardized for the assessment of myopaenia and sarcopaenia. These include basic (clinical) anthropometry, bioelectrical impedance analysis (BIA), total body water (TBW) measurement (hydrodensitometry), quantitative computed tomography (qCT) scanning, magnetic resonance imaging and dual energy X-ray absorptiometry (DXA).

Techniques for body composition analysis range from BIA to more advanced techniques using CT imaging and software developed specifically for identifying different tissue compartments (Slice-O-Matic).

Clinical anthropometry is the most basic method of assessing body composition using body mass, height and BMI. Anthropometry includes measuring skinfolds, waist circumference and hip ratios. The main issue with these measurements is that body weight may be altered significantly by changing proportions of muscle and fat mass which results in anthropometry being less accurate in certain populations such as the obese.

The analysis of body composition by bioelectrical impedance produces estimates of total body water (TBW), fat-free mass (FFM), and fat mass by measuring the resistance of the body as a conductor to a very small alternating electrical current.¹⁹⁹ BIA has been applied to overweight or obese samples in only a few studies;²⁰⁰ thus, the available BIA prediction equations are not necessarily applicable to overweight or obese children or adults. The ability of BIA to predict adiposity in the obese is difficult because obese patients have a greater proportion of body mass and body water accounted for by the trunk, the hydration of FFM is lower in the obese, and the ratio of extracellular water to intracellular water is increased in the obese.²⁰¹ Bioelectrical impedance analysis validity and its estimates of body composition are significant issues even for normal weight individuals. BIA is useful in describing mean body composition for groups of individuals, but large errors for an individual limit its clinical application, especially among the obese.^{202,203}

Total body water is simple to measure because it does not require undressing or any real physical participation. Water is the most abundant molecule in the body, and TBW volume is measured by isotope dilution. As with the other methods mentioned earlier, the TBW method is limited in the obese. The major assumption is that FFM is estimated from TBW based on an assumed average proportion of TBW in FFM of 73%, but this proportion ranges from 67 to 80%.²⁰⁴ In addition, about 15 to 30% of TBW is present in adipose tissue as extracellular fluid, and this proportion increases with the degree of adiposity.²⁰⁵ These proportions tend to be higher in women than in men as well as higher in the obese, and therefore produce underestimates of FFM and overestimates of adiposity.

Dual energy X-ray absorptiometry is the most popular method for quantifying fat, lean, and bone tissues. The two low-energy levels used in DXA and their

differential attenuation through the body allow the discrimination of total body adipose and soft tissue, in addition to bone mineral content and bone mineral density. DXA is fast and user-friendly for the subject and the operator.²⁰⁶ Mathematical algorithms allow calculation of the separation components using various physical and biological models.

Dual energy X-ray absorptiometry estimates of body composition are also affected by differences among manufacturers in the technology, models, and software employed, methodological problems, and intra- and intermachine differences.²⁰⁷ Additionally, some studies indicate that DXA may not be as reliable in extreme populations, including the obese.²⁰⁶ DXA is a convenient method for measuring body composition in much of the population and is currently included in the ongoing National Health and Nutrition Examination Survey (NHANES).

1.4.5 Computed Tomography and Magnetic Resonance Imaging

The other imaging modalities, such as CT and MRI, are gaining in popularity and represent important new techniques for body composition assessment. CT is able to accommodate large body sizes but has high radiation exposures and, as such, is inappropriate for whole body assessments, but it has been used to measure intra-abdominal fat. In many instances, MRI is not able to accommodate large body sizes but can be used for whole body assessments in normal weight or moderately overweight individuals. Both these methods require additional time and software to provide whole body quantities of fat and lean tissue.

In addition to its imaging capabilities, CT can also distinguish body tissues based on signal attenuation. This technique is especially useful for assessing non-adipose fat or the fatty infiltration of skeletal muscle or liver tissue.²⁰⁸

1.4.6 Techniques for Assessment and monitoring of body composition in IBD

A total of 25 studies have thus far investigated the impact of disease phenotype and stage on body composition with a total of 1331 patients. Of these, six hundred and twelve (46%) were male. The mean age for the study cohort was 36 years (see Tables 3 and 4).

Studies evaluating different body composition parameters in relation to IBD phenotype are detailed in Table 3 for CD and Table 4 for UC. Nine hundred and thirty (76%) patients had CD and 292 (24%) had UC. Activity of disease was documented in 15 studies with 683 being in clinical remission (88%) as defined by CDAI <150 or MAYO score <2. Of those with active disease (196), the vast majority had CD (86%).

The techniques of body composition analysis within the studies was heterogeneous with 9 studies using a combination of imaging (DEXA) and anthropometry while 2 studies included functional assessments of muscle strength. BIA was utilized in 10 studies, anthropometric measurements in 13 studies while the 3 remaining studies^{190,209,210} used a combination of mean fat index from qCT, arm muscle measurement and isotopic dilution with total body water (TBW).

Healthy, sex and age-matched controls were identified in 15 studies^{164,165,189,210-221} with 3 studies having no controls^{189,200,222} while 8 studies included IBD patients in clinical remission.^{165,189,211,212,214,215,223,224}

Table 3. Studies of body composition in Crohn's disease.

Author, year	Patients	Gender (% male)	Age (mean, years)	Type of study	Control type	control population	CD	UC	CD active	Total number with record of activity	Measure of activity	Technique	Functional assessment	BMI		FFM		FM (kg)	
														Control	Patients	Control	Patients	Control	Patients
Gong, 2015	75	N/A	33 (med)	Cross sectional study	IBD remission	none	67	0	48	67	CDAI	BIA, FFM, FM, body cell mass, SMM. Tricep skinfold, arm circumference	No	18.1 +/- 2.6	17.4 +/- 3.0 *	43.5 +/- 7.9	42.4 +/- 8.8	6.9 +/- 4.8	7.6 +/- 4.5
Bryant, 2015	137	56	31 (med)	Cross sectional study	Healthy control	75	95	42	20	137	CDAI > 150	DXA, ASM, FM, FMI, grip strength	anatomical function grip strength > 1sd below mean	N/A	24.8 (22.5–29.9)	N/A	N/A	N/A	N/A
Erhayiem, 2011	50	N/A	49.3 (mean)	Cross sectional study	Inflammatory CD vs complicated CD (fistulising/stricturing)	21	50	0	N/A			MFI	No	N/A	N/A	N/A	N/A	N/A	N/A
Benjamin, 2011	123	56	36.4	Cross sectional study	IBD remission	100	123	0		43	N/A	Anthropometry, BIA	No	Remission: 21.6 +/- 5.0	Active: 18.8 +/- 3.6 *	Remission: 43.3 +/- 10.4	Active: 40.7 +/- 8.5	Remission: 13.4 +/- 10.6	Active: 8.2 +/- 5.9
Rocha, 2009	102	34	41.2	Prospective, observational cohort	IBD remission	74	50	52	16	102	CDAI > 150	TST, SST, AMA	No	25.06 CD, 23.22 UC	20.09 (CD), 21.1 (UC)	N/A	N/A	N/A	N/A
Leslie, 2009	101	42	47	Prospective observational cohort study	No control	none	56	45			N/A	DXA (FFM, bone density, body fat)	No	N/A	N/A	N/A	N/A	N/A	N/A
Valentini, 2008	47	13	38	Cross sectional study	Healthy control	47	30	17			CDAI, UCAI	BIA, anthropometry	Hand grip	CD 21.8 (20.2–23.7)	Crohn: 22.0 (20.2–23.7)	Crohn: 45.8 (42.3–48.4)	Crohn: 43.9 (39.5–46.6) *	Crohn: 15.1 (13.5–20.1)	Crohn: 17.5 (14.7–21.5)
Vaisman, 2006	8	50	30	Cross sectional study	Crohn's remission	8	8	0			CDAI < 150	DXA	No	21.1 +/- 1.8	17.0 +/- 1.2 *	49.6 +/- 7.9	39.6 +/- 9.8 *	N/A	N/A
Jahnsen, 2003	120	40	37	Cross sectional study	Healthy control	60	60	60			N/A	DXA, anthropometry	No	23.4 (22.6–24.2)	Crohn: 23.3 (22.2–24.4)	48.8 (45.9–51.8)	44.5 (42–47) *	20 (18.2–21.8)	Crohn: 20.8 (18.6–23.1)
Tjellesen, 1998	31	42	40	Cross sectional study	Healthy control	88	31	0				DXA	No	Women: 22.4 (20.7–24.1) Men: 23.9 (22.4–25.4)	Women: 20.7 (19.6–21.8) Men: 23.5 (21.9–25.1)	Women > 50: 41.2 (38.9–43.5) Men: 62.2 (59.7–64.7)	Women > 50: 34.1 (31.2–37.0) Men: 51.8 (47.5–56.1) *	Women > 50: 23.8 (23.7–26.9) Men: 19.2 (16.5–21.9)	Women > 50: 21.5 (18–25) Men: 20.3 (17.2–23.4)
Capristo, 1998	43	53	33.8	Cross sectional study	Healthy control	60	43	0			SCDAI < 3	anthropometry, BIA (indirect calorimetry used for expenditure)	No	23.7 +/- 1.3	21.5 +/- 1.5 *	Male: 54.8 +/- 3.9 Female: 46.5 +/- 4.5	Male: 53.9 +/- 5.9 Female: 43.9 +/- 5.6 *	Male: 17.1 +/- 3.4 Female: 16.9 +/- 2.8	Male: 10.9 +/- 2.5 Female: 13.6 +/- 1.9
Mingrone, 1996	20	40	29.5	Cross sectional study	Healthy control	16	20	0			CDAI < 120	Anthropometry	No	24.77 +/- 0.49	19.89 +/- 0.71 *	52.2 +/- 2.06	45.72 +/- 1.64 *	18.30 +/- 0.89	9.97 +/- 1.38 *

Author, year	Patients	Gender (% male)	Age (mean, years)	Type of study	Control type	control population	CD	UC	CD active	Total number with record of activity	Measure of activity	Technique	Functional assessment	BMI		FFM		FM (kg)	
														Control	Patients	Control	Patients	Control	Patients
Geerling, 1998	32	44	40	Cross sectional study	Healthy control	32	32	0			CDAI 139 (86–193)	Anthropometry (BMI, percent body fat, waist to hip ratio, mid arm circumference, upper leg circumference)	No	24.6+/-3.6	23.2+/-3.7	49.7+/-11.6	48.6+/-9.3	19.7+/-6.3	17.6+/-7.9
Capristo, 1998	34	56	36.8	Cross sectional study	Healthy control	20	18	16			SCDAI<3	Anthropometry inc waist/hip/midarm circumference/triceps skinfold thickness	No	23.6 (range 19.4–26.0)	20.5 *	49.6 (range 36.7–60.9)	CD: 45.6	17.4 (range 9.88–24.2)	CD: 12.6 *
Mingrone, 1999	12	56	35.3	Cross sectional study	Healthy control	18	12	0			CDAI<150	Anthropometry, isotopic dilution	No	23.8+/-1.8	21.6+/-2.9*	47.4+/-6.1	48.0+/-7.1	19.0+/-3.5	13.8+/-5.6 *
Capristo, 1999	102	57	N/A	Cross sectional study	Healthy control		63	39	29	102	CDAI > 150 in 29%	Anthropometry inc biceps/triceps/subscapular thickness and chest/waist/hip circumference	No	UC M: 23.3+/-2.6	CD M: 21.6+/-3.0	UC M: 54.5+/-7.5	CD M: 52.4+/-6.6	UC M: 15.4+/-5.2	CD M: 12.6+/-7.0
Katznelson, 2003	20	100	45	Cross sectional study	Healthy control	20	20	0			mean 101 (SD 12)	quantitative CT, BIA	No	23.31 (SD 0.35)	24.24 (CD 0.50)	N/A	N/A	N/A	N/A
Filippi, 2006	54	48	39	Cross sectional study	Healthy control	25	54	0	0	54	CDAI<150	Anthropometry, BIA	No	22.1+/-0.5	22.1+/-0.5	46.7+/-2.0	49.2+/-1.4	16.6+/-0.9	14.4+/-0.8 *
Schneider, 2008	82	48	36	Cross sectional study	Healthy control	50	82	0	0	82	CDAI<150	DXA, Anthropometry	No	22.2+/-2.5	21.1+/-3.4 *	46.7+/-10.1	43.8+/-9.4	16.1+/-4.9	16.2+/-8.9
Cuoco, 2008	13	69	31	Cross sectional study	Healthy control	20	13	0	13	13	CDAI>=230	DXA, Anthropometry	No	23.4+/-1.1	19.8+/-1.2 *	49.6+/-4.4	35.8+/-4.1 *	19.6+/-2.4	21.2+/-1.4

* = significant p<0.05

CDAI, Crohn's disease activity index; med, median; BIA, bioelectrical impedance; FFM, fat free mass; FM, fat mass; FMI, fat mass index; BMD, bone mineral content; DXA, dual energy X-ray absorptiometry; BMI, body mass index; TST, tricep skinfold thickness;

Table 4. Studies of body composition in ulcerative colitis.

Author, year	Patients	Gender (% male)	Age (mean, years)	Type of study	Control type	control population	CD	UC	UC active	Active disease	Total number with record of activity	Measure of activity	Technique	Functional assessment	BMI		FFM		FM (kg)	
															Controls	Patients	Controls	Patients	Controls	Patients
Zaltman, 2014	23	0	43.9 (mean)	Cross sectional study	UC remission	43.9 (mean)	0	23	0	15	23	MAYO	QS, HGS, BIA, anthropometry		28 +/- 6.8	29.5 +/- 6.6	45.2 +/- 6.3	44.9 +/- 3.5	24.3 +/- 12.3	28.6 +/- 13.2
Rocha, 2009	102	34	41.2 (mean)	Prospective observational cohort.	IBD remission	74	50	52	16	28	102	MAYO	TST, SST, AMA	not measured	25.06 CD, 23.22 UC	20.09 (CD), 21.1 (UC)	N/A	N/A	N/A	N/A
Leslie, 2009	101	42	47 (mean)	Prospective observational cohort study	None		56	45				mixed	DXA (FFM, bone density, body fat)							
Jahnsen, 2003	120	40	37	Cross sectional study	Healthy controls	60	60	60				None	DXA, anthropometry		23.4 (22.6–24.2)	Crohn: 23.3 (22.2–24.4)	48.8 (45.9–51.8)	Crohn: 44.5 (42–47) *	20 (18.2–21.8)	Crohn: 20.8 (18.6–23.1)
Ulivieri, 2001	43	49	35.9 (calculated mean)	Prospective observational cohort	Healthy controls	111	0	43				"mild"	DXA		31 Men: 23.5 +/- 2.7	21 Men: 24.7 +/- 4	Men: 57.5 +/- 5.6	Men: 56.8 +/- 5.8 *	Men: 13.8 +/- 5.4	Men: 13.2 +/- 4.3
Capristo, 1998	34	56	36.8	Cross sectional study	Healthy volunteers matched for age sex and height	20	18	16				MAYO	Anthropometry inc waist/hip/midarm circumference/triceps skinfold thickness		23.6 (range 19.4–26.0)	CD:20.5 *	49.6 (range 36.7–60.9)	CD: 45.6	17.4 (9.88–24.2)	UC: 12.6*
Capristo, 1999	102	57		Cross sectional study	None		63	39	29	29	102	MAYO	Anthropometry with biceps/triceps/subscapular thickness and chest/waist/hip circumference		UC M: 23.3+/-2.6	CD M: 21.6+/-3.0	UC M: 54.5+/-7.5	CD M: 52.4+/-6.6	UC M:15.4+/-5.2	CD M: 12.6+/-7.0

* = significant p<0.05

med, median; BIA, bioelectrical impedance; FFM, fat free mass; FM, fat mass; FMI, fat mass index; BMD, bone mineral content; DXA, dual energy X-ray absorptiometry; BMI, body mass index; TST, tricep skinfold thickness

1.4.6.1 Active Disease Versus Clinical Remission and Association with Body Composition

Clinical remission in Crohn's disease has been the primary outcome measure of many clinical trials. However, it is known that this does not constitute deep remission and new targets include endoscopic remission. Body composition parameters are altered dramatically during active flares of disease but also in patients with clinical remission.²²⁵

There have been 6 studies (n=278)^{189,214,217-219,223,226} which have described reductions in fat mass with one study in those in steroid free clinical remission for at least 3 months.²¹⁴ A total of 683 patients were in clinical remission with IBD. The definition of clinical remission has varied among the studies with a Crohn's disease activity index (CDAI) < 150 in 6 studies,^{165,189,211,212,215,223} one study using a definition of simplified Crohn's disease activity index (SCDAI) < 3,²¹⁴ and one study with MAYO < 2²²⁴.

Those with active CD were analysed in 3 studies^{211,215,223} which found reductions in FM that was also associated with malnutrition as measured by body mass index < 20.²¹¹

Patients who were in clinical remission had ongoing reductions in fat free mass while patients who had active CD had reduction in fat mass and fat free mass compared to those in remission and healthy controls. One study showed that CD patients had lower FM than those with UC.²¹⁴

With regard to other body composition parameters, one study demonstrated that visceral adiposity was higher in those with CD and the ratio of visceral to total body fat was higher in CD while no difference was noted between those with active disease from those who were in remission.

1.4.6.2 Impact of Body Composition Changes on Muscle Strength in IBD Patients

Alterations in body composition may result in lower muscle mass and subsequently reduced strength.^{164,210,211,224} The method of assessment of strength has varied in studies to date with hand grip strength assessed in 3 studies^{164,211,224} and hamstring and quadriceps strength evaluated in a separate

study.²¹⁰ Sarcopaenia was found in 72% of patients with IBD including those with active disease and disease in remission. Geerling *et al.* also observed higher fat free mass amongst males compared with females (60.5 $\pm$ 8.9kg vs 1.2 $\pm$ 2.9kg, $p<0.05$) with IBD.²¹⁰ However, in the latter longitudinal study, there were no significant changes in FFM compared with controls as there was recovery of limb FFM over time relative to baseline measurements. Zaltman *et al.* concluded from BIA, anthropometric measures and a validated exercise questionnaire that higher BMI was associated with lower quadriceps strength in a cohort of women with UC which they found was linked to decreased physical activity.²²⁴

1.4.6.3 Relationship Between Medical Therapy and Body Composition

Variable alterations to body composition may result from the use of different classes of medications in the treatment of IBD. Data pertaining to the impact of medications on body composition is available for steroids and anti-TNF therapy.

Steroids are known to cause muscle atrophy, increase adiposity in patients with chronic disease on high-dose treatment and lower bone mineral density (BMD).²²⁷ Data on the impact of steroid therapy on FFM and FM are limited to three studies. Steroids have been found to decrease BMD in CD patients ($n=120$).²¹² Corticosteroid therapy has been found to be associated with lower FFM²¹² whereas two studies have found increased FM in females²¹⁰ and CD patients.²²⁸

Both primary non response and secondary loss of response rates to anti-TNF therapy have been found to be higher among patients with a raised BMI.¹²³ According to Csontos *et al.*¹⁷⁰ it is parameters such as fat free mass and skeletal muscle index which were inversely correlated with adalimumab trough levels (FFMI: $r=-0.494$ $p=0.045$, SMI $r=-0.508$, $p=0.038$). Trough levels did not correlate with fat parameters. Furthermore, a recent study investigating body composition parameters and impact on anti-TNF therapy identified that myopenia was associated with primary non response but not secondary loss of response with an HR;4.73(1.81–12.39), $p=0.002$.²²⁹

More recently, Subramaniam *et al.*²³⁰ demonstrated a decrease in inflammatory sarcopenia following administration of infliximab in a prospective cohort study of 19 adult CD patients. Muscle volume (measured by computer analysis of

quadriceps on MRI) and strength increased from baseline to week 25 and correlated with male gender ($p=0.0003$) with a decrease in pro-inflammatory cytokine IL-6 ($p=0.037$).

1.4.6.4 Incidence of Muscle Depletion in IBD

The rates of myopaenia in IBD vary due to the heterogeneity of patient populations and modality used to measure the underlying tissue. It is thought that patients in remission also suffer from myopaenia at rates of between 50–60%^{190,220,231}. Decreased bone size and osteopenia due to inadequate trabeculation of bone are potential effects of disease activity and myopaenia, which are factors particularly important to bone development in the paediatric population.²³²

Body composition is also known fluctuate during a patient's phase of disease from remission to active disease, with demonstration of fat mass and muscle mass depletion during an active flare of IBD.¹⁹⁰ A study by Rocha *et al.* of 102 patients (50CD, 52 UC) demonstrated that muscle mass depletion was detected in half of CD and UC patients, with CD patients having lower muscle mass according to arm muscle area and triceps skinfold thickness.

Patients with IBD in clinical remission are at greater risk of sarcopaenia.²²⁰ A publication by Schneider *et al.* demonstrated that of 82 patients with CD, 60% were found to be sarcopenic and 30% osteopenic in patients in clinical remission compared to healthy controls measured by dual-energy x-ray absorptiometry. 91% of sarcopenic patients were also found to be osteopenic (T-score < -1.0).²²⁰

1.4.7 Management

Body composition in IBD patients can be altered favourably with nutrition, physical exercise, rehabilitation, and medication in combination with close monitoring.

1.4.7.1 Nutrition

Appetite regulation, together with level of physical activity are the major determinants of energy balance and body composition changes. It has been suggested that inflammatory mediators play a vital role in appetite regulation. In the majority of patients, systemic inflammation results in anorexia and fat loss in combination with myopenia.¹⁸⁰ In others, appetite may be sustained despite

activation of systemic inflammation, resulting in myopenia with increased BMI; a condition described as myopenic obesity.²³³ This broad phenotypic variability within the same disease therefore requires careful tailoring of nutritional interventions. Protein intake should be increased in order to counteract the anabolic resistance associated with inflammation and inactivity.¹⁷⁶

Nutrition support is integral to supporting IBD patients through acute severe flares of disease. Nutrition support may require hospitalization for enteral and/or total parental nutrition, which has been associated with improved perioperative morbidity and mortality in patients with CD requiring resectional surgery.¹⁶⁸

1.4.7.2 Physical Activity and Exercise

Physical activity in the pre-illness period has been found protective against the onset of IBD,²³⁴ particularly among patients with CD compared with UC.²³⁵ Studies on sedentary patients with inactive or mildly active CD have shown that moderate exercise such as walking programs or yoga results in significant improvements in the measures of the quality of life and stress levels.²³⁶ In an online survey of almost 1,000 IBD patients (54% with CD and 46% with UC) from the UK the majority of respondents were undertaking regular exercise, and exercise was found to be beneficial for the symptoms of IBD. However, most of the respondents needed to stop exercising at some point because of the severity of their symptoms.¹⁸⁷

Physical activity may alter outcomes in IBD, with resistance exercise shown to prevent and even reverse the progression of sarcopenia.²³⁷ Resistance exercise has been demonstrated to increase muscle cell proliferation, protein synthesis and translational efficiency acutely, while increasing cell numbers as well as the number of myonuclear domains per myofiber chronically. Exercise can also ameliorate the severity of a flare in CD and accompanying anorexia while modifying the release of adipokines and ghrelin.²³⁸

1.4.7.3 Rehabilitation

Rehabilitation medicine, with its focus on optimizing function after treatment despite a patient's symptom burden or impairments, involves strategies that may be beneficial in patients with myopaenia. Further optimising physical

improvement before treatment (prehabilitation) might result in better outcomes as seen in oncology but this remains to be evaluated in IBD.²³⁹

1.4.7.4 Medical Therapy

Treatment of the underlying disease has been found to halt and reverse sarcopenia in IBD patients. In a recent small study of 19 patients treated with infliximab, there was an increase in muscle volume in both legs with reversal of sarcopenia, which resulted in an increase in muscle strength.²³⁰ Concomitant exclusive enteral nutrition along with infliximab in Crohn's patients has been shown to be more effective than infliximab alone on multivariate analysis.²⁴⁰ Enteral nutrition (EN) >600kcal/day (elemental and polymeric diet) has also been associated with a lower likelihood of loss of response to infliximab compared with controls, with an odds ratio: 0.23; p=0.0043.²⁴¹

1.4.8 Discussion

The majority of patients with IBD experience an alteration in their body composition. The most common alterations include a reduction in fat mass irrespective of disease activity, whereas changes in lean body mass (FFM) independent of BMI tend to be observed in those with active disease. CD has been associated with greater changes in FM and FFM compared with UC. These changes have impact on surgical outcomes with increased post-operative morbidity and may alter outcomes of anti-TNF therapy.

The literature to date has been inconsistent in its use of the terms myopenia and sarcopenia and have often used the terms interchangeably. However, myopenia refers to a loss of muscle mass and sarcopenia refers to clinically significant muscle wasting with impaired functional capacity. Ideally both parameters require evaluation to provide an accurate assessment of body composition.

Although the pathogenesis behind altered body composition in IBD requires elucidation, emerging evidence suggests that muscle and fat are metabolically active organs which interact with each other and appear to have an impact on disease outcome and influence response to anti-TNF therapy. Fat (adipose tissue) plays a role in the regulation of inflammatory activity. It also appears that

skeletal muscles play a protective role against worsening IBD via anti-inflammatory effects that inhibit proinflammatory mediators from visceral fat.

Accurate assessment and monitoring of body composition in IBD are evolving. Conventional anthropometry, BIA, total body water and DEXA are limited by their inability to accurately determine body composition and reflect fat and muscle mass in obese patients. There are a variety of methods available to assess body composition, but these have been variably applied and several novel methods lack validation. Novel software packages which segment fat and muscle compartments may facilitate more accurate measurements of body composition and overcome the limitations associated with conventional assessment tools.

Alterations in fat and muscle mass occur regardless of IBD subtype and irrespective of disease activity and appear to influence response to drug therapy. However, evaluating the extent to which body composition alters in the setting of disease activity and treatment has been difficult to assess. In particular a major limitation of this review related to the heterogeneity of the data available within study groups and small sample sizes in some cohorts, which precluded quantitative analysis. Not all studies included data on diet, exercise, smoking or underlying disease profile, which are known confounders that have an impact on body composition. There also remains a paucity of data on the impact of medical therapy on body composition parameters.

A variety of nutritional and physical modalities are likely to be helpful in positively influencing body composition in IBD; however, the evidence base is lacking. Further studies are required to investigate to what extent individual treatment modalities are likely to result in favourable alterations to body composition in IBD such as a prevention of loss of muscle mass and avoidance of a gain in visceral fat.

Body composition analysis in IBD appears to have implications beyond nutritional assessment alone. Whilst the metabolic activity of fat is increasingly being recognized muscle also appears to have a metabolic role. The extent to which fat and muscle influence therapeutic response to drug therapy warrants further exploration as it may influence the pharmacodynamic and pharmacokinetic handling of IBD therapeutics. Future studies should employ standardized clinical outcome

measures within well-defined patient cohorts in order to identify body composition parameters which may be associated with response to anti-TNF therapy.

1.5 Supplementary Text

1.5.1 Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the process followed *a priori* established protocol. (Figure 4)²⁴²

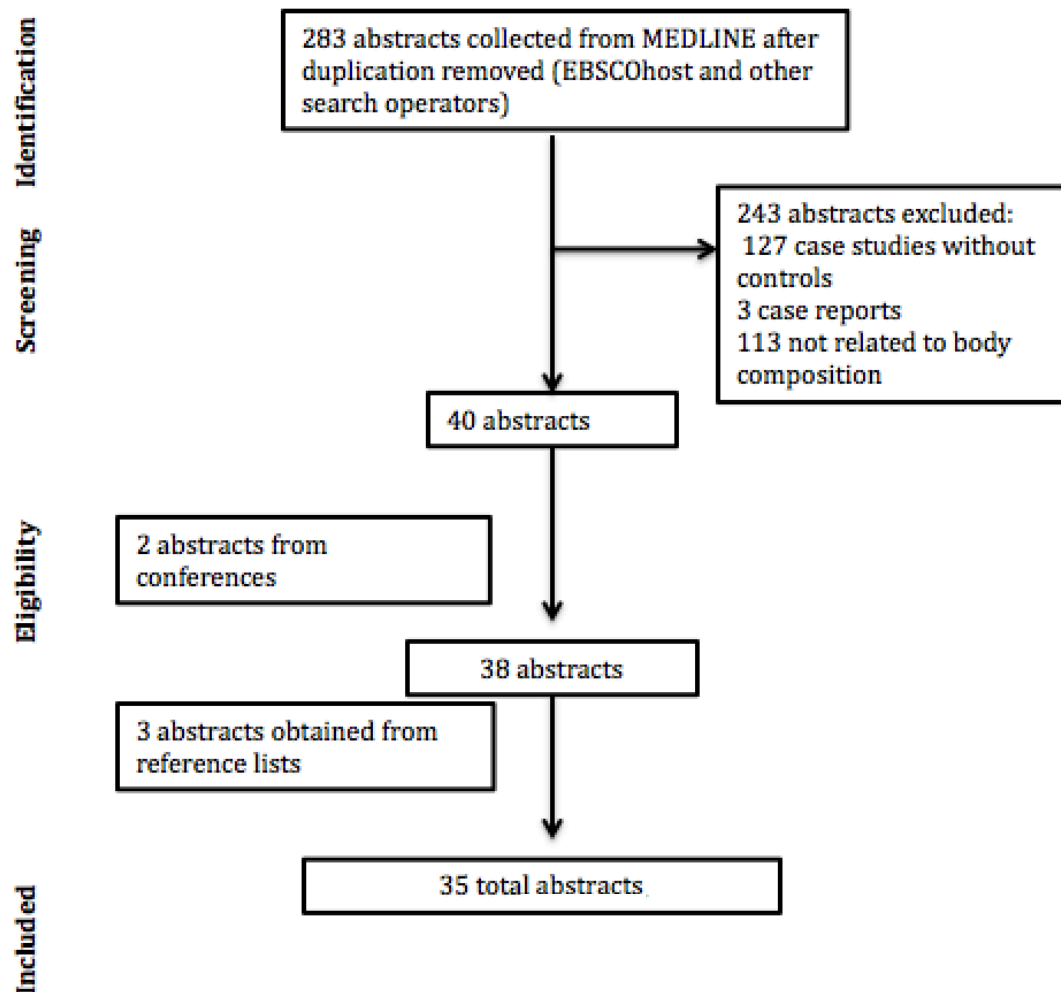


Figure 5. PRISMA flow chart detailing study design.

1.5.2 Selection Criteria

Studies included in the systematic review met the following inclusion criteria: (a) cohort studies, case-control, cross-sectional studies, case series and randomized controlled trials in (b) adult patients with established inflammatory bowel disease with (c) reported effects on body composition.

We excluded the following studies: (a) studies with insufficient follow-up on the outcome, paediatric patients, studies on stomas and review articles.

1.5.3 Search Strategy

We conducted a comprehensive search of multiple electronic databases from inception to January 2017 in adults. The databases included: Ovid Medline In-Process & Other Non-Indexed Citations, Ovid databases (MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Web of Science, and Scopus. The search strategy was Medical Library Subject Heading (MeSH) terms 'body composition', 'muscle', 'muscle strength' and 'sarcopaenia', 'sarcopenia', combined with the set operator 'AND' plus each of 'Inflammatory Bowel Diseases', 'Crohn disease', 'Colitis, Ulcerative' or 'colitis'. Articles were limited to those published in English, between the years 1992 and present, limited to human subjects. The details of the search strategy are included in the Supplementary Appendix. The title and abstract of studies identified in the search were reviewed by two authors independently (NSD, IAB) to exclude studies that did not address the research question of interest, based on pre-specified inclusion and exclusion criteria. The full text of the remaining articles was examined to determine whether it contained relevant information. Next, the bibliographies of the selected articles and review articles on the topic were manually searched for additional studies. Third, a manual search of conference proceedings of major gastroenterology conferences (Digestive Disease Week, American College of Gastroenterology annual meeting, Advances in Inflammatory Bowel Diseases meeting organized by the Crohn's and Colitis Foundation of America, and European Crohn's and Colitis Organization annual meeting) between 2010–2015 was conducted to identify additional studies published only in the abstract form.

1.5.4 Data Extraction

Data on the following study-, patient- and treatment-related characteristics were abstracted onto a standardized form, by two authors independently (NSD, IAB): (a) study characteristics – primary author, time period of study/year of publication; (b) patient characteristics – age, sex, body mass index, disease phenotype, duration; technique of assessment with significance and (c) outcomes as defined by:

1. Type of study, control population, definition of active disease or remission.
2. Rates of body composition alteration (myopenia, fat-free mass, sarcopenia, visceral and subcutaneous adiposity in remission and active disease.
3. Effects of body composition on response to medical therapy and surgical outcome.

Any discrepancies were addressed by a joint re-evaluation of the original article. A meta-analysis was not performed due to the heterogeneity of the studies.

1.6 Metabolic Profiling in Gastroenterology and IBD

Beyond the metabolic potential of fat and muscle it has become apparent that man has evolved with the microbiome as a supre-organism which has significant metabolic potential in its own right. Metabolic profiling represents functional characterisation of the microbiome and its composition and offers the potential to find biomarkers that reflect the unique interactions between the host and microbiome that culminate in disease.¹³

There are increasing uses for “omics” technologies in gastrointestinal disease to identify novel diagnostic targets and gain insights into the underlying molecular mechanisms of IBD pathogenesis. Metabonomics or metabolite profiling overcomes the limitations imposed by other “omic” approaches such as genomics, transcriptomics or proteomics by integrating information about gene regulation, post-translational modification and pathway interaction. Therefore, metabolites can act as an immense readout of the cell phenotype.^{230,231} In addition, this synthesis of diverse upstream “omics” signals in metabolites makes them useful for detecting subtle changes in metabolic pathways before the phenotypic changes occur.²³¹ Metabolomics is defined as “the unbiased identification and quantitative measurement of metabolites in a biological system”,²³² whereas metabonomics is defined as “the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification”, addressing the dynamic changes.²³³ Both terms are used and are often interchangeable.^{230,234}

Metabolites reflect host physiological and pathological states providing a picture of the host phenotype or metabotype.²³⁵ Bodily fluids encompassing serum or

plasma, urine, faeces, and tissues provide adequate matrices for metabolite profiling. Depending on the nature of the chosen matrix, metabolites of different origins, such as the host and/or the microbiota, can be identified and quantified. For example, faecal water extracts provide a pool of microbiota-derived and dietary metabolites, whereas the host metabolites prevail in the serum and tissue samples. The metabolites that are derived from the interactions between the host and the microbiota are known as host-microbial co-metabolites²³⁰ e.g. hippurate (a glycine conjugate of benzoic acid).

1.6.1 Metabolic Profiling Methods and Workflow

Many analytical platforms are available for metabolic profiling, such as Gas Chromatography-Mass Spectrometry (GC-MS), High/Ultra Performance Liquid Chromatography coupled with MS (H/UPLC-MS), Nuclear Magnetic Resonance (NMR) spectroscopy,^{230,231} Fourier-Transform Infrared spectroscopy (FT-IR), and Capillary Electrophoresis (CE) coupled to MS. Moreover, Mass Spectrometry Imaging (MSI) is a powerful tool that can be used to visualize the spatial distribution of preselected metabolites in the tissue samples²³¹. Among these analytical platforms, proton NMR spectroscopy and UPLC-MS are the most widely used instrumentation in metabolic profiling. NMR spectroscopy exploits the spin property of nuclei, where the nuclei can absorb and re-emit electromagnetic radiation in a magnetic field.^{232,236} The NMR spectra provide rich information on the molecular structures and it is both qualitative and quantitative.^{230,233} NMR spectroscopy is extremely robust with high reproducibility, making it valuable in studying large sample sets from longitudinal or epidemiological studies, which require a high instrumental power. However, NMR has a relatively low sensitivity and may not detect the compounds with very low concentrations, i.e. at the sub-micromolar level. In contrast, UPLC-MS is more sensitive and provides retention time and mass-to-charge ratios (m/z) of metabolites.^{232,236} The UPLC system separates a complex sample into multiple simpler fractions and each fraction is in turn analysed by mass spectrometry in positive or negative mode depending on the positive/negative charges of the molecules. Another advantage of UPLC-MS-based metabolic profiling is that the UPLC methods can be modified to target a specific set of molecules, e.g. bile acids, lipids, and amino acids.²³⁰ In contrast, the targeted analyses, which are

based on a defined group of metabolites and often require prior knowledge of the metabolites of interest, can be applied to investigate specific pathways.²³⁰

The typical workflow of metabolic profiling consists of (1) experimental design, (2) sample collection and processing, (3) sample preparation and analysis, (4) data treatment and modelling, (5) metabolite identification and (6) result interpretation and validation.²³⁷⁻²⁴⁰ Throughout the workflow, it is essential to follow the standard operating protocols to ensure the quality of both samples and the data. For example, consistent sampling protocols should be used at multiple centres and samples should be randomised prior to the analysis. Quality control samples, which are usually formed by pooling a small volume from each of the analytical samples, should be analysed together with the analytical samples to check the stability of the instrument over the entire data acquisition time.

Appropriate data pre-processing methods need to be applied to the metabolic datasets obtained from various analytical platforms before statistical analyses. Typically, these pre-processing methods include calibration, phasing, baseline correction, peak picking and alignment, normalisation and scaling.^{241,242} Multivariate statistical analysis methods, such as unsupervised principal component analysis (PCA) and supervised orthogonal signal corrected-projection to latent structure-discriminant analysis (OPLS-DA), can subsequently be applied to model the data.^{243,244} The key discriminant features (e.g. chemical shift in NMR spectra, retention time-mass to charge ratio in UPLC-MS data) can be identified as metabolites for biological interpretation.

Metabolite identification is a challenging step in untargeted metabolic profiling and often requires additional experiments to identify the molecular structures. The techniques often used in metabolite identification, in combination with various databases, include 2-dimensional (2-D) NMR spectroscopy, Statistical Correlation Spectroscopy (STOCSY), MS / MS or molecular fragment patterns, hyphenated HPLC-NMR-MS systems and spike-in authentic compounds.^{237,245-247} Two-dimensional NMR spectroscopy, such as ^1H - ^1H correlation spectroscopy, ^1H - ^1H total correlation spectroscopy, ^1H - ^{13}C heteronuclear single-quantum correlation and ^1H - ^{13}C heteronuclear multiple bond correlation spectroscopy, show the connectivity of atoms in the molecules and provide structural information of the

molecules of interest. STOCSY is a statistical approach and calculated based on a large set of 1-dimensional NMR spectra. It provides correlation information between the spectral peaks derived from both the same molecule and the compounds involved in the same pathway. The hyphenated HPLC-NMR-MS technique allows one to combine the information from both NMR spectroscopy and MS. Tandem MS / MS is often used to obtain the structural information by breaking the parent ion into multiple fragments, providing information on the substructure of a molecule. These identified metabolites can not only provide mechanistic understanding of the biological processes, but also could be used as biomarkers for disease diagnosis and prognosis, assessment of therapeutic treatment and patient stratification.

1.6.2 Application of Metabolic Phenotyping in Gastrointestinal Diseases

Metabolic profiling is ideally suited as a platform to investigate the effects of diet and environmental factors in gastroenterology due to alterations in the host-gut microbiome environment resulting in dysbiosis.

Other examples of microbial-related differences in the phenotype include the finding that malignant gastrointestinal tumours which carry differential 16S ribosomal RNA bacterial profiles.²⁴³ Critical biosynthetic pathways that substantially extend host metabolic capacity are introduced by microbes, for example affecting the biosynthesis of a number of B vitamins.²⁴⁴ Metagenomic analysis can be used to map the microbial community of faecal or intestinal samples. Parallel metabolic profiling of biofluids such as urine, plasma or faecal water can provide a window to investigate the functionality of the microbiota and assess consequences on human health.

Furthermore, there have been applications of nuclear magnetic resonance (NMR) in other disease areas such as colorectal cancer with MR and mass spectrometry having expanded the knowledge of tumour pathogenesis by enabling metabolical profiling of solid CRC tumours.²⁴⁵ Although tumour tissue generates informative profiles, tissue samples are by nature less suitable as a means to arrive at a diagnosis. Using a combined analytical strategy comprising NMR and gas chromatography-mass spectrometry (GC-MS), clear metabolic differences in tumour tissues obtained from colon and rectal locations can be identified, based

on eicosanoid, lipid and steroid metabolism.²⁴⁵ Other NMR- based studies have identified elevated lactate, threonine, serine, acetate, succinate, formate and tyrosine levels with lower concentrations of taurine, creatine, betaine and myoinositol in tumour tissue compared with adjacent nonmalignant tissue.²⁴⁶

1.6.3 Application of Metabolic Phenotyping in IBD

The first metabolite profiling study that was able to discriminate IBD patients from healthy individuals took place in 2007 and was based on ¹H NMR analysis of faecal water extracts²⁴⁸. Since then numerous studies have been performed and have consistently shown that the metabolic phenotype of IBD differs from a healthy state.²⁴⁸⁻²⁵⁰ Interestingly, different subtypes of IBD such as Crohn's disease (CD) and Ulcerative colitis (UC) can also be distinguished.^{248,250} Bacterial metabolites including short chain fatty acids (SCFAs), methylamine and trimethylamine were depleted in the faecal water extracts from IBD patients compared with healthy controls, whereas amounts of faecal amino acids were elevated suggesting a possible malabsorption in the intestine due to mucosal injury resulting from inflammation.²⁴⁸ Methylamine and trimethylamine are products of bacterial degradation of dietary compounds including choline and carnitine, while SCFAs are derived from bacterial fermentation of indigestible dietary fibre.²⁵¹ Furthermore, the depletion of SCFAs was correlated with reduced abundance of *Clostridium coccoides* and *Clostridium leptum* in IBD patients, which are implicated in SCFAs production.²⁴⁸ A growing body of evidence has shown a reduction in the levels of SCFAs in IBD, highlighting their importance.^{250,252} SCFAs, which are 1–6 carbons in length, comprise mainly acetate, butyrate and propionate and have a role in the regulation of host metabolism and immune system homeostasis.²⁵³ In particular, butyrate is the main energy source of colonocytes and has a role in the maintenance of intestinal epithelial barrier integrity.^{251,254} Additionally, butyrate has been brought into the frontline of IBD research since it exerts an anti-inflammatory role suppressing inflammation through a range of mechanisms.^{253,255-257} Treatment of UC patients with butyrate enemas has been found to ameliorate intestinal inflammation²⁵⁸ and addition of butyrate in intestinal biopsy specimens from CD patients has been found to reduce the expression of pro-inflammatory cytokines.²⁵⁹ A reduction in the levels of SCFAs, particularly butyrate, is associated with an altered microbial

composition and presumably a decrease in butyrate-producing bacteria such as *Clostridia*.^{248,255,256,260}

Metabolite profiling can discriminate IBD patients depending on the affected area of the gut (ileum or colon).²⁴⁹ A study on identical twins, including healthy individuals and twin pairs discordant or concordant for CD, differentiated CD from healthy controls and further stratified CD cases to affected tissue, i.e. ileum or colon.²⁴⁹ Differences in faecal metabolites within the pathways of amino acid metabolism (in particular tyrosine, tryptophan and phenylalanine), bile acids and fatty acids synthesis were identified. Higher concentrations of tyrosine and tryptophan, bile acids, and saturated and unsaturated fatty acids were indicative of ileal CD phenotype.²⁴⁹ The levels of bile acids, a family of cholesterol-derived molecules that are produced in the liver with a role in fat breakdown to release dietary lipids and lipid-soluble vitamins,²⁵³ are elevated in IBD.²⁴⁹ Bile acids are concentrated in the gallbladder, and in response to diet-dependent signals are emptied in the small intestine to perform their detergent-like role.^{253,261} After that, they are re-absorbed and directed back to the liver.²⁶² Inflammation and increased intestinal permeability, which are hallmarks of IBD, hinders their reabsorption leading to increased levels.²⁴⁹ Metabolism of bile acids is dependent on deconjugation (removal of glycine and taurine residues) and dehydroxylation processes, which are directed by gut microbiota.^{253,261} IBD patients have high levels of conjugated bile acids and sulphated bile acids, but low proportions of secondary bile acids in the gut lumen compared with healthy individuals.²⁶³ Of note, secondary bile acids (such as deoxycholic, lithocholic and muricholic acids) are derived from the transformation of primary bile acids (cholic and chenodeoxycholic acids).²⁵³ Therefore, these modifications in the luminal bile acid pool are directed by defective deconjugation, transformation and desulphation activities of the altered IBD microbiota composition. Given that secondary bile acids such as deoxycholic acid and lithocholic acid exert anti-inflammatory effects on the intestinal mucosa, alterations in bile acid metabolism enhance inflammatory pathways leading to a feed forward loop perpetuating inflammation.²⁶³

The aforementioned studies have been primarily conducted on faecal samples. Other biofluids and tissues that have been used for metabolite profiling in IBD

comprise urine, serum, plasma and colonic mucosa biopsies. Metabolic profiling studies based on these matrices were also able to discriminate IBD patients from healthy controls²⁶⁴⁻²⁷⁴ as well as the different subtypes of IBD.²⁶⁹ These studies have further revealed alterations in amino acid, lipid and energy metabolism pathways as evidenced by the decreased levels of amino acids (such as glutamine), lipoproteins (mainly low density lipoprotein) and tricarboxylic acid (TCA) cycle intermediates (such as succinate and citrate). These metabolic changes indicate enhanced energy requirements and rapid utilization of metabolites that feed energy producing pathways during intestinal inflammation.^{264,275} A common finding especially in urine metabolic profiling studies is the lower levels of hippurate in IBD, suggesting its potential use as a biomarker. Hippurate or N-benzoglycine is a metabolite generated by bacterial fermentation of dietary aromatic compounds (such as polyphenols, purines or aromatic amino acids) and hence its low levels may reflect altered gut microbiota composition, given that hippurate levels positively correlate with *Clostridia* levels in the gut.²⁷⁶

While the vast majority of metabolite profiling studies has focused on IBD in adults, there is a scarcity of data on paediatric IBD. Understanding the metabolic dysregulation in children with IBD is particularly important, since children with IBD suffer from growth failure and delayed puberty in addition to the known pathological features of the disease.^{277,278} A study on newly diagnosed paediatric IBD reported differences in faecal metabolite profiles between IBD patients and healthy individuals and categorized IBD cases in CD and UC.²⁷⁹ Metabolite pathways involved in amino acid metabolism, sphingolipid metabolism, urea cycle and bile acid biosynthesis were perturbed in paediatric IBD.²⁷⁹ A recent study following IBD paediatric patients over a year reported differences in amino acids with an emphasis on glycine metabolism, bile acids, urea cycle, metabolites of energy metabolism, signalling molecules (such as dopamine and gamma-aminobutyric acid (GABA)) and gut microbial metabolites.²⁸⁰ In particular, urinary levels of pyroglutamic acid, glutamic acid, glycine, cysteine, as well as fumarate, isocitrate, 2-hydroxyglutaric acid, methylsuccinic acid, methionine and tyrosine were elevated in paediatric IBD patients during the course of the study, whereas hippurate appeared in lower concentrations.²⁸⁰ Metabolite phenotyping provides

insight in differential metabolic requirements in children with IBD, which could lead to better disease management.

Metabolite profiling discriminates active disease from remission phase in IBD.^{252,268,281,282} Discriminating metabolites include *N*-acetylated compounds, phenylalanine, glycine lipoproteins and acetoacetate. The levels of the medium chain fatty acid, hexanoate, were negatively correlated with disease status in CD whereas the benzenoid compound styrene, which is produced by protein fermentation, was positively correlated with the status of UC.²⁵² Identification of metabolites whose variation is associated with certain stages of disease activity holds promise for the discovery of IBD biomarkers in the future.

1.7 Overall Thesis Hypothesis

- There are clinical, endoscopic, histologic, body composition and metabolic biomarkers which may be predictive of response to anti-TNF Therapy in Crohn's disease.

1.8 Overall Thesis Aims

- To identify clinical factors which may be associated with response to anti-TNF therapy in Crohn's disease
- To identify treatment factors which may be associated with decrease recurrence of disease activity in patients with complex anastomotic stricturing Crohn's disease
- To identify a metabonomic profile which can be predictive of response to anti-TNF therapy in patients with Crohn's disease.

1.9 Details of the Studies in this Thesis

Chapter 2: Body composition

A retrospective, longitudinal cohort study of the body composition parameters for patients with luminal Crohn's disease receiving anti-TNF therapy and their impact on response in Crohn's disease.

Chapter 3: Crohn's anastomotic dilatation and anti-TNF therapy response

A retrospective longitudinal cohort study of patients who have had prior anastomotic strictures. Analysed data identified which clinical or endoscopic factors are predictive of future need for dilatation or surgery.

Chapter 4: Endoscopy and histologic predictors of response

A retrospective cohort study of patients with luminal Crohn's disease requiring anti-TNF therapy. Analysed data identified the impact of histology taken from biopsies and endoscopic factors prior to induction therapy which affect response to anti-TNF therapy.

Chapter 5: Metabonomic predictors of response to anti-TNF therapy

A prospective, longitudinal cohort study of biomarkers from metabolic profiling of faeces, urine or serum in patients receiving anti-TNF therapy with Crohn's disease. The metabolic profiling involved assays measuring bile acid, fatty acid and amino acids.

Chapter 2: Body Composition Profile is Associated with Response to Anti-TNF Therapy in Crohn's Disease and may offer an Alternative Dosing Paradigm

This chapter explores whether specific body composition parameters are associated with response to anti-TNF therapy in patients with Crohn's disease. It also seeks to explore whether body compartment modelling can be used to explain the mechanism behind primary non-response.

2.1 Introduction

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease (IBD) of multifactorial aetiology. It has long been identified that patients with IBD have altered body composition during various disease states.^{165,220,247} This may be due to a multitude of factors such as malnutrition, a catabolic state and malabsorption which alters body composition.^{189,211} Decreased muscle mass or myopenia is prevalent in patients with CD who are in clinical remission.²²⁰ Furthermore, recent studies have shown that body composition influences outcomes in Crohn's patients with surgery, attracting more post-operative complications.^{168,248} CD phenotype may also play a role in a patient's body composition with luminal CD contributing to decreased lean body mass.²⁴⁹

Anti-TNF therapy forms the backbone of treatment in patients with moderate to severe Crohn's disease. However, it has a primary nonresponse rate of 10–30% in clinical practice and secondary loss of response rate of about 5% per patient-year,^{112,250} potentially exposing patients to side-effects with minimal therapeutic benefit. BMI has been linked to primary nonresponse in observational cohort studies and may be due to pharmacokinetic effects on drug levels.⁵⁶ Skeletal muscle and adipose tissue have demonstrated either pro or anti-inflammatory properties through the release of cytokines¹⁸⁶. The recent discovery of the role of creeping fat in IBD via Substance P by releasing IL-17 in pre-adipocytes and increasing IL-17A receptors in the IBD colon, has shown the important role of visceral adiposity in the development of inflammatory bowel disease.¹⁹⁶ It has been observed that inter-patient variability of anti-TNF levels was poorly associated with BMI, but intra-patient anti-TNF levels were stable.⁷ However, in a small population of anti-TNF naïve patients, the body composition parameters

that correlated with anti-TNF trough levels were body surface area, fat-free mass index (FFMI) and skeletal muscle index (SMI).¹⁷⁰ Therefore, we hypothesized that the composition of a patient's body mass may demonstrate a more refined method of analysis and provide better understanding of the causes of primary nonresponse and secondary loss of response.

We aim to demonstrate that using a validated tool for assessing body composition, we can predict primary nonresponse or secondary loss of response in patients receiving anti-TNF therapy with moderate to severe Crohn's disease using a patient's muscle and fat content.

2.2 Methods

2.2.1 Patient Population

A total of 650 consecutive anti-TNF naïve patients undergoing anti-TNF therapy at St Mark's Hospital, London, between January 2007 and June 2012 were identified from a database. Patients were selected if: an abdominal CT scan was performed within 6 months of commencement of anti-TNF therapy for moderate to severe luminal Crohn's disease and images retrievable in an electronic format; at least 3-year follow-up data on therapy outcomes was available; patient height and weight data were recorded. Patients were excluded if the indication for their initial anti-TNF application was perianal fistulising disease.

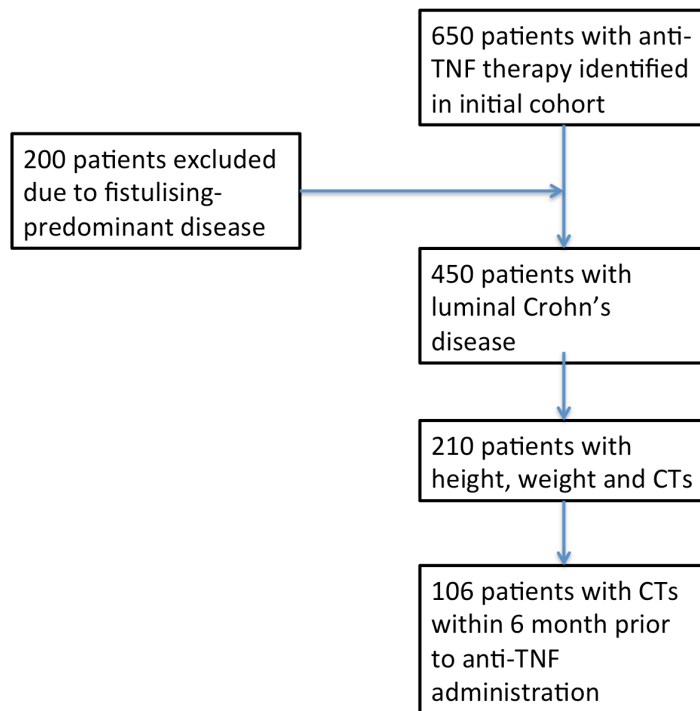


Figure 6. Flowchart of study protocol.

Demographics including age, sex and BMI were collected. Patient's Montreal classification at diagnosis was identified and severity of disease was assessed with levels of CRP and albumin at time of induction therapy. An albumin level less than 30g/L was considered to be low. Primary nonresponse was defined as cessation of anti-TNF within 6 months of first administration as deemed by their treating gastroenterologist using global physician assessment. Patients who had cessation of therapy due to drug intolerance were not included as primary non-responders. Secondary loss of response was defined as a recurrence of patient's symptoms after a period of improvement with subsequent need for cessation or switch of anti-TNF therapy. At the time, therapeutic drug monitoring was not routine practice. Endpoints for analysis were primary nonresponse and secondary loss of response. To demonstrate the wide variations in potential drug levels due to inter-patient variability in body composition, a hypothetical dose of 5mg/kg of infliximab was given with calculated drug levels from fat free mass.

2.3 Computed Tomography Image Analysis

Images were retrieved from digital storage in the Picture Archiving and Communication System. Computed tomographic image analysis was performed

using Slice-O-Matic V4.3 software (Tomovision, Montreal, Canada) as described previously.¹⁷⁴ The study was limited to CT given it was the predominant modality during the study period with Slice-o-Matic being validated for use in CT and MRI imaging. Briefly, total skeletal muscle and adipose tissue surface area (cm²) were evaluated on a single image at the third lumbar vertebra (L3) level as per previous validated data¹⁷² using Hounsfield unit (HU) thresholds of -29 to 150 for skeletal muscle, -150 to 50 for visceral and subcutaneous adipose tissue (see Figure S1). Visceral, subcutaneous fat area (VFA, SFA), and skeletal muscle area (SMA) or skeletal muscle index form the taxonomies used. The sum of skeletal cross-sectional muscle areas was normalized for stature (m²) and reported as lumbar skeletal muscle index (LSMI) (cm²m²). Muscle attenuation (MA) in HUs was reported for the whole muscle area. Reduced LSMI (myopenia) and low MA (myosteatorsis) were defined. The database is registered with the UK National Research Ethics Committee (reference number: 14/LO/2010).

2.4 Statistical Analysis

SPSS v19.0 (IBM SPSS Statistics, Ontario, Canada) was used for statistical analysis. Body composition parameters are presented as median values and interquartile ranges (IQR) and are categorized into sex-specific groups. The definition of myopenia and myosteatorsis were the lowest quartiles of LSMI and MA, while visceral obesity was defined as the highest quartiles of VFA. This is a validated method from previous work without a reference cohort, performed in previous cancer populations.^{171,172,251} Mann-Whitney and Fisher exact tests were used to test for differences in continuous and categorical variables, respectively. Univariate and multivariate logistic regression were used to assess the relationship between muscle depletion and myosteatorsis and multiple categorical variables with the dichotomous outcomes of primary nonresponse and secondary loss of response.

2.5 Results

650 patients who were administered their first dose of anti-TNF therapy were included with 250 patients having CT studies prior to their first dose. Of these, 106 patients had CT studies within 6 months of anti-TNF commencement (Figure 6).

47 of 106 (44%) were male with a median age of 44.8 years (Interquartile range (IQR) 34.2–53.6) and 13 of 106 (12.3%) were considered obese (BMI> 30). Primary nonresponse occurred in 26 (24.5%) patients with secondary loss of response in 29 (27.4%) and continued usage at the time of census in 50 patients (47.2%). Study cohort details including Montreal classification, baseline biochemistry data and medical therapy are summarized in Table 5. The median duration of anti-TNF therapy was 22.7 months (range 6.1–37.7). There were 65 (61.3%) patients on combination therapy with immunomodulator at time of induction of anti-TNF.

Table 5. Background and demographics of study cohort.

Demographics		
Sex	Male N (%)	47 (44.3)
	Female N (%)	59 (55.7)
Age in years (Med (IQR))		44.84 (34.16–53.55)
BMI in kg/m ² (Med (IQR))		22.31 (19.60–26.87)
BMI categories	Obese (BMI >30)	13 (12.3)
	Overweight (BMI 25–30)	23 (21.7)
	Normal (BMI 20–25)	39 (36.8)
	Underweight (BMI <20)	31 (29.2)
Montreal classification N (%)		
	A1	17 (16.0)
	A2	69 (65.1)
	A3	15 (14.1)
	L1 – terminal ileum	7 (6.6)
	L2 – colon	16 (15.1)
	L3 – ileocolonic	87 (82.1)
	L4 – upper GI	0 (0)
	B1 – inflammatory disease	31 (29.2)
	B2 – stricturing disease	21 (19.9)
	B3 – penetrating disease	49 (46.2)
Biochemistry (Med (IQR))		
	CRP (mg/L)	5.0 (1.0–19.7)
	ESR mm/hr	10.5 (3.0–26.3)
	Albumin (g/L)	37 (31–40)
Medical therapy N (%)		
	Immunomodulator	65 (61.3)
Anti-TNF	Infliximab	73 (68.9)
	Adalimumab	33 (31.1)
Outcome N (%)		
	PNR	26 (24.5)
	SLOR	29 (27.4)
	Ongoing	50 (47.2)
	Remission	1 (0.9)
	Months on anti-TNF (Med (IQR))	22.65 (6.05–37.68)

PNR = Primary Nonresponse

SLOR = Secondary Loss of Response

The sex-specific cut-offs for L3 skeletal muscle index ascertained by the lowest quartile were 31.0 cm²/m² for men and 32.37 cm²/m² for women with patients

below these values being classified as having myopenia. Lumbar visceral fat index (LVFI) was calculated with cut-offs being the 75th centile (40.7 for males and 31.8 for females) with values above this being classified as visceral obesity. Muscle attenuation (MA) was measured in Hounsfield units (HU) with cut-offs being the 25th centile (31.0 for males, 29.5 for females), which was defined as myosteatorsis.

2.5.1 Primary Nonresponse

There were 26 patients (24.5%) who experienced primary nonresponse. Of these, 15 (57.7%) were myopenic. There were 6 (25%) patients with nonvisceral obesity and 5 (25%) with myosteatorsis.

On univariate analysis, presence of myopenia [Odds ratio (OR) 4.69;1.83–12.01, $p=0.001$] and albumin (OR 0.94;0.88–0.99, $p=0.04$) predicted primary nonresponse. When myopenia and albumin were entered into multivariable analysis, only presence of myopenia was significant (OR 2.93;1.28–6.71, $p=0.01$) (Figure 7). No other factors, including BMI or inflammatory markers (C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)) at induction were shown to be significant (Table 6). There were no statistical differences between patients who were myopenic and non-myopenic in regard to their demographics (disease duration, phenotype, age or biochemical markers of severity). The rates of primary nonresponse with each quartile of lumbar skeletal muscle index (LSMI) correlated with the lowest quartile of lumbar skeletal muscle index (myopenic patients). The rate of primary nonresponse in the lowest quartile was 11 (40.7%) and decreased to 7 (25.9%) in the 2nd quartile, 3(11.5%) in the 3rd quartile and 5(19.2%) in the fourth quartile (Table 7).

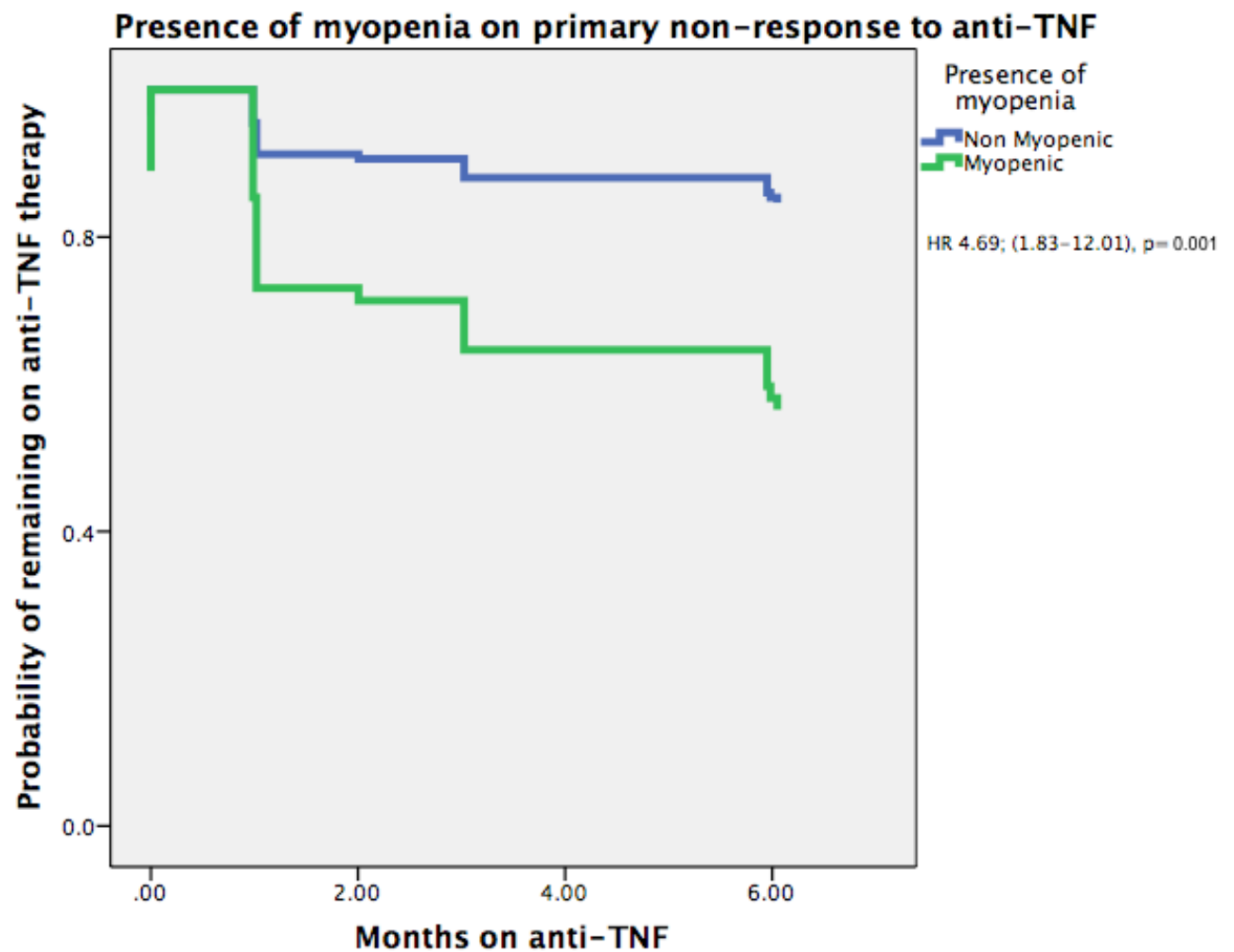


Figure 7. Kaplan-Meier plots showing overall rates of primary nonresponse to anti-TNF therapy by myopenia status.

Table 6. Univariate and multivariate analysis of patient demographics, medical therapy, biochemistry and body composition factors on outcome of primary nonresponse (PNR) to anti-TNF therapy.

Variables		Univariate analysis		Multivariate analysis	
		OR 95% CI	p-value	OR 95% CI	p-value
Gender	Male	1			
	Female	1.37 (0.56–3.40)	0.49		
Age	(b=-0.002)	0.99 (0.96–1.03)	0.91		
BMI	BMI < 30	1			
	BMI > 30	0.52 (0.11–2.52)	0.42		
Medical therapy					
Immunomodulators*	Absent	1			
	Present	0.54 (0.22–1.32)	0.09		
Anti TNF drug					
Infliximab	1	1			
Adalimumab	2	0.32 (0.10–1.02)	0.052		
Biochemistry					
CRP (mean)		1.012 (0.99–1.03)	0.28		
Albumin (mean) #		0.94 (0.88–0.99)	0.04	0.97(0.92–1.01)	0.15
Body composition factors					
Myopenia #	Absent	1		1	
	Present	4.69(1.83–12.01)	0.001	2.93(1.28–6.71)	0.01
Visceral obesity	Absent	1			
	Present	1.03 (0.36–2.96)	0.95		
Myosteatorsis	Absent	1			
	Present	1.13 (0.33–3.17)	0.92		
Myopenic obesity	Absent	1			
	Present	0.52 (0.11–2.53)	0.42		

*azathioprine, mercaptopurine or methotrexate

significant at p<0.05 on univariate analysis

Table 7. Primary nonresponse rates to anti-TNF therapy with each quartile of muscle mass with correlation between lean skeletal muscle index and primary nonresponse.

Degree of myopaenia	PNR (%)		Chi-squared, p	Total
	No	Yes		
1st (<25%)	16 (59.3)	11 (40.7)	0.02 (against no myopaenia)	27
2nd (25–49%)	20 (74.1)	7 (25.9)	0.25 (against 1st quartile)	27
3rd (50–74%)	23 (88.5)	3 (11.5)	0.02 (against 1st quartile)	26
4th (>75%)	21 (80.8)	5 (19.2)	0.09 (against 1st quartile)	26
No myopaenia (i.e. >25%)	64 (81.0)	15 (19.0)	0.02 (against 1st quartile)	79
Total	80	26	N/A	106

2.5.2 Secondary Loss of Response

In our cohort, 29 (27.4%) patients had secondary loss of response. The reason for the switch or cessation of anti-TNF therapy was due to recurrence in symptoms after a period of clinical remission. Of the patients that had secondary loss of response, 7 (24.1%) were myopenic, 8 (27.6%) had visceral obesity and 5 (17.3%) had myosteatorsis. On univariate analysis (Table 8), the use of immunomodulator was protective for secondary loss of response (OR 0.48; 0.23–0.98, $p=0.04$) (see Figure 8). However, none of the body composition factors or biochemistry at induction were associated with secondary loss of response.

Table 8. Univariate and multivariate analysis of patient demographics, medical therapy, biochemistry and body composition factors on outcome of secondary loss of response to anti-TNF therapy.

Variables		Univariate analysis	
		OR 95% CI	p-value
Gender	Male	1	
	Female	0.80 (0.34–1.90)	0.62
Age	(b=-0.009)	1.01 (0.98–1.04)	0.59
BMI	BMI < 30	1	
	BMI > 30	1.80 (0.54–6.02)	0.32
Medical therapy			
Immunomodulators* #	Absent	1	
	Present	0.48 (0.23–0.98)	0.04
Anti TNF drug			
Infliximab	1	1	
Adalimumab	2	1.88 (0.77–4.59)	0.165
Biochemistry			
CRP (mean)		0.97 (0.94–1.01)	0.10
Albumin (mean)		1.06 (0.99–1.13)	0.10
Body composition factors			
Myopenia	Absent	1	
	Present	0.62 (0.24–1.65)	0.34
Visceral obesity	Absent	1	
	Present	1.45 (0.54–3.88)	0.45
Myosteatorsis	Absent	1	
	Present	0.86 (0.28–2.63)	0.79
Myopenic obesity	Absent	1	
	Present	1.79 (0.54–6.03)	0.34

*azathioprine, mercaptopurine or methotrexate

significant at p<0.05 on univariate analysis

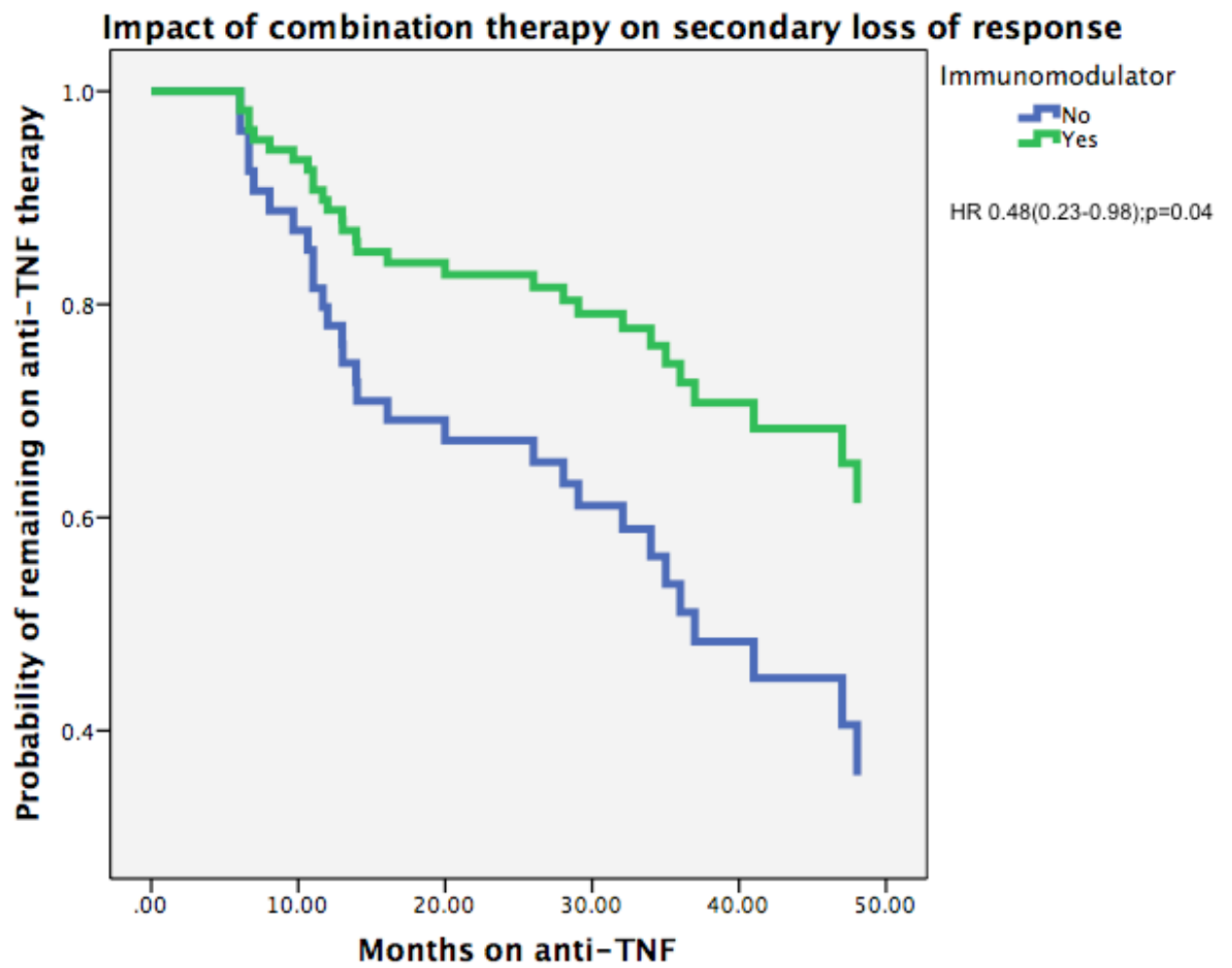


Figure 8. Kaplan-Meier plots showing overall rates of secondary loss of response to anti-TNF therapy depending on immunomodulator therapy

2.5.3 Obesity

BMI > 30, defined obesity, did not predict for primary nonresponse or secondary loss of response and was poorly correlated with all composite markers of body composition (LSMI, LVFI and MA). There were 13 (12.3%) patients who were classified as obese (BMI> 30) and 31 (29.2%) being underweight (BMI<20). Of the obese patients, only 5 did not meet criteria for having visceral obesity.

2.5.4 Pharmacokinetic Sub-study

The association between estimated total body fat-free mass and BMI was poor ($r^2=0.15$; $p=0.54$). A consequence of high fat mass or low fat free mass would be a larger volume of distribution of anti-TNF therapy. This effectively means higher doses are required to obtain a particular drug level. A simulated hypothetical dose of anti-TNF at 5 mg/kg of body weight was used to calculate the variation in the resulting anti-TNF per kg estimated fat-free mass. The overall distribution of estimated anti-TNF per kg fat-free mass varied greater than six-times (range 14.36– 97.92 mg anti- TNF per kg) (Figure 9A).

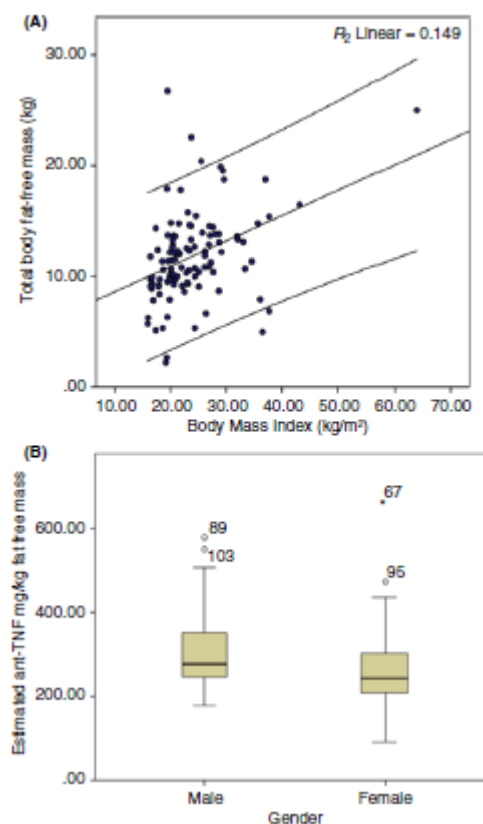


Figure 9. (A) Association between lean body mass and BMI in patients with anti-TNF therapy, (B) Estimated anti-TNF per kg fat-free mass in male and female patients based on 5 mg/kg anti-TNF dosing; all patients ranked from lowest to highest anti-TNF dose per kg fat-free mass.

Men were more likely to have a lower fat-free mass in proportion to their BMI than women ($p=0.05$), and thus a larger proportion of men than women were in the highest ranges of anti-TNF per kg fat-free mass. This is demonstrated in Figure 9B.

2.6 Discussion

Myopenia is associated with primary nonresponse to anti-TNF therapy in patients with moderate to severe luminal Crohn's disease. This study also demonstrates that there is wide variation in body composition profile, thus tailoring medication to each patient is important. This may be due to a multitude of factors including underlying luminal disease causing malabsorption^{189,220,221,252} and the interplay between pro-inflammatory and anti-inflammatory cytokines released by adipocytes or muscle.^{167,253} Body composition is important in determining the volume of distribution of medications such as anti-TNF therapy and it is plausible that this contributes to pharmacokinetic failure due to inadequate dosing.¹⁹

The mechanisms of primary nonresponse as laid out in a review by Ding *et al.* include pharmacokinetic, pharmacodynamic or immunogenic failure.²⁵⁰ Following administration, anti-TNF is distributed centrally to extracellular fluid with a volume of distribution of about 0.1l/kg.^{254,255} The most likely explanation for nonresponse due to myopenia would be inadequate dosing due to increased clearance or decreased uptake resulting in pharmacokinetic failure. Unfortunately, drug levels were not available at the time of this study but would be an area of future research. Furthermore, taking into account patient's disease phenotype and severity at time of induction, myopenia was associated with primary nonresponse to anti-TNF therapy. However secondary loss of response was not associated with any of the body composition factors. Rather, combined immunomodulator at time of induction was protective of secondary loss of response as shown in Figure 9B, which complements the existing literature.^{116,256} The lack of association may be due to anti-TNF therapy improving inflammatory myopenia after induction therapy as demonstrated in a prospective observational study.²³⁰ The finding that combination therapy with an immunomodulator and anti-TNF is protective of secondary loss of response demonstrates that this may prevent immunogenic failure through the inhibition of antibody formation.²⁵⁶

Intra-individual variation in anti-TNF level is dramatic given that anti-TNF therapy is absorbed and distributed centrally throughout the body.²⁵⁷ Data from oncology utilising a similar pharmacokinetic study with FFM as the volume of distribution of chemotherapy drugs rather than body-surface area, showed similar variations in drug level within the cohort.²⁵¹ Therefore, the composition of an individual's mass is important in determining correct dosing. Body surface area has been shown to have a negative correlation with adalimumab trough levels and is correlated to muscle content (FFMI: $r=-0.494$, $p=0.045$), but not with fat parameters (BFMI: $r=-0.099$, $p=0.708$).¹⁷⁰ Our study in this large cohort confirms these findings, as pharmacokinetic failure may result from myopenia, but no correlation was identified with visceral obesity. The median albumin in our cohort was 37 g/L (IQR 37–40). Our finding that albumin (less than 30g/L) was associated with primary nonresponse on univariate analysis (OR 0.94; 0.88–0.99, $p=0.04$) also supports the current literature and demonstrates that patients with severe disease may require higher doses to induce remission.⁵⁶

A consistent finding is that there is loss of lean mass with preserved fat mass.²⁴⁷ Other possible causes of pharmacokinetic failure may be due to increased drug clearance in patients with higher (>120kg) and low (<40kg) body weight.¹²⁵ Our hypothetical scenario of anti-TNF infusion at a dose of 5mg/kg when taking into account patient's lean body mass revealed six-fold variation in anti-TNF per kg fat-free mass.

The wide variation in body composition profiles of patients with Crohn's disease and the additional data that can be obtained from CT images can be used in personalizing medical therapy. Within our cohort, there were 33 (31.1%) patients who had myopenia and 24 (22.6%) with visceral obesity. BMI did not predict for primary nonresponse or secondary loss of response. Furthermore, BMI was poorly correlated with lean body mass ($r=-0.15$, $p=0.54$). It is important to note that patients who are obese have a wide variation in body composition profile and 38.5% of those who had BMI > 30 did not have visceral obesity.

A large proportion of patients with Crohn's disease are also affected by sarcopenia or the functional loss of muscle with rates of up to 60% as demonstrated in a study of those in clinical remission.²²⁰ Infliximab has been

shown to reverse inflammatory sarcopenia in Crohn's disease in a recent publication.²³⁰ This study demonstrated that those receiving infliximab had increased muscle volume at the right quadriceps femoris from 1505 cm³ at baseline to 1607 cm³ (p=0.012) following induction at week 16. There was a coinciding increase in muscle strength with a reduction in pro-inflammatory cytokine Il-6 from 4.195ng/ml at week 1 to 0.175ng/ml at week 25 (p=0.037). These findings suggest that patients with Crohn's have loss of muscle mass and that subsequent treatment with anti-TNF therapy may possibly reverse this.

One possible explanation for lower muscle mass in CD patients is poor nutritional status. In CD, selected micro and macro-nutrient deficits, loss of body cell mass and muscle strength are frequent even in remission.²¹¹ In a recent publication by van Langenberg *et al.*¹⁸⁶ there was a 54% lower phosphorylated:total Akt ratio in muscle samples (p<0.05) showing a key decrease in the hypertrophy signalling pathway. However, there were no differences in the atrophy signalling targets MuRF-1 or atrogin-1 mRNA protein levels.

Therefore, the rate of replacement of muscle protein may not be adequate to meet the rate of muscle proteolysis in an inflammatory state.²⁵⁸ In an in vivo animal model, it was shown that colitis increased albumin synthesis at the expense of muscle protein synthesis in macronutrient-restricted piglets.¹⁸³

Demonstrating that myopenia is prevalent in Crohn's patients is important for its functional effects on patients including weakness and quality of life.^{211,224,259} Much of the focus on anti-TNF therapy has been on goals of therapy particularly mucosal healing.^{63,69,112} However, early work by Wiese *et al.* demonstrated that infliximab improved nutritional indices along with inflammatory markers with possible decrease lipolysis and resultant weight gain.¹⁹⁹ Much of this may be attributed to the decrease in inflammatory sarcopenia.²³⁰

The main limitation of this retrospective observational study design is the inability to determine causality. Other clinical parameters such as smoking could not be obtained given the retrospective nature of the data. Also, therapeutic drug monitoring was not available during the time of this study and therefore the end-point of anti-TNF cessation or switch was used as the definition of secondary loss of response. However, myopenia was identified to be a significant factor after

adjusting for biomarkers of disease severity such as CRP and albumin. Although albumin may be a marker for chronic disease, this was not seen to be significant on multivariable analysis. The use of CT in the cohort was selected to provide uniform imaging for segmentation analysis. However, segmentation would also be possible with MRI examination and if the study were replicated within the last 5 years, MRI examination would have been selected. The lack of anti-TNF drug levels also limits the conclusions about the possible pharmacokinetic effects of primary nonresponse. Furthermore, this work will lead onto further studies detailing adequate dosage adjustments which may be made with body composition markers as the main determinant rather than body mass index and with the measurement of drug levels, which will have implications for therapy. Potentially, measuring body composition in patients prior to anti-TNF therapy may allow for a more accurate dosing paradigm in personalizing medical therapy.

2.7 Conclusion

Body composition is important in analysis of patients with Crohn's disease. Our study has identified myopenia prior to anti-TNF administration was associated with primary nonresponse of anti-TNF therapy in Crohn's patients. Adjusting dosage and undertaking a holistic management approach with nutrition and muscle-building exercises may improve therapeutic outcomes in patients commencing anti-TNF therapy.

Chapter 3: Crohn's Anastomotic Dilatation and Anti-TNF Therapy Response

Having established myopenia as a clinical factor associated with primary non-response in our previous study into body composition, we sought to evaluate factors associated with primary non-response in a separate clinical cohort. In this chapter we sought to evaluate the need for balloon dilatation or re-operation in patients with known anastomotic strictures who were treated with anti-TNF therapy in order to determine factors associated with treatment success or failure.

3.1 Introduction

Crohn's disease results in surgical resection in over 50% of patients within the first 10 years of diagnosis.²⁶⁰ Within this cohort of patients, 90% will develop disease recurrence at the anastomosis due to ongoing inflammatory activity.²⁶¹ Attempts to attenuate this with escalation in medical therapy for high-risk patients have demonstrated improved outcomes in disease activity.^{262,263} However, up to seventy percent of patients with prior surgery will require a further resection.²⁶⁴

Assessing recurrence of disease and possible luminal narrowing using radiology with computer tomography and magnetic resonance imaging, may help to demonstrate pre-stenotic dilatation and transmural activity of disease as shown by bowel wall thickening and mucosal enhancement in the pre-stenotic lumen.^{265,266}

Control of moderate to severe Crohn's disease using combination therapy with immunomodulators and anti-TNF medications have been demonstrated to be an effective regimen.²⁵⁶ In Crohn's disease patients who have undergone ileocolonic resection and anastomosis, the addition of azathioprine or anti-tumour necrosis factor-alpha therapy when there are high risk lesions present at a 6 month colonoscopy is associated with better disease control.²⁶⁷ Patients who have progressed to stricturing at the anastomosis, fall into a high-risk category, as it is usually due to disease recurrence at the anastomotic site. However, limited data is available on whether escalation of medical therapy following dilatation of anastomotic strictures, may prevent the need for repeat dilatation or surgery. In a recent meta-analysis, short-term success rates of endoscopic balloon dilatation in avoiding surgery are estimated at 78% with a complication rate of 2%.²⁶⁸ Long-

term outcomes in many studies are only limited to a follow-up period of 3 years with small cohort sizes. Moreover, endoscopic disease, radiologic and serologic markers of disease activity at the time of dilatation along with the effect of alterations in medical therapy on outcome of stricture dilatation is not known. The primary aim of the study was to demonstrate the long-term efficacy and safety of endoscopic balloon dilatation of Crohn's anastomotic strictures in a large referral centre cohort. We also aimed to show the effect of medical therapy and disease activity on long-term clinical outcomes.

3.2 Methods

Patients were identified via a search of the hospital endoscopy database using the terms "endoscopic dilatation", "balloon", "dilatation", "CRE" and "wire". Manual checks were performed to ensure the inclusion criteria had been met, with *de novo* Crohn's strictures excluded. Dilatations performed between 2004 and 2009 were extracted. A detailed review of the clinical notes provided retrospective data on site of stricture, initial surgery, medical management and clinical outcomes. Ethics was obtained via Brent REC North West London Hospital R&D department 08/H0717/24.

3.2.1 Management Protocol

Patients at our institution had endoscopic dilatation performed due to obstructive symptoms including nausea, vomiting and abdominal pain. Typically, dilatations were performed via standard colonoscopy or flexible sigmoidoscopy with a 'through-the-scope' balloon (Rigidflex or Controlled Radial Expansion Wire guided Balloon Dilatation CatheterTM, Boston Scientific, Boston, MA, United States), with diameters from 8 mm to 20 mm on inflation and lengths of 30–80 mm. The balloon was filled with water under visual control and graduated dilatation under direct vision performed, typically with a one-minute dilatation time at each set diameter; no more than a total of increment of 3mm was usually dilated at any one sitting. Therapeutic success was defined as the ability to pass the scope through the stricture following dilatation.

A modified Rutgeerts score²⁶⁷ was graded by a single experienced endoscopist (AH) blinded to the outcome viewing images retrospectively from the initial endoscopy with activity of disease at the anastomosis site being the most

relevant. Therefore, no recurrence was defined as i0 (no lesions) or i1 (≤ 5 aphthous lesions); recurrence was defined as i2 (>5 aphthous lesions or larger lesions confined to anastomosis), i3 (diffuse ileitis), or i4 (diffuse inflammation with large ulcers at anastomotic site). Histology of the biopsied anastomosis was reviewed from those available with particular attention to fibrosis and activity of disease.

Patients had escalation of medical therapy, which was defined as commencement of a thiopurine or anti-TNF within 6 months of the first dilatation as determined by global physician assessment. Combination therapy was defined as the use of a thiopurine and anti-TNF drug (Figure 10).

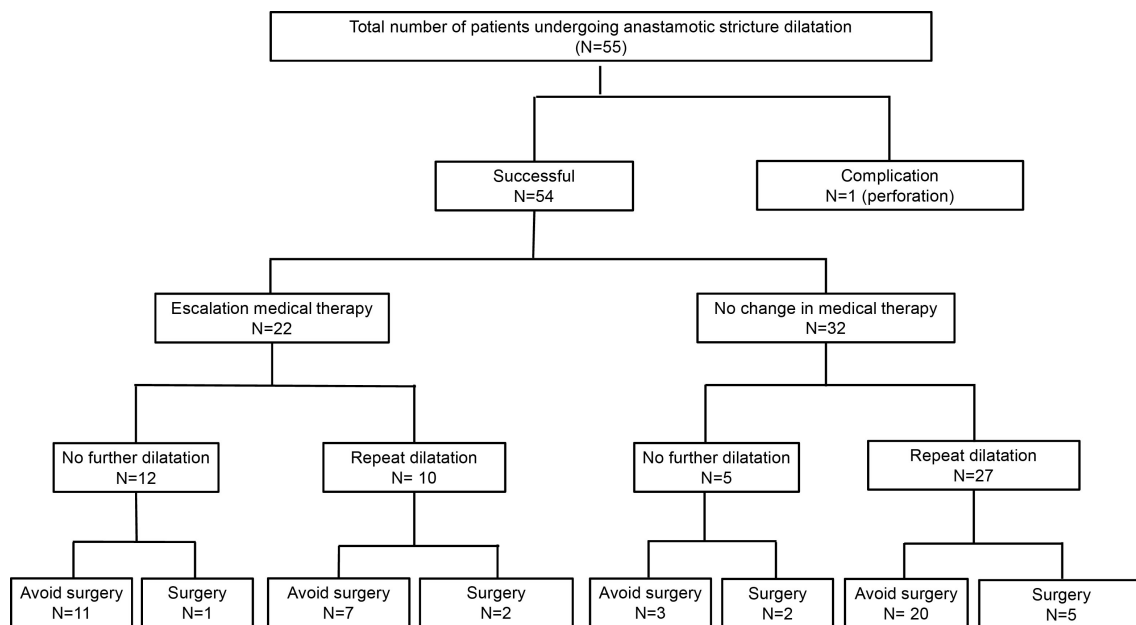


Figure 10. Flowchart with study design.

3.2.2 Outcomes Measures

Immediate therapeutic success was defined as the ability to pass the scope through the stricture with clinical success being defined as improvement of obstructive symptoms. If persistent pain following dilatation occurred, this was assessed clinically and possible imaging with onward surgical management sought. Patients were followed up until one of the primary endpoints were met 1) resection of anastomotic stricture, 2) last clinic follow-up, or 3) censor date of 1/6/2014. Long-term efficacy was defined as avoidance of surgical resection or repeat dilatation after the first dilatation. The long term (>5 yrs) follow up data was

analysed to assess the impact of endoscopic, histologic and radiological disease activity and escalation of medical therapy on repeat dilatation and resection rates.

3.2.3 Statistics

Descriptive statistics were used to analyse patient demographics. Chi-squared test was used for differences in proportions of patients experiencing a given outcome. Kaplan- Meier survival analysis with log rank statistics was used to assess event-free survival and Cox conditional proportional hazards regression analysis used to assess predictors of recurrence, including endoscopic and biochemical disease activity. All continuous variables were reported with median and interquartile range (IQR) and dichotomized for analysis using the median value as the cut-off. Analysis of long-term outcomes was performed on patients who had a successful initial dilatation and did not suffer from perforation.

3.3 Results

3.3.1 Patient Characteristics

A total of 54 patients were identified, median age was 52 years (IQR, 46–62 years) and 21/54 (39%) were male. The median follow-up period was 6 years (IQR, 5–7 years) with a median disease duration of 28 years (IQR, 19–32 years). Patient's demographics are listed in Table 9. All dilatations were performed at the anastomosis with the underlying surgery being ileo-caecal resection in 30 (55.5%), right hemi-colectomy in 22 (40.7%) and segmental resection in 2 (3.7%). Eighty-seven per cent of patients were on some form of medical therapy at the time of dilatation: 8(14.8%) on 5-ASA; 33 (61.1%) on thiopurines; 3 (5.6%) on anti-TNF medication.

Table 9. Demographics of patients in cohort.

Demographics	
Number of patients	54
Number of dilatations	151
Gender (M/F)	21/33
Median age Med (IQR)	53 years (46–62)
Disease duration (IQR)	24 years (17–30)
Median follow-up (IQR)	5.8 years (5.0–8,9)
Original surgery	
Ileocaecal resection	40 (55%)
Right hemicolectomy	22 (40%)
Other segmental resection	1 (2%)
Small bowel resection	1 (2%)
Disease phenotype at diagnosis	
Location of disease	
L1 – terminal ileum	16 (29.6)
L2 – colon	16 (29.6)
L3 – ileocolonic	22 (40.7)
L4 – upper GI	0 (0)
Behaviour of Crohn's disease	
B1 – inflammatory disease	29 (53.7)
B2 – stricturing disease	9 (16.7)
B3 – penetrating disease	16 (29.6)
No of prior surgeries, n (range)	2 (1–4)
Medical therapy at first dilatation	
5-ASA	8 (14.8%)
Immunomodulator	33 (61.1%)
Anti-TNF	3 (5.6%)
Activity of disease (out of 50 graded)	
I0–i1	10/50 (20%)
I0–i2	36/50 (72%)
I3–i4	14/50 (28%)

3.3.2 Disease Characteristics

MRI, CT, or barium follow through was used to assess the stricture in all cases: median length was 20mm (IQR 10–30 mm) with features of active mucosal inflammation described at the anastomosis in 38/54 (70%) and upstream dilatation in 25/54 (46%). Endoscopic images were used to determine active disease and assessment with Rutgeerts scoring was possible in 50/54 cases with

a median of i2 (range 1–4); endoscopic images were not available from four of the index colonoscopies.

3.3.3 Efficacy of Endoscopic Dilatation

Repeat dilatation was required in 37/54 (69%) of patients. The median number of dilatations was two (IQR 1–4) with a median time to first repeat dilatation of 23 months (IQR 7.2–56.9). Therapeutic success (ability to pass colonoscope through stricture after dilatation) was achieved in 48/54 (89%) cases with a median balloon dilatation of 15mmHg and a clinical success of 98%.

3.3.4 Effect of Clinical and Endoscopic Factors on Repeat Dilatation

On univariate analysis, stricture length >40mm (HR 1.38 1.23–4.51 p=0.04) was associated with a need for repeat dilatation. Other factors such as gender, age, duration of disease and disease activity (Rutgeerts score $\geq$ 2) at time of first endoscopy did not predict for repeat dilatation (Table 10).

Table 10. Univariate analysis of clinical, radiologic, endoscopic and medical therapy on outcome of repeat dilatation.

	HR	95% CI	p-value
Age > 53	0.91	0.48–1.73	0.77
Sex (M)	1.66	0.87–3.17	0.12
Duration of disease >24*	1.08	0.56–2.08	0.82
Radiologic			
Length >40mm *	1.38	1.23–4.51	0.04
Mucosal enhancement	2.05	0.93–4.52	0.08
Upstream dilatation	1.54	0.80–2.96	0.19
Medical therapy			
Before initial dilatation			
Steroids	0.93	0.33–2.64	0.89
Immunomodulator	1.14	0.57–2.26	0.71
5-ASA	1.88	0.66–5.33	0.24
Anti-TNF	1.67	0.40–7.13	0.48
< 6 months after initial dilatation			
Immunomodulator	0.68	0.32–1.45	0.32
5-ASA	1.11	0.39–3.15	0.45
Anti-TNF	0.36	0.16–0.83	0.02
Combination therapy* #	0.26	0.09–0.75	0.01
Endoscopic factors			
Size of balloon:			
<15mm	ref		
≥15mm	1.49	0.58–3.83	0.41
Modified Rutgeerts score			
i3-i4*	1.46	0.66–3.21	0.35
i4	1.05	0.45–2.42	0.92

*Variables included in the final multivariate model

anti-TNF therapy and immunomodulators

3.3.5 Effect of Medical Therapy on Repeat Dilatation

Following initial endoscopy and dilatation, five (9.2%) patients were on a 5-ASA with 43 (79.6%) on an immunomodulator and 16 (29.6%) on an anti-TNF. There was escalation of medical therapy in 22 (40.7%) with combination therapy being used in 12 (22.2%). The use of combination medical therapy (HR 0.26 95%CI 0.09–0.75 p=0.01) was found to decrease the need for repeat dilatation (Table 10). In further subgroup-analysis, anti-TNF therapy was the only medication found to be significantly associated with a decreased risk of repeat dilatation (HR 0.36; 0.16–0.83; p=0.02).

Four variables were chosen due to existing evidence predicting outcomes in Crohn's disease and entered into a multivariate model: duration of disease; stricture length; activity of disease and combination therapy (see Table 11).^{32,268,269} Combination therapy was significantly associated with a reduced need for repeat dilatation (HR, 0.23; 95% CI, 0.07–0.67; $p=0.01$) (see Figure 11). The remaining 32/54 (59.3%) who did not have escalation in medical therapy received a repeat dilatation prior to any changes in medications with a time to repeat dilatation of 19 months (IQR 5.75–46.58).

Table 11. Multivariate analysis – repeat dilatation outcomes.

	HR	95% CI	<i>p</i> -value
Length > 40mm	1.40	0.40–4.80	0.61
Combination therapy	0.227	0.07–0.67	0.01
Duration of disease > 24 years	1.10	0.54–2.26	0.79
Rutgeerts score > i3	1.31	0.59–2.94	0.51

Of the patients who had repeat dilatation, longer duration of disease (>24 years) was associated with a shorter time to second dilatation ($p=0.048$).

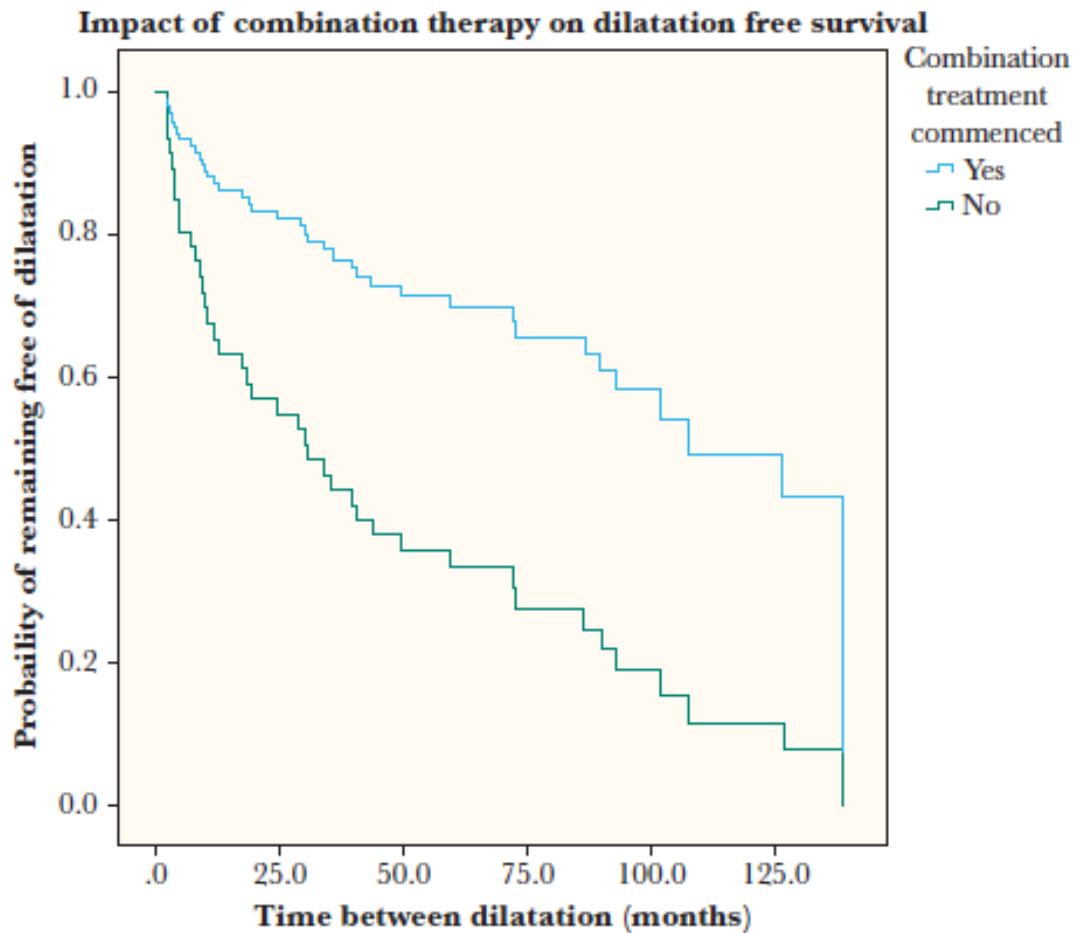


Figure 11. Impact of combination therapy on dilatation-free survival.

3.3.6 Effect of Clinical, Radiologic, Endoscopic Factors and Medical Therapy on Surgery

After initial dilatation, 10 (18%) patients progressed to surgical resection of anastomotic stricture due to worsening or recurrence of symptoms. The median time to progression to surgery was 2.4 years (1.8–3.9). On univariate analysis, the factor which predicted for surgery was Rutgeerts i4 (HR, 3.33; 95%CI, 1.27–8.74; $p=0.03$) (see Table 12).

Table 12. Univariate analysis of clinical, radiologic, endoscopic and medical therapy on outcome of surgical resection.

	HR	95% CI	p-value
Age > 53	0.90	0.238–3.429	0.88
Sex (M)	2.14	0.57–8.03	0.26
Duration of disease >24yrs	6.00	0.74–48.61	0.12
Radiologic			
Length >40mm *	1.19	0.15–9.60	0.09
Mucosal enhancement	4.80	0.59–39.12	0.14
Upstream dilatation*	1.76	1.12–2.76	0.08
Medical therapy			
Post			
Immunomodulator	2.00	0.25–16.01	0.52
5-ASA	1.13	0.35–1.25	0.57
Anti-TNF*	0.167	0.021–1.362	0.09
Combination therapy	0.25	0.03–2.02	0.19
Endoscopic activity			
i3-i4	2.93	1.32–6.50	0.28
i4 *	3.33	1.27–8.74	0.03

* multivariate analysis performed on these factors

On multivariate analysis (See Table 13), using variables with $p < 0.1$ on univariate analysis (anti-TNF therapy, Rutgeerts i4, upstream dilatation, stricture >40mm), Rutgeerts i4 was correlated with increased likelihood of surgery (HR, 4.55; 95%CI 1.08–19.29; $p = 0.04$).

Table 13. Multivariate analysis – surgical outcomes.

	HR	95% CI	p-value
Upstream dilatation	1.27	0.29–5.52	0.75
Stricture > 40mm	2.17	0.43–10.90	0.35
Anti-TNF	1.83	0.34–9.82	0.48
Rutgeerts score >i3	4.55	1.08–19.29	0.04

3.3.7 Radiology and Correlation with Endoscopic Disease Activity

All images were reviewed with length of stricture, underlying mucosal enhancement or suspected signs of chronicity noted on intestinal imaging modalities. Radiological findings that included signs of active disease (T2-weighted hyperintensity) did not significantly correlate with active disease as

observed at time of endoscopy with an R value of -0.11 and kappa of -0.04 $p=0.57$.

3.3.8 Complication

There was one perforation, identified within 24 hours of the procedure where the patient had worsening abdominal pain and confirmatory cross-sectional imaging. This resulted in a resection of the anastomosis and temporary ileostomy, which was reversed 12 months later.

3.4 Discussion

Post-operative anastomotic stricturing Crohn's disease can be treated effectively with repeated balloon dilatation in the long-term as demonstrated by our long-term data. Our data shows that escalation of medical therapy to include an anti-TNF following initial dilatation may decrease the need for repeat dilatation. Stricture length >40mm was shown on univariate analysis to be a significant predictor for further dilatation. Severe disease as graded by Rutgeert's i4 disease at the time of initial endoscopy, increased the risk of repeat surgery. This data suggests that the presence of inflammatory disease at the site of an anastomotic stricture may benefit from an escalation of medical therapy to at least an anti-TNF, or more effectively combination therapy, to prevent the need for repeat intervention.

After bowel resection for Crohn's disease, the anastomosis is the most common location of recurrence.²⁶⁷ Disease progression results in luminal narrowing and stricturing disease.²⁷⁰ Avoidance of further resection is possible with the use of balloon dilatation.⁸⁴ As far as we are aware, this patient cohort represents the longest follow-up period in the biological era where use of anti-TNF therapy was routine, in a scheduled fashion and with the likelihood of concomitant immunomodulator therapy. No association between activity of disease at time of initial endoscopy and future outcomes has been identified. However, our study shows that disease activity was associated with future surgical resection.

Our data demonstrates that longer disease duration was associated with a shorter time to repeat dilatation. This may be due to the underlying pathology being a fibrotic, post-surgical stricture with healing occurring in a defined pattern,

which may progress to luminal narrowing despite escalation of medical therapy.²⁶⁷

We demonstrate that combination therapy with immunomodulator and anti-TNF may decrease the need for repeat dilatation and improve long-term outcomes in conjunction with endoscopic dilatation. Van Assche *et al.*²⁷¹ found no significant predictors of re-intervention in terms of CRP or endoscopic activity, nor was there any influence due to concomitant medications. Patients receiving anti-TNF in the Van Assche study may also have had more severe disease as demonstrated by the rate of repeat dilatation being high at 13.8% per patient-year.²⁷¹ Furthermore, the patients on anti-TNF therapy in their study had the shortest follow-up period out of all groups of medications prescribed (2.8 years). This may not be enough to demonstrate the longer-term effects of anti-TNFs on strictures post-dilatation. We found no significant difference in follow-up time between those who had escalation in medical therapy versus those who had no change to medical therapy.

Furthermore, severity of disease activity at initial endoscopy (Rutgeert's i4) was found to predict a greater risk of future surgery with our standardized grading of disease activity. In this cohort of patients, our data would suggest that, where active inflammation is present, escalation of therapy to include at least anti-TNF is necessary to augment the natural history of Crohn's disease and improve clinical outcomes.

Nanda *et al.*²⁷², in a smaller cohort of 31 patients with a shorter follow-up period (median of 46 months), demonstrated that 21% avoided further dilatation or surgery with a median time to repeat dilatation of 13.8 months. Again, in this study no difference was noted between those that needed repeat dilatation or not when placed on immunomodulators or anti-TNF therapy. 8% of patients had anti-TNF therapy prior to initial dilatation, which increased to 45% following dilatation in their cohort, which concurs with the proportions who were escalated in our study.

A systematic review by Hassan *et al.*²⁶⁸ of 13 studies reported a similar technical success rate of 86% and long term efficacy of 58% with a major complication rate of 2% overall. It also stated that stricture length <40mm was associated with

surgery-free outcome. Similarly, stricture length < 40mm was associated with decreased risk of re-dilatation in our cohort on univariate analysis.

The only previous published study that reported a significant difference in terms of medical therapy was by Honzawa *et al.* in a small cohort of 25 patients.²⁷³ Their data showed patients who were commenced on immunomodulator therapy prior to initial dilatation had fewer repeat dilatations than those commenced post-dilatation (1.6 vs 4.8 p=0.04), however the intervals between dilatations were not significantly different.

The data surrounding the use of anti-TNF therapy in stricturing Crohn's disease has differed widely. Initial studies suggested that anti-TNF worsened stricturing as the inflammatory component is replaced by fibrosis with luminal narrowing.¹⁴⁷ Van Assche *et al.*⁸⁴ also reported that those treated with anti-TNF had higher rates of surgery. However, more recent data from ileal strictures shows that the progression to surgery is the same.³² The successful treatment of symptomatic strictures with anti-TNF therapy in several studies means that the inflammatory component may heal without stricturing.^{129,274}

Endoscopic dilatation techniques differ between previous published studies and the reported immediate success rates vary between 73% and 100%. In our study we showed an excellent 53/54 (98%) immediate success without need for repeat dilatation in 17/54 (31%) or surgery in 82% of patients after a first dilatation.

There was a significant difference in the need for repeat dilatation between patients receiving escalation of medical therapy to combination treatment and those having no change to their medical therapy (45% vs 84% respectively).

Our study has some limitations. Due to its retrospective nature, similar to previous studies, it lacks a control group (patients going direct to surgery) and patients were escalated as part of medical therapy, as determined by the clinician. The degree of luminal narrowing caused by inflammation versus fibrosis is unknown and escalation of medical treatment would have been biased toward those having active inflammatory disease. Therefore, the patients with active disease were likely selected to escalate to combination therapy with anti-TNFs and immunosuppressives, which may explain the positive effect on surgery and re-

dilatation free survival. A further limitation was that Rutgeerts scoring was graded from endoscopic images, which would have precluded the accurate grading in those where the scope could not be passed through the stricture (11%). Two out of three patients underwent surgery after initial dilatation without escalation in medical therapy. After removing these patients, (HR,0.34; 95% CI 0.13–0.87, $p=0.024$) combination therapy was still significant.

In a recent European Crohn's and Colitis Organisation (ECCO) statement on the topic, it was concluded that endoscopic balloon dilatation was safe and effective at avoiding surgery in patients with anastomotic stricture.²⁷⁵ Infliximab injected locally with dilatation had significantly better outcomes with avoidance of surgery in all twelve patients versus three out of eleven in the group that had dilatation alone.²⁷⁶ The true benefit of these types of novel endoscopic treatments is still unknown, and further data is needed.

3.5 Conclusion

In summary, the long-term data from our cohort demonstrates that Crohn's anastomotic strictures can be successfully managed with endoscopic balloon dilatation and may avoid the need for further surgery. Escalation of medical therapy in those with active inflammatory disease at the site of the anastomosis to include combination therapy with a thiopurine and anti-TNF appears to reduce the need for repeat dilatation and surgery. Moreover, severe active inflammation (Rutgeerts) at the anastomosis may predict the need for further surgery, thus escalating medical therapy early in this group could possibly alter this outcome in the long-term.

Chapter 4: Endoscopic and Histologic Predictors of Response to Anti-TNF Therapy in Crohn's Disease

Despite the advent of thiopurines and biologic therapies the risk of surgery remains high in patients with Crohn's disease. This chapter aimed to explore whether there was any association between endoscopic and histologic disease activity and subsequent outcome in a study of anti-TNF treated patients with Crohn's disease.

4.1 Introduction

The main aim of treatment in Crohn's patients is to induce and maintain remission over time. In order to assess this, symptom reduction and improvement in quality of life is measured. Alongside the measurements in symptoms, endoscopic assessment for mucosal healing has become an important target.¹⁰

4.2 Assessment of Severity and Healing

Objective assessment of disease severity is currently performed in everyday practice and across clinical trials using the Crohn's disease activity index (CDAI).^{277,278} Rutgeerts *et al.*²⁷⁹ surmised the importance of "complete healing of bowel ulcerations in parallel with clinical remission." Currently, mucosal healing can be assessed using the validated Crohn's disease endoscopic index of severity (CDEIS). Unfortunately inter-observer differences also exist in the CDEIS scoring system.²⁸⁰ and according to Cellier *et al.* in a prospective multicentre study (n=121),²⁸¹ very little correlation exists between the CDAI and the CDEIS in Crohn's disease patients.

Inflammation from Crohn's disease is transmural and may damage the continuous layer of cells in the mucosa, leading to ulcers of various sizes. It therefore follows that the best way to define mucosal healing is by the restoration of tissue architecture back to its original state.²⁸² Therefore, a deeper form of healing may be defined using histologic endpoints. Whilst a specific histological definition of mucosal healing is lacking, mucosa with limited architectural abnormalities, but normally differentiated epithelial cells and no signs of active inflammation (presence of neutrophils) or an increased density of lymphocytes and plasma cells, has been suggested as evidence of histological healing.²⁸³ This

definition incorporates a lack of inflammation and as such would be the ideal endpoint measurement in treating an inflammatory bowel disease. Unfortunately, there is very little evidence regarding histologic measurements for mucosal healing in Crohn's disease due to the presence of skip lesions and sampling error in biopsies, resulting in the lack of a validated scoring system.^{2,10,282}

4.3 Medical Management

4.3.1.1 Evidence for Biological Therapy

The advent of biological therapy, particularly anti-TNF drugs have revolutionised the treatment of Crohn's disease due to its efficacy. An endoscopic study performed on a subset of ACCENT I patients (n=99) by Rutgeerts *et al.* which reviewed mucosal healing following induction IFX therapy,^{24,284} demonstrated that mucosal healing was associated with fewer hospitalisations, and maintenance IFX led to greater rates of mucosal healing in the long term. A cohort of 214 patients with Crohn's disease followed up over 3 years demonstrated that mucosal healing on endoscopy at a median of 6 months, was associated with sustained long-term clinical benefit with regards to significantly better disease-free survival when compared to patients who lacked mucosal healing (76.9% versus 61%).²⁸⁵ Patients who received concomitant corticosteroid and biological therapy had lower rates of mucosal healing than those who only had biological therapy.

Mucosal healing in patients receiving adalimumab was evaluated in the EXTEND study, which showed significant mucosal healing in patients who were on maintenance therapy.²⁸⁶ This seminal study was the first to use mucosal healing as the primary endpoint.

4.3.1.2 Histologic or Endoscopic Predictors of Primary Nonresponse or Loss of Response

D'haens *et al.* investigated histological healing in 30 CD patients treated with infliximab. The infliximab treated patients achieved improvement in inflammatory infiltrate compared to the placebo group after initial infusion.²⁸⁷ Immunohistochemical stains also revealed a decrease in inflammatory cells due to a reduction in CD4+ and CD8+ T lymphocytes.²⁸⁸ The body of evidence demonstrating that histologic remission is achievable, has been largely derived

from studies involving patients with ulcerative colitis. This is due to the underlying disease factors such as the affect on mucosal layers of the colonic lumen, and the proximal extension of disease in a contiguous pattern from the rectum, which creates less sampling error. Histologic remission was shown to be possible from biologic therapy in a cohort of 646 UC patients, with 40% achieving histologic quiescent disease while 10% having histologic normalization. Histologic normalization was associated with increased odds of relapse-free survival therapy (HR, 4.31; 95% CI, 1.48–12.46; $p=.007$).¹²

4.4 Aim

The aim of this study was to identify any endoscopic or histologic parameters which may be predictive of primary nonresponse or secondary loss of response to anti-TNF therapy in patients with Crohn's disease.

4.5 Method

This was a retrospective cohort study of Crohn's disease patients who underwent anti-TNF treatment at St. Marks Hospital, London between January 2007 to June 2014. The patients were from the IBD department with confirmation performed from the hospital pharmacy database. This produced a total number of 583 patients. Each patient was then reviewed individually, and 80 patients were excluded due to the final diagnosis not being Crohn's disease. Electronic notes were reviewed for the remaining 503 patients. Of the 503 patients, a further six were excluded due to a lack of anti-TNF start date, 62 had no endoscopic records, 184 did not have an endoscopy prior to anti-TNF therapy, and one had a computed tomography (CT) virtual colonoscopy. The endoscopy records for the remaining 250 patients were acquired and added to the database with further exclusion of 13 patients due to incomplete endoscopic data (Figure 12).

Clinical and biochemical information retrieved from the electronic notes were used in combination with anti-TNF data to determine whether or not a patient fell under the category of response, PNR or SLOR. PNR was defined as 'Lack of improvement within 3–6 months of commencement of anti-TNF therapy determined by global assessment.' This was performed by a gastroenterologist using clinical information regarding symptoms and biochemical information, specifically markers of inflammation such as C-reactive protein (CRP),

erythrocyte sedimentation rate (ESR) and faecal calprotectin. SLOR was defined as cessation of anti-TNF in view of clinical deterioration as assessed by their treating gastroenterologist. Endoscopy records were also collected where available.

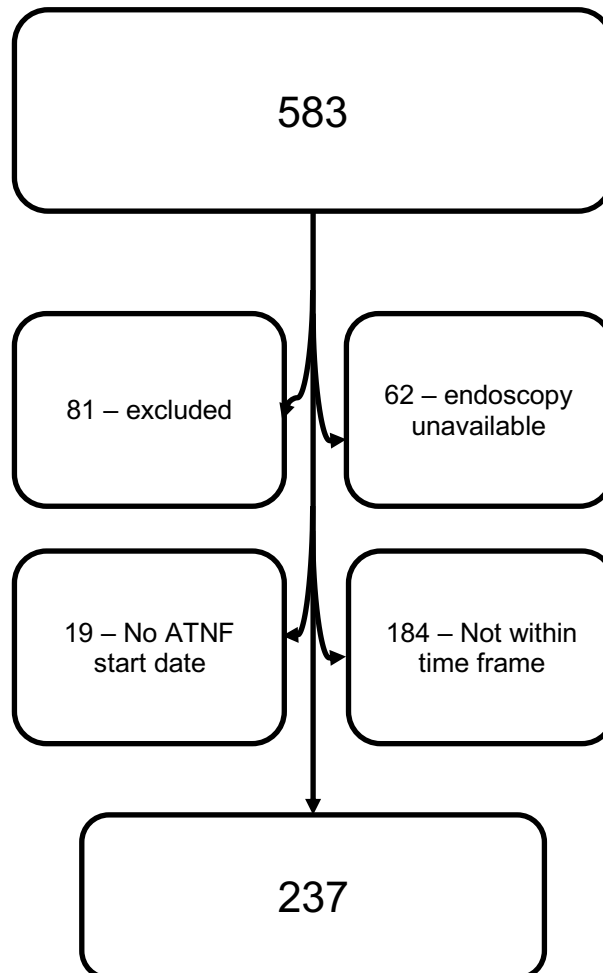


Figure 12. Flowchart detailing study design

Of the 237 patients who had endoscopy records up to one year prior to their first date of treatment, 198 had an associated histology report. 83/237 also had an endoscopy 6–12 months after the start of their treatment, with 48 patients with histologic data. Each of the endoscopic reports were then reviewed and the data coded into variables; specifically, the location of the disease, behaviour of the disease, presence of ulcers and/or evidence of previous surgery (Table 9).

Histologic data was coded according to:

- Granuloma
- Lymphoid aggregates

- Mucin depletion
- Cryptitis
- Crypt abscess
- Presence or inflammation (or infiltration by inflammatory cells)
- Crypt architecture distortion

4.5.1 Statistics

Data were analysed using SPSS statistics software (Version 20.0. Armonk, NY: IBM Corp). Univariate analysis was assessed with the use of Pearson χ^2 and Fishers exact tests, Wilcoxon rank-sum test was used to determine non-parametric variables, and Kaplan-Meier survival curves was used for length of time to SLOR. Cox regression and binary logistic regression tests were used for multivariate analysis. The 3 outcome variables were PNR, SLOR and PNR/SLOR.

4.6 Results

A total of 237 patients were eligible for data analysis. 136/237 (57%) were female with a median age of 30 (IQR 24–68). 76% had IFX treatment with 11% of the cohort having PNR while 21% had SLOR (Table 14).

Table 14. Basic demographic information for the 237 patients.

Demographics	N (%)
Sex (M/F)	101/136
Infliximab/adalimumab	180/57
Status	
PNR	24 (11)
Secondary LOR	44 (21)
Ongoing	122 (58)
Remission	17 (8)

4.6.1 Endoscopic and Histologic Predictors

The most common segment of disease activity was ileal in 89 (38%) of the cohort (Table 15). The median age was 34 years with 101 (43%) males and 180 (75%) on infliximab therapy.

Table 15. Endoscopic and histologic parameters identified at baseline colonoscopy.

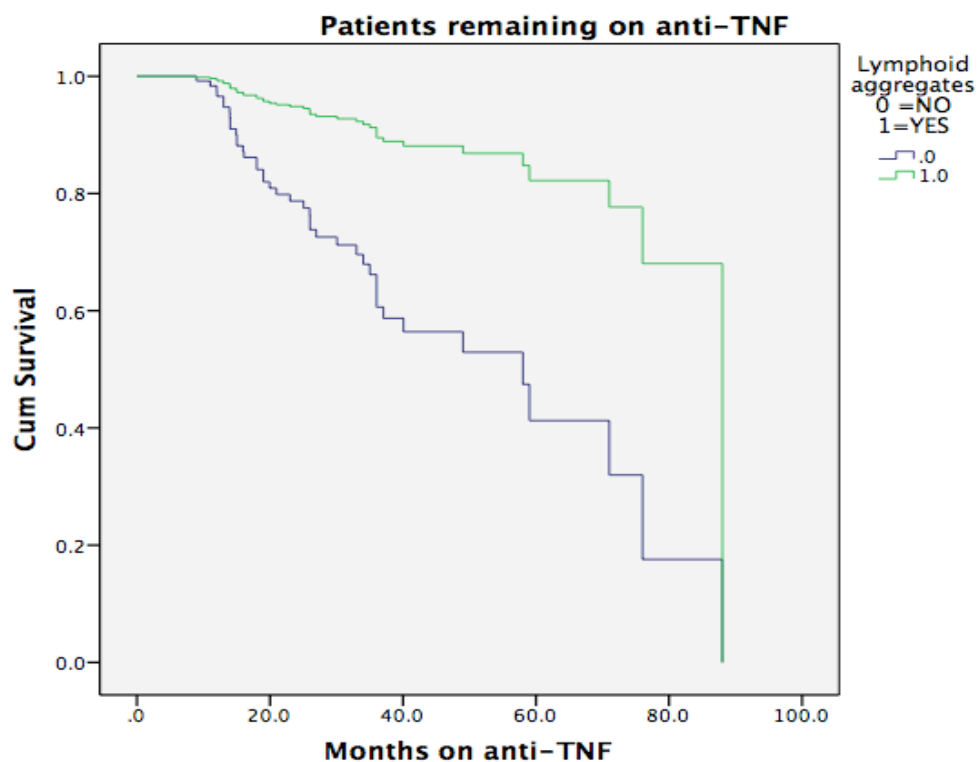
	N (%)
Endoscopic variables	
Ileal	89 (38%)
> 5 segments of disease	50 (21%)
Right sided	47 (19%)
Left sided	30 (12%)
Histologic variables	
Granuloma	20 (8%)
Lymphoid aggregates	35 (15%)
Mucin depletion	33 (14%)
Cryptitis	178 (75%)
Crypt abscess	134 (57%)

4.6.1.1 Endoscopy and Histologic Activity Affecting Response to Anti-TNF Therapy

When considering all variables for endoscopic and histologic activity, on univariate analysis for PNR, the presence of ileal involvement as assessed by endoscopy (OR:0.56, $p=0.046$) and presence of granulomas were significantly associated with lower rates of primary non response (OR: 1.93, $p=0.04$) while >5 segments of disease at colonoscopy (OR:2.1, $p=0.012$) were associated with primary nonresponse. The results for endoscopy and histology are shown in Table 15. Smoking was shown to be significant on univariate analysis for predicting both PNR ($p=0.048$) and SLOR ($p=0.018$). A Kaplan-Meier (K-M) curve demonstrated a significant ($p=0.038$ LogRank) protective effect of lymphoid aggregates for SLOR as shown in Figure 13. No other histologic or endoscopic variables were significant for secondary loss of response other than smoking.

Table 16. Table showing multivariate analysis for PNR

Variables	OR	p-value
Endoscopic variables		
Ileum	0.56	0.046
>5 segments of disease at colonoscopy	2.10	0.012
Right vs Left-sided	1.18	0.89
Histologic variables		
Lymphoid aggregates	1.04	0.56
Granuloma(s)	1.93	0.04
Crypt architecture distortion	1.23	0.78
Mucin depletion	1.14	0.23
Presence of inflammatory infiltrates	1.38	0.31

**Figure 13.** Survival curve demonstrating that lymphoid aggregates are protective against SLOR.

4.7 Discussion

Anti-TNF therapy remains the mainstay of treatment in patients with moderate to severe Crohn's disease despite newer biologic agents being approved. Prior to the commencement of anti-TNF therapy, we sought to identify endoscopic or histologic parameters which may be predictive of nonresponse and loss of response to anti-TNF in patients.

The rate of PNR in our cohort was 11%, which is similar to other cohort studies assessing non response⁶⁸. This is likely due to the stringent exclusion criteria in RCT's, which may not be truly representative of real-world figures. Another retrospective cohort study conducted in the UK (Leeds) with similar numbers (n=210) reported a PNR rate of 8% and SLOR rate of 15.2% compared to the 21% in our study.²⁷

Ileal disease was found to be a predictor for better response to anti-TNF treatment in our cohort but was not seen in previously published studies by Vermeire *et al.*⁵⁸ and Laharie *et al.*²⁸⁹ whose findings suggested that isolated colonic disease better predicts improved response rates compared to ileal involvement which predicted non response in the Belgian cohort (OR 0.36, p=0.004). The underlying reason for patients with >5 segments of disease being associated with PNR may be to the need for higher drug levels to heal the severely ulcerated mucosa or the leakage of drug across the mucosa.

In the literature, granulomas in Crohn's disease is reported to be seen in 30–70% of surgical specimens.^{290,291} Within our cohort, the presence of granulomas was seen in only 8% which is likely due to the collection of tissue samples for histology coming from mucosal biopsies. This under-representation may be due to a greater number of granulomas which may exist in the submucosa and serosa. Furthermore, errors in sampling may contribute to the small numbers, as biopsies taken from the ulcer edge are far more representative of the underlying disease activity than in the centre of the ulcers. Whilst there has been conflicting evidence supporting the theory of granulomas predicting a poorer outcome in Crohn's disease, a meta-analysis by Simillis *et al.* showed that granulomas are associated with greater risk of recurrence and shorter time to recurrence of Crohn's disease.²⁹² Granulomas have also been found to be associated with extra-intestinal manifestations of disease which have been known to be negatively impact disease outcomes.²⁹⁰ The results of this study indicate that granulomas predict PNR, but it is difficult to theorise why this may be the case due to a lack of understanding of both Crohn's disease and granuloma formation within the disease.

The presence of lymphoid aggregates was the only histologic or endoscopic variable protective associated with lower rates of SLOR. Crohn's disease results in changes to lymphoid aggregates as was demonstrated in a cohort of 77 patients undergoing resection with the various underlying diagnoses including colorectal cancer, ulcerative colitis and Crohn's disease. It was noted that the density of lymphoid aggregates in Crohn's specimens was increased in diseased specimens with a transmural distribution.²⁹³ One hypothesis for loss of response to biological therapy is that the underlying immunologic pathway for inflammation shifts after prolonged exposure to anti-TNF drugs.²¹

The main strength of the study comes from the inclusion of real-world patients with Crohn's disease within a specialised quaternary centre for IBD and the extended follow-up period which allowed for greater data capture. However, a potential weakness was the changes in practice during this period (from episodic to sustained maintenance therapy during the follow-up period). The retrospective nature of the study also resulted in the inability to control for inter-observer variability in reporting with 10 pathologists reporting all the histologic specimens; although 3 pathologists reported over 50% of the histologic specimens. Furthermore, findings on endoscopy and histology would have affected clinical decisions regarding altering therapy which may have confounded the results and therefore impacted the conclusions that can be drawn from these data.

In conclusion, predictors for primary nonresponse include; patients with ileal disease, >5 segments of disease activity, granulomas within biopsy specimen and smoking, while lymphoid aggregates present within the biopsy specimen have been shown to protect against loss of response. Further investigation and validation of these results is required in a prospective observational cohort study.

Chapter 5: Metabonomics and the Gut Microbiome Predict Response to Anti-TNF Therapy in Inflammatory Bowel Diseases

In the previous chapters we have undertaken an evaluation of invasive predictors of response to anti-TNF therapy using endoscopy and histology. In this chapter we sought to evaluate non-invasive biomarkers of anti-TNF response in Crohn's disease using metabolic profiling.

5.1 Introduction

Inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are lifelong and life-limiting, relapsing and remitting inflammatory disorders that affect the intestine.²⁹⁴ The aetiology is poorly defined, but it is clear that both environmental and genetic factors predispose an individual to the development of IBD.^{295,296} With regard to the contribution of genomics to the field of IBD, over 250 host susceptibility loci have been identified to date.²⁹⁷⁻³⁰⁰ It is clear that in addition to these loci, there are environmental triggers and other host factors that play an important role in pathogenesis and in determining specific phenotypes which predict response to IBD therapies.²⁹⁹

There are numerous new therapeutic targets for IBD^{301,302} with recent therapeutic strategies demonstrating that early initiation of treatment results in greater response.²⁵⁶ Therefore, personalising treatments to select the optimal therapy for disease control is crucial as the likelihood of response may decrease with each sequential biologic agent.³⁰² Anti-tumour necrosis-alpha (TNF- α) remains the backbone of treatment for patients with moderate to severe CD or UC, with primary response rates of 60–70% in initial controlled trials.²⁵⁰ Currently, there are no robust biomarkers for predicting response to biologic therapy when using clinical factors such as age, duration of disease, or BMI.¹⁵ There is the suggestion that colonic expression of α 4 β 7 is predictive of response to etrolizumab, an α E β 7 anti-integrin.³⁰³ Recently, in a prospective cohort of IBD patients starting therapy with vedolizumab, an α 4 β 7 anti-integrin (42 CD and 43 UC), the composition of the microbiota in the colonic microbiome, at baseline, was used to predict response to vedolizumab and the researchers demonstrated that *Roseburia inulinivorans* and a *Burkholderiales* sp. were more abundant at baseline among those CD patients achieving week 14 remission.¹⁰¹ Additionally,

community α -diversity at baseline was significantly higher in CD patients who achieved remission, but β -diversity was lower at baseline amongst remitters. Changes in bacterial function may be associated with response to biologic therapy. Specifically, 13 pathways including branched chain amino acid synthesis, were enriched in CD patients achieving remission on vedolizumab.

The microbiota is involved in driving inflammation in IBD² and a dysregulation of the microbiome has been reported in both CD and UC, with reduced diversity and temporal instability in the dominant taxa compared to healthy individuals.³⁰⁴ For example, reduced relative abundance of *Firmicutes* and anti-inflammatory microorganisms (*Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis* and *Dialister invisus*) have been demonstrated in analysis of faecal microbiota from CD patients.^{2,305-307} Microbiota sampling using a longitudinal approach has been shown to partially overcome the effects of microbial instability and more accurately classify CD patients based on their microbiota signature.³⁰⁸ Metabolic profiling of patients with IBD has also indicated that medium-chain fatty acid such as pentanoate, hexanoate, heptanoate, octanoate and nonanoate are important biomarkers of disease activity in IBD.^{244,304,309}

Metabolic phenotyping by high-resolution spectroscopy, either liquid chromatography-mass spectrometry (LC-MS) or nuclear magnetic resonance spectroscopy (NMR) can provide an additional tool for establishing the functionality of the gut microbiome and allows clinicians and scientists to gain new insights into the knowledge gap which exists in assessing the enteric microbial composition and their function. This metabonomic approach allows the detection of metabolites in IBD upon which hypotheses about their role in disease activity or health can be generated. Gut microbiota compositional differences in IBD patients has been linked to changes in metabolite concentrations and diversity such as bile acids (BA), lipids (including fatty acids), amino acids, and eicosanoids, all of which are important in inflammation, energy and signalling.³⁰⁹⁻³¹¹ For example, bile acid metabolism has been demonstrated in IBD patients to result in alteration in gut barrier homeostasis which predisposes patients to intestinal inflammation.^{304,312}

Using a prospectively recruited cohort of IBD patients prior to commencing anti-TNF therapy, faecal urine and serum samples from IBD patients were used to identify metabonomic and microbiota predictors of response to anti-TNF treatment. We classified patients according to objective markers of inflammation with C-reactive protein (CRP), faecal calprotectin (FC) and endoscopy/ imaging which were performed as part of routine care; as well as a longitudinal assessment of metabonomic biomarkers found in patients' samples to assess alterations in response and remission.

5.2 Methods

5.2.1 Sample Collection

Serum, urine and faecal samples were obtained from three cohorts; patients with CD (n=76), UC (n=23) (Figure 14 and healthy participants (HC, n=13). Patients with CD had induction doses of anti-TNF therapy starting at day 0 with either adalimumab (Humira®) or infliximab (Remicade®) (Figure 14A). Patients with UC were inpatients requiring salvage therapy for severe acute colitis without prior anti-TNF exposure. An aliquot of samples (urine, 5 mL), faeces (2 g) and serum (6 mL) were immediately frozen at -20°C and transported within 24 hours to a -80°C freezer for storage prior to analysis. Ethics was obtained from Brent NRES Committee London with HREC 08/H0717/24.

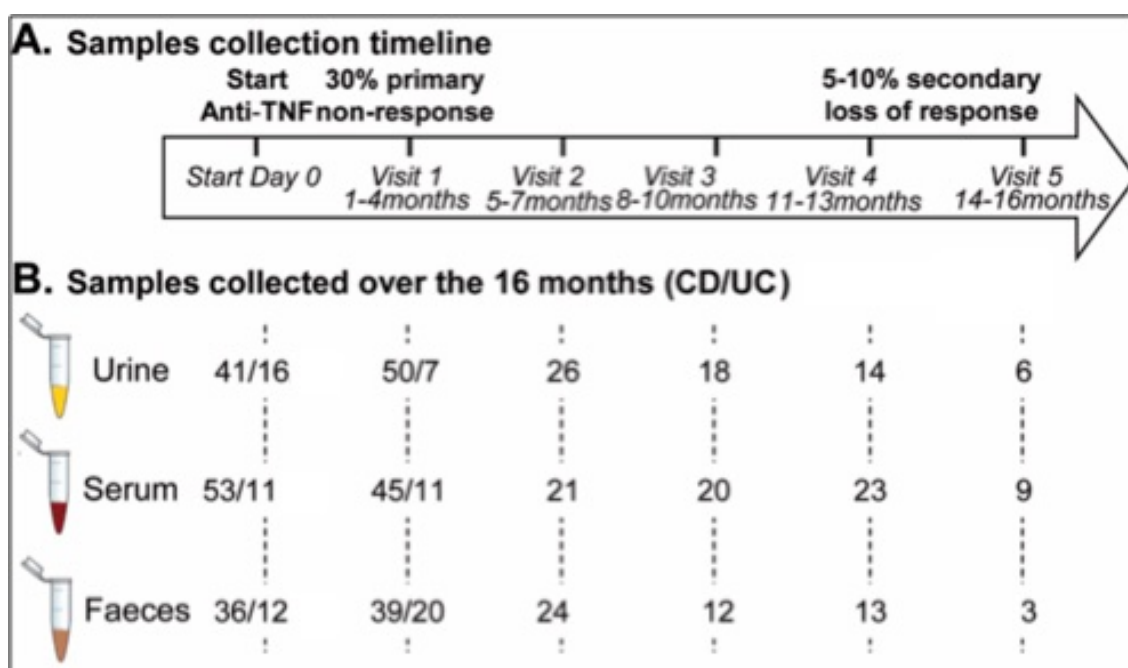


Figure 14. Longitudinal study on IBD patients' response to anti-TNF treatment (A). Urine (n=181), serum (n=214) and faecal (n=147) samples were collected from CD and UC with patient samples over 16 months (B).

5.2.2 Baseline Disease Activity Assessment

Participants in the control group had no prior history of gastrointestinal or metabolic disease and had not used antibiotics or undergone medical therapy influencing gut transit or microbiota in the preceding 6 months. Diagnosis of IBD (CD and UC) was confirmed by endoscopy, histology and radiological techniques. In addition, disease activity was assessed for the CD group applying clinical indices with CD Activity Index (CDAI) scores and for the UC cohort, the partial Mayo score.^{278,313} All patients had an objective measure of disease activity with FC and CRP at baseline. Exclusion criteria included any patient with a prior history of surgical resection of the gastrointestinal tract. Participants who met the control and IBD group criteria were included in this study and provided written informed consent. Clinical and biochemical characteristics of IBD patients are summarized in Table 17.

Table 17. Summary of clinical and biochemical characteristics of IBD patients.

		Normal range	CD	UC	p-value
	Age (IQR)	* 30.5 2.94(27–35)	38.49 14.61(19–82)	35.52 17.34 (19–70)	0.45
	% Female	* 54%	53%	48%	0.56
	Haemoglobin (g/L) (IQR)	120–175	127.71 14.52(87–159)	123.06 17.91 (98–152)	0.37
	White cell count ($\times 10^9/L$) (IQR)	4.5–10	7.51 2.92(2.9–20.1)	10.3 4.35 (4.4–18.1)	0.03
	Platelet count ($\times 10^9/L$) (IQR)	150–450	326.23 94.74(113–561)	352.33 83.78 (244–544)	0.31
	ESR (mm/hr) (IQR)	0–29	17.28 18.15(1–95)	11 9.91(2–21)	0.22
Inflammatory biomarkers	C-reactive protein (mg/L) (IQR)	< 5	12.49 14.85(1–78)	7.35 6.46 (1–17)	0.06
	Calprotectin ($\mu g/g$) (IQR)	0–250	699.22 655.78(7–2000)	534.21 446.9 (5.3–1170)	0.34
Liver function	Bilirubin (μM) (IQR)	3–25	7.55 8.22(2–59)	6 2.85 (3–14)	0.25
	Alkaline phosphatase (IU/L) (IQR)	44–147	83.89 29.79(41–190)	80.5 26.52 (48–156)	0.68
	Alanine aminotransferase (IU/L) (IQR)	20–60	25.56 15.26(7–73)	31.35 33.34 (11–114)	0.53
	Albumin (g/L) (IQR)	35–55	42.58 4.23(28–55)	40.64 6.89 (28–49)	0.33
Renal function	Creatinine (μM) (IQR)	45–110	69.12 16.62(34–113)	61.21 13.65 (35–86)	0.08
	CDAI/ MAYO index (IQR)	-	490 (409–501)	12 (10–12)	-
Response index classification	Responder (5–7)/non responder (0–1)	-	11/37	5/5	-

* Figures represent age and sex of healthy controls

5.2.3 Response Index to Anti-TNF Treatment Using Objective Markers of Inflammation

A “response index” for the response to anti-TNF treatment was created using a composite of FC, inflammatory biomarkers with *C-reactive protein* (CRP) and endoscopic or radiologic evaluation as was performed in clinical practice (Table 18). The criteria for defining response to anti-TNF were: a fall in FC by > 50% or a decrease of CRP to < 5mg/L with the inclusion of various time points at 2–4 months and 4–6 months. The initial 2–4 month time point was chosen to reflect patients who responded rapidly with resolution of CRP and/or FC. However, this does not capture patients who may still respond to anti-TNF therapy but due to

high inflammatory burden, require a longer timeframe for assessment. Therefore, those who are likely to respond to therapy had further assessment of objective markers of CRP and/or FC at 4–6 months (see Table 18). The combined total score out of 7 was calculated for each patient. In this study, non-responders to anti-TNF had a score ranging from 0–1, partial responders 2–4 and responders 5–7. Patients had evidence of mucosal healing confirmed with endoscopic assessment and radiology with MRI-small bowel organised as part of standard of care within 6 months depending on the location of CD. All responders based on our objective index also correlated with response according to more subjective measures with a decrease in CDAI of 100 points by 3 months.

Table 18. Scoring system of response index using objective markers of mucosal healing and inflammation on CD and UC patients. Seven criteria were selected to classify response of CD patients to anti-TNF therapy (Table 18). Each criteria is given a score of 1 if the patient meets the criteria and 0 if not with the sum totalling the response index. CD patients are classified as 0–1 non-responders, 2–4 partial responders and 5–7 responders according to the response index.

Biochemical response		C-reactive protein (CRP)
	Criteria 1	CRP decrease by 50% at month 2–4
	Criteria 2	CRP $\leq$ 5 mg/l at month 2–4
		Faecal calprotectin (FC)
	Criteria 3	FC decrease by 50% at month 2–4
	Criteria 4	FC decrease by 50% at month 4–6
	Criteria 5	FC < 50 μ g/g at month 2–4
	Criteria 6	FC < 50 μ g/g at month 4–6
Mucosal response	Criteria 7	Radiological/ endoscopic evidence of improved inflammation
Total score		Total out of 7

The following are two examples of “response index” scoring.

Example 1: 52-year-old patient presenting with Crohn’s colitis (CDAI 500). The patient had induction and maintenance infliximab commenced in combination with mercaptopurine. They had the following parameters at baseline: CRP 20mg/L, FC 1534 mcg/g; at 2–4 months: CRP 9mg/L, FC 500mcg/g and at 4–6 months: CRP 1mg/L, FC 20 mcg/g. They had evidence of mucosal healing on colonoscopy at 6 months. This patient had a response index of 5/7 and was classified as a responder.

Example 2: 63-year-old patient with Crohn’s colitis has initiation of infliximab in combination with azathioprine. They have the following parameters at baseline:

CRP 15mg/L, FC 734mcg/g; at 2–4 months: CRP 16mg/L, FC 723mcg/g and at 4–6 months: CRP 14mg/L, FC 674mcg/g. They had minor erosions in the descending colon and sigmoid colon on colonoscopy at 6 months. This patient had a response index of 0/7 and was classified as a non-responder.

Chemical and reagents for metabonomic profiling assays. Organic solvents (HPLC grade) used for sample preparation were obtained from Sigma-Aldrich (Dorset, UK). Mobile phases were prepared with grade LC-MS solvents and modifiers from Sigma-Aldrich (Dorset, UK).

Metataxonomics (16S rRNA gene sequencing). Sequencing of the 16S rRNA gene was implemented to characterise the bacterial composition of the faeces. DNA was extracted from 250 mg of faeces using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) following manufacturer's instructions with the following modification: samples were homogenised in a Bullet Blender Storm bead beater (Chembio Ltd, St. Albans, UK) for 3 min at speed 8 to aid in bacterial cell lysis. DNA was aliquoted and stored at -80°C until ready to use. Sample libraries were prepared following Illumina's 16S Metagenomic Sequencing Library Preparation Protocol.³¹⁴ The SequalPrep Normalization Plate Kit (Life Technologies, Paisley, UK) was used to purify and normalise the sample libraries following the index PCR amplification step. Also, the NEBNext Library Quant Kit for Illumina (New England Biolabs, Hitchin, UK) was used to quantify the sample libraries. Pooled sample libraries were sequenced using the Illumina MiSeq platform (Illumina Inc., Saffron Walden, UK) and the MiSeq Reagent Kit v3 (Illumina) using paired-end 300bp chemistry.

16S rRNA gene sequencing data was pre-processed using the Mothur package version v.1.35.0 following the MiSeq SOP Pipeline.³⁵⁹ Sequence alignments were performed using the Silva bacterial database (www.arb-silva.de/) and sequences were classified using the RDP database reference sequence files and the Wang method.³⁶⁰ Comparison of pre- vs post- anti-TNF therapy samples (taken 5–7 months after starting therapy) was performed using the Statistical Analysis of Metagenomic Profiles (STAMP) software package with White's non-parametric t-test and Benjamini-Hochberg correction to adjust for false discovery rate.³⁶¹

Metabolic profiling of serum, urine and faeces. Three analytical platforms were implemented to achieve broad metabolome coverage; polar metabolites (hydrophilic interaction liquid chromatography, Hilic), lipids and bile acids ultra-performance LC (UPLC)-MS profiling, combined with 16S rRNA gene sequencing. A comprehensive analysis of metabolites was performed on serum, urine and faecal samples from the 112 participants (Figure 14) using UPLC-MS profiling analyses.

Sample preparation for metabolic profiling of serum. Prior to analysis, lipids/polar metabolites and bile acids were extracted respectively with pre-chilled isopropanol and methanol from 50µL serum (1 aliquot/3 solvent).³¹⁵ Aliquots were vortexed and centrifuged at 14,000g for 20 min at -4°C. 20µL of supernatant was transferred into vials for the lipid and bile acid assay. The supernatant for the polar metabolites assay, was evaporated at room temperature in a vacuum concentrator (Eppendorf Concentrator Plus), reconstituted in acetonitrile/H₂O (90:10) and transferred into vials. In addition, quality control (QC) samples were prepared from pooled serum samples (20µL from each sample). Samples were stored at -80°C awaiting UPLC-MS analysis.

Sample preparation for urine. Aliquots (200µL) were evaporated and reconstituted with H₂O/isopropanol/acetonitrile (2:1:1) for the bile acid assay and H₂O/acetonitrile (10:90) for the Hilic polar metabolite assay. Samples were vortexed and centrifuged at 14,000g for 20 min at -4°C. Sample supernatants were transferred into vials and the QC samples were prepared from pooled urine samples (20µL from each sample). Samples were stored at -80°C awaiting UPLC-MS analysis.

Sample preparation for faeces. Prior to analysis, faecal samples were weighed (~150mg) and dried. For the lipid, bile acid and polar metabolite profiling assays, samples were extracted with mixture solvents of H₂O/isopropanol/acetonitrile (respectively 1:1:2, 2:1:1 and 1:1:1). Samples were vortexed and centrifuged at 14,000g for 20 min at -4°C. The supernatant of samples prepared for the polar metabolite assay was evaporated and reconstituted with acetonitrile/H₂O (90:10). For each sample, the supernatant fraction was transferred into a vial and a QC

sample was prepared from pooled faeces samples (20µL from each sample). Samples were stored at -80°C awaiting UPLC-MS analysis.

Metabolic profiling assays. Aqueous and organic extractions were performed for serum, urine and faecal samples. All samples were transferred into vials with QC samples prepared from pooled faecal samples (20µL from each sample). These samples were analysed using reversed phase-UPLC-MS lipid profiling, HILIC UPLC-MS profiling and bile acid UPLC-MS profiling.³¹⁵ The QC samples were injected every 12 samples throughout the analytical run. Samples were stored at -80°C awaiting UPLC-MS analysis.

Data pre-processing and statistical analysis of MS data. The UPLC-MS raw data were converted to netCDF format using MassLynx™ software (Waters Corporation, Milford, USA). Data processing was implemented using the XCMS package in R programming software. The dataset was normalized to total spectral area. Multivariate data analysis was performed using the SIMCA package (v.13.0.2, Umetrics, Umeå, Sweden). Multivariate Pareto scaled data were modelled using principal components analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). The OPLS-DA parameters (R²X, R²Y, Q²Y and cross validated CV ANOVA p-value) aimed to assess the inherent metabolic variation and robustness of each model. The p-value was adjusted for multiple testing. The two-tailed t-test was applied to the main discriminatory features of each model (Microsoft Excel 2007). A Python script was developed to plot the receiver operating characteristic (ROC) and calculate the area under the curve (AUC) with confusion matrix parameters (accuracy, sensitivity and specificity) of each biomarker.

Metabolite assignment. Metabolite identification by MS was conducted by matching accurate *m/z* measurements of detected chromatographic peaks to theoretical values from in-house databases and on-line databases such as the human metabolite database (HMDB, <http://www.hmdb.ca>), KEGG (<http://www.genome.jp/kegg/ligand.html>), LIPID MAPS (<http://www.lipidmaps.org/tools/index.html>), and METLIN (<http://metlin.scripps.edu>). Tandem MS fragmentation patterns were obtained for further structural elucidation and confirmed with an authentic standards matching for retention time and *m/z*.

5.3 Results

5.3.1 Demographics

From a total of 86 IBD patients included in this study (76 CD, 10 UC) who were anti-TNF naïve, the median age was 38 years for CD and 35 years for UC patients. There were no significant differences in demographics compared with controls (Table 17). CD patients had a baseline CDAI of 490 [IQR (409–501)] with a median CRP of 12.5 mg/L and FC of 699 µg/g. UC patients had a baseline MAYO score of 12 (10–12); CRP of 7.3 mg/L and FC of 534 µg/g. At week 102, 56% of Crohn's patients remained in remission according to objective markers of disease activity (CRP and FC). Thirty-seven CD participants were classified as primary non-responders to anti-TNF therapy using the response index with objective markers previously described with 11 responders and 28 partial responders in the CD cohort. All patients who were classified as responders with our response index were also responders according to CDAI criteria with a decrease in CDAI of 100 points.

Due to the existing literature demonstrating clear differences in metabolic profiling between CD and UC, we performed separate analysis on these cohorts. However, due to the small sample size of UC patients, analysis was only performed to demonstrate differences at baseline between the two groups. Predictive metabolic profiling analysis for primary nonresponse was only undertaken for CD patients.

5.3.2 Classification of Patient Response to Anti-TNF Therapy According to an Objective Response Index

An index was used to assess response versus nonresponse to anti-TNF therapy using objective markers of inflammation, including FC and/or CRP, in addition to radiologic or endoscopic parameters of response. These two groups separated patients with evidence of biochemical /mucosal response to anti-TNF therapy from those with no demonstrable change in objective markers of inflammation. The unclassified or partial response group (score of 2, 3, 4) were not analysed in relation to predictive metabolites or microbiome due to the heterogeneity of response within this group.

5.3.3 Differences in Metabolic Profiles Observed Between Healthy Controls, CD Patients and UC Patients using UPLC-MS Metabonomic Approaches

Robust OPLS-DA models, as assessed by the QC sample distribution and model validation statistics (Q^2Y , permutation testing)³¹⁶ were built from lipid, polar metabolites and bile acid profiles and demonstrated significant metabolic differences between the three groups; healthy controls, CD and UC (Figures S1–3).

When comparing CD patients to healthy controls on Day 0, before induction of anti-TNF therapy, metabolic profiles generated across the three UPLC-MS profiling assays and across the three biofluids; faecal, serum and urine showed clear discrimination of all sample groups with a R^2Y of 0.957; 0.601 (max;min) and a Q^2Y of 0.610; 0.214. For both CD and UC, the strongest model was generated from polar metabolite profiling (Hilic) of serum samples. Based on the OPLS-DA coefficient plots, the features that contributed to the differentiation between healthy controls and CD or UC were identified (Table S1, Figures S1–3). Venn diagrams were used to assess matching features between CD and UC when compared with healthy controls. Whilst serum contained a high degree of overlap between the UC and CD phenotypes in terms of number of core metabolites differentiating between healthy controls and IBD, there was less overlap between the urine and faecal CD and UC phenotypes (Figure S1).

When comparing CD to UC, most of the serum OPLS-DA models were not valid and presented non-significant p-values (Table S1). Robust models for CD vs UC were only achieved for faecal and urine samples analysed by polar metabolite profiling (Hilic) and bile acid profiling (Figures S4 and S5).

Differences in metataxonomic profiles observed between healthy controls and CD patients. Profound changes in gut microbiota composition between HC and CD patients were identified from the metataxonomic analysis of faecal samples (Figure 15).

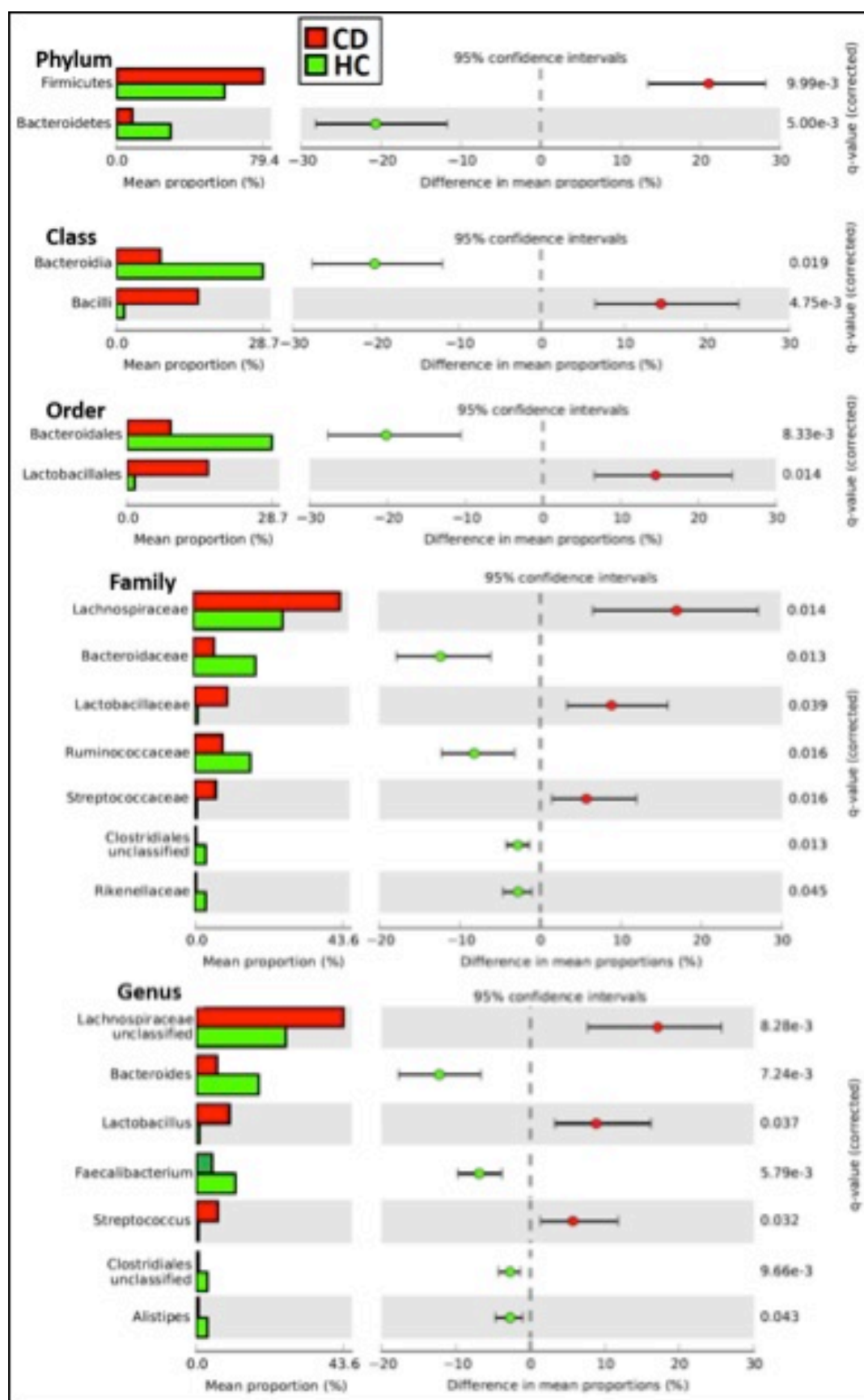


Figure 15. Significant variations in faecal bacterial composition of healthy control (HC) and Crohn's disease (CD) patients observed at all taxonomic levels. Faecal samples were analysed by 16S rRNA gene sequencing, and data were evaluated using White's non-parametric t-test with Benjamini-Hochberg FDR.

The gut microbiota from CD patients was characterised by higher relative abundance of the phylum *Firmicutes* and a lower relative abundance of the phylum *Bacteroidetes* compared to healthy controls (Figure 15). At the family level, there was a significant decrease in the relative proportion of the families *Bacteroidaceae*, *Ruminococcaceae*, unclassified *Clostriales* and *Rikenellaceae*, combined with higher relative proportions of the family *Lachnospiraceae* and *Lactobacillaceae* as observed in CD patients. There was also a significant reduction in the relative abundances of the genera *Bacteroides*, *Faecalibacterium*, unclassified *Clostriales* and *Alistipes* as well as an increase in the relative proportion of unclassified *Lachnospiraceae* and *Lactobacillus* in CD patients compared to healthy controls (Figure 15).

5.4 Metabolic Predictors of Anti-TNF Response Using UPLC-MS Profiling in CD Patients

OPLS-DA was carried out to determine if there was a metabolic response to the anti-TNF treatment of CD patients. The “response index” to anti-TNF was calculated as discussed with objective markers using biochemical data to classify response in CD patients. OPLS-DA models were built from metabolic markers associated with the “response index”. The subsequent identified metabolites were found to be predictive biomarkers of anti-TNF response as determined by the “response index”.

The “response index” that aimed to classify the response of CD patients to anti-TNF treatment, showed good correlation with metabolic profiles (Table S3, Figures S6–9). The OPLS-DA models built from all the visits were validated with p-value <0.05 across all UPLC-MS assays and biofluids. Based on baseline samples in comparison with later time points (visit 4 to visit 6 which were at 11 to 16 months), OPLS-DA models were significant for bile acid profiling in serum and urine, Hilic profiling in serum and lipid profiling in faeces for responders and non-responders

Bile acid metabolism was investigated by UPLC-MS profiling. Several compounds from faeces (n=5 bile acid markers), serum (n=5 bile acid markers) and urine (n=3 bile acid markers) differentiated between patients responding and not responding to anti-TNF treatment (Table S4, Figures S8–9). Bile acid markers associated with the sulfate/taurine, glycine and sulfate/glycine pathway were

respectively altered in faeces (Figure 16A), serum (Figure 16B) and urine (Figure 16C). Although the bile acid profiles from all three biofluids discriminated between responders and non-responders, each biofluid manifested a distinct bile acid signature (Figure 16 and Table S4). Secondary (deoxycholic acid) and tertiary bile acid markers were significantly elevated and highly represented in serum of patients responding to anti-TNF treatment. Patients who did not respond to the anti-TNF treatment exhibited significantly higher levels of sulfate and glycine-conjugated primary bile acids. From a combination of the 3 faecal bile acid markers with the highest discriminating ability, an AUROC of 0.81 ± 0.17 was generated, demonstrating a model with good predictive ability for anti-TNF treatment response (Figure 17C); 5 serum bile acid markers produced a model with AUC 0.74 ± 0.15 (Figure 17B) and urine BA of 0.70 ± 0.17 (Figure 17E). Combining the three biofluid matrices did not substantially improve the prediction of response.

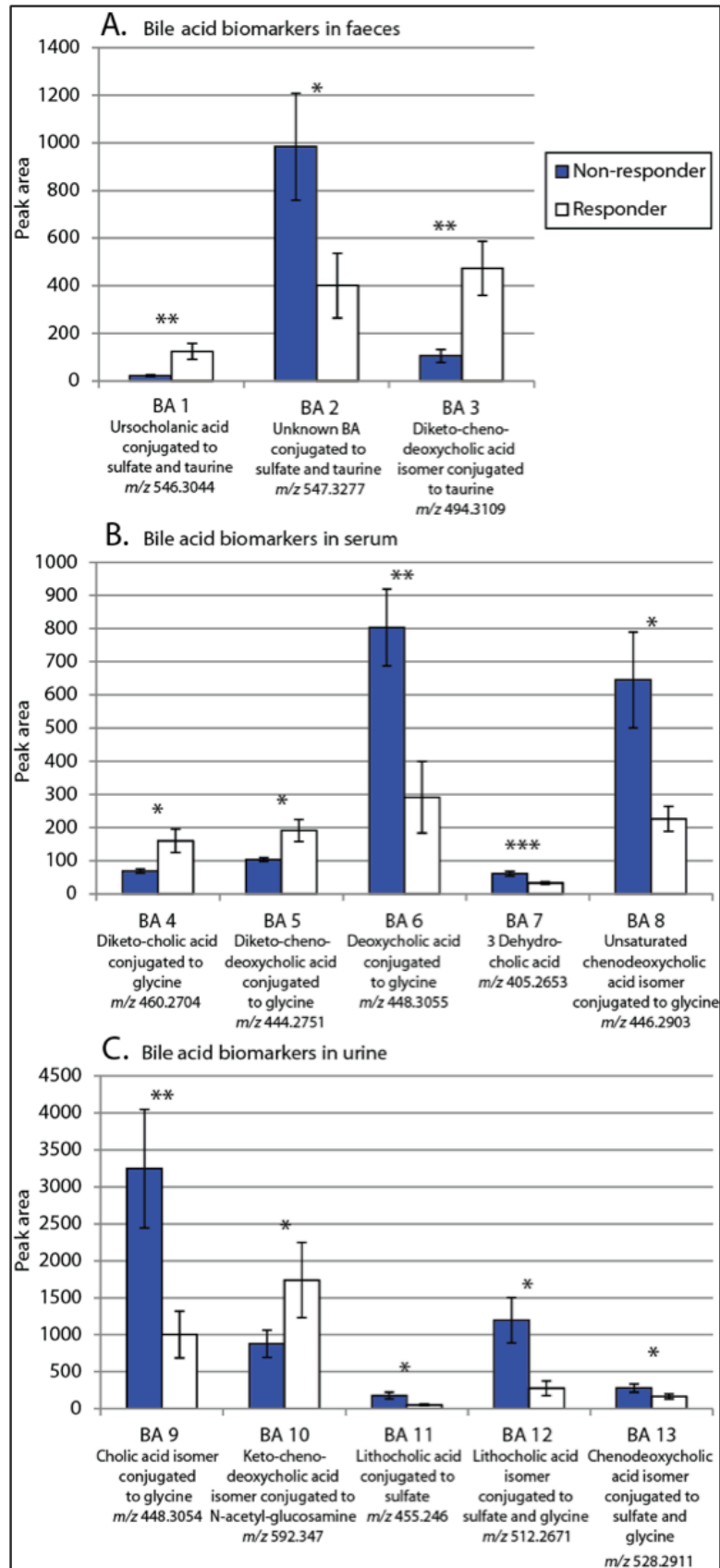


Figure 16. BA metabolites in urine, serum and faeces correlate with the longitudinal response of CD patients to anti-TNF treatment. BA markers vary between responders and non-responders to anti-TNF therapy in faeces (A), serum (B) and urine samples (C). Two-tailed Student's *t*-test * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Supplementary information is available in Table S4.

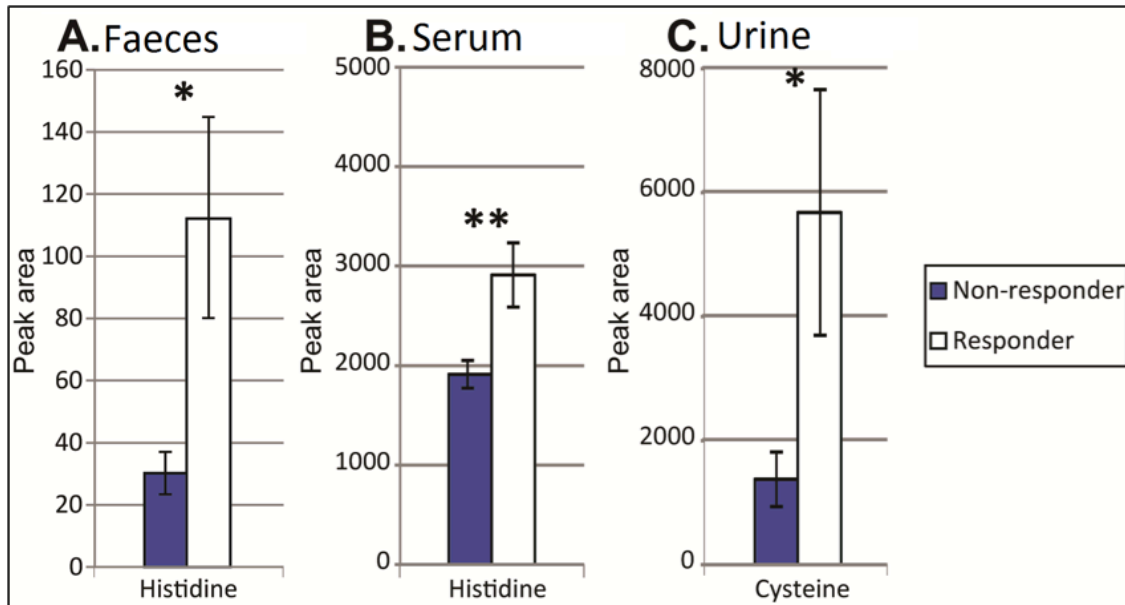


Figure 17. Predictive model demonstrating area under curve for classifying non-responders to anti-TNF therapy from lipid, bile acid and Hilic profiling biomarkers. (A) Serum lipid, (B) Faecal lipid, (C) Faecal bile acid, (D) Serum bile acid, (E) Urine bile acid, (F) Faecal histidine, (G) Serum histidine and (H) Urinary cysteine biomarkers for predicting nonresponse to anti-TNF therapy. Confusion matrix parameters are true positive (TP), true negative (TN), false negative (FN) and false positive (FP) with calculated accuracy, specificity and sensitivity.

The polar metabolite (Hilic) profiling of serum, urine and faeces indicated significant differences between responders and non-responders to anti-TNF (Table S3, Figures S6–9). Histidine levels in faeces and serum was identified to be significantly higher in anti-TNF responders (Figure 18A, 18B), together with a number of unidentified metabolites. Urinary cysteine was also elevated in responders (Figure 18C). ROC analysis demonstrated a good model for predicting anti-TNF response for urinary cysteine (AUC 0.7+/-0.14) (Figure 17H) but not for histidine in faeces (AUC=0.63+/-0.28) (Figure 17F) or histidine from serum (AUC=0.48+/-0.18) (Figure 17G).

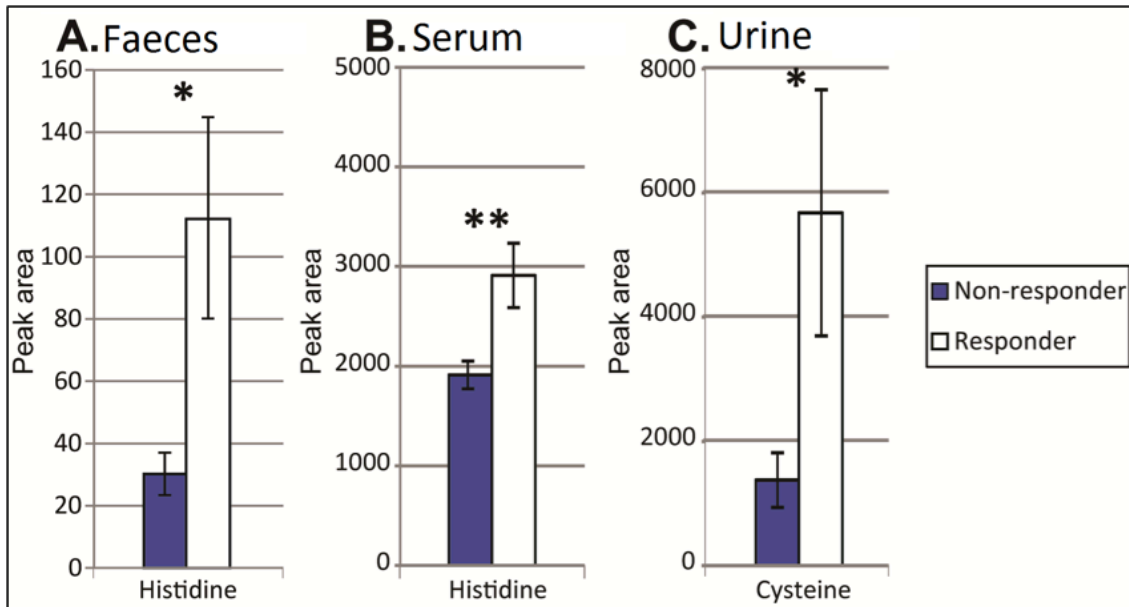


Figure 18. Polar metabolites (Hilic) correlating with the longitudinal response of CD patient's anti-TNF treatment. Histidine and cysteine vary between patient that did and did not respond to anti-TNF treatment in faeces (A), serum (B) and urine samples (C). Two-tailed Student's *t*-test * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Metabolic variations between responders and non-responders to anti-TNF treatment were also observed in the lipid profiles (Table S3, Figures S6–9). Circulatory lipid markers, phosphatidylcholine (PC), ceramides (Cer), sphingomyelins (SM) and triglycerides (TG) were present in significantly lower concentrations in patients that responded to the treatment (Figure 19B). Conversely, increased level of phosphocholines and triglycerides were detected in the faeces of responders (Figure 19A). ROC analysis demonstrated a good model for predicting anti-TNF response for serum lipid (AUC 0.78 $\pm$ 0.12) (Figure 17A) and a very high predictive accuracy for faecal lipid profiling (AUC=0.94 $\pm$ 0.10) (Figure 17B).

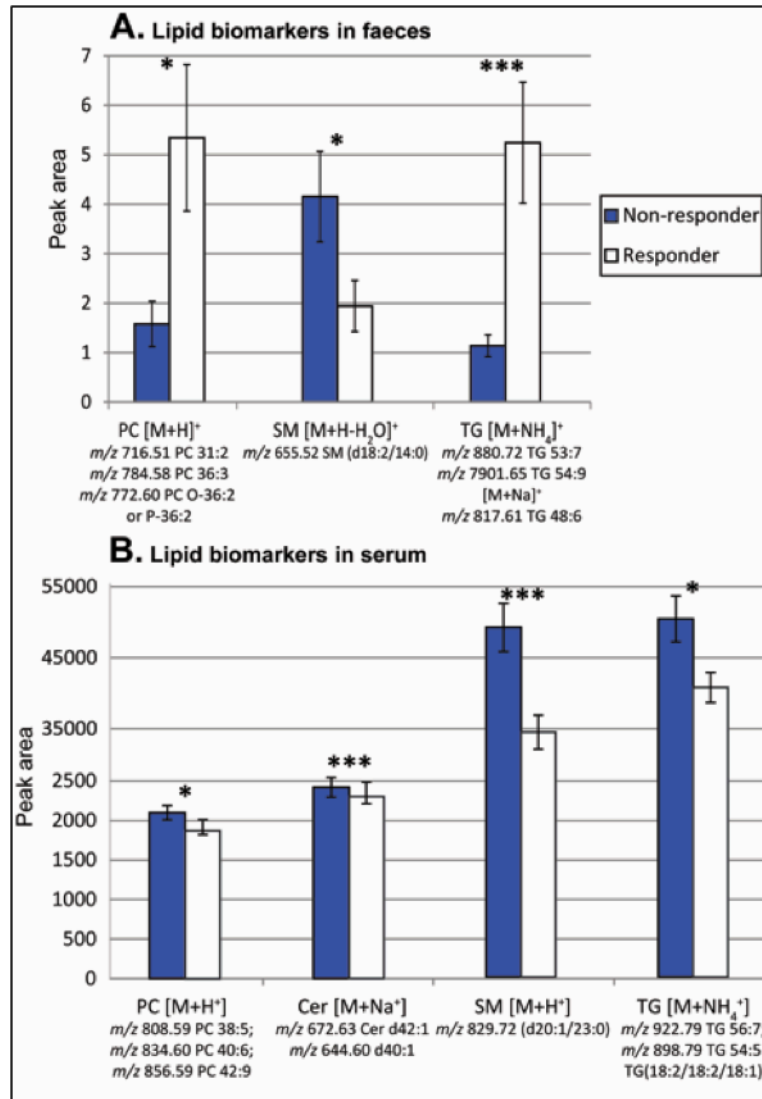


Figure 19. Lipid classes correlating with the longitudinal response of CD patients to anti-TNF therapy. Mean levels of lipid markers according to classes (PC, Cer, SM and TG) which vary between patients responders and non-responders to anti-TNF treatment in faeces (A) and serum samples (B). Two-tailed Student's *t*-test **p*<0.05*, ***p*<0.01 and ****p*<0.001.

5.4.1 Exploration of 16S rRNA Gene Sequencing of Longitudinal Samples with Respect to Response to Anti-TNF Therapy

An analysis of all samples from IBD patients pre-anti-TNF compared to all IBD patients post-anti-TNF (including responders, non-responders, and partial responders) showed that there was a decrease in the phylum *Bacteroidetes* (*p*=0.020), corresponding to the class *Bacteroidia* (*p*=0.038) following anti-TNF therapy (Figure 20). However, we did not find any differences in the gut microbiota profiles of responders compared to non-responders, either before or after anti-TNF therapy. This is most likely due to sample size limitations.

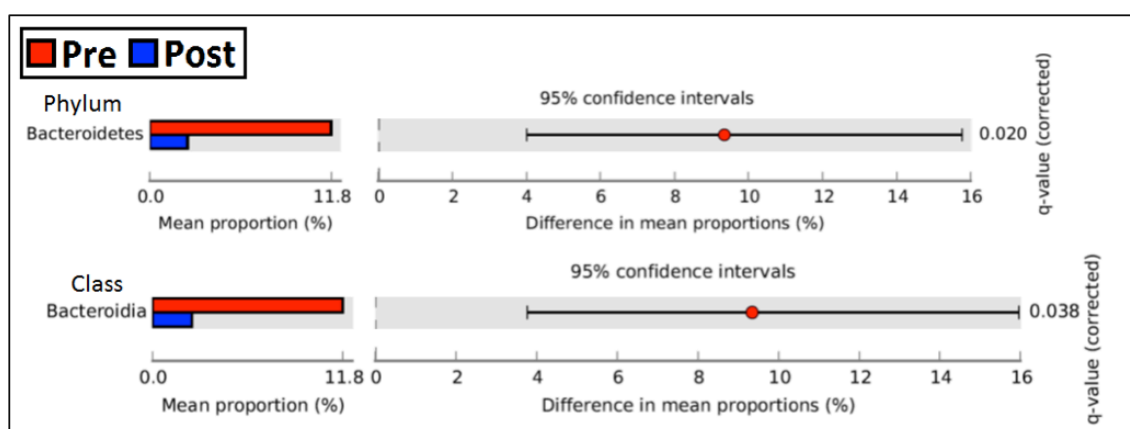


Figure 20. Differences in bacterial composition of IBD patients before and after receiving anti-TNF therapy. IBD patients included responders, non-responders, and intermediate responders, and post samples were taken from one time point 5–7 months after treatment.

5.5 Discussion

Anti-TNF therapy primary nonresponse in patients with IBD remains an issue with rates of 30% in the reported literature.³¹⁷ Biomarkers for the prediction of anti-TNF therapy response have hitherto been lacking.²⁵⁰ We identified metabolic profiles, which are predictive of primary nonresponse in IBD patients undergoing anti-TNF therapy with alterations in bile acids, amino acid and lipid metabolites. In addition to identifying a serum metabolic phenotype associated with anti-TNF response, we demonstrated that we were also able to classify responders to anti-TNF therapy with urinary cysteine and bile acid-specific profiles in serum, urine and faeces.

5.5.1 IBD Biofluid Metabolite and Metataxonomic Profiles Exhibit a Distinct Metabolic Phenotype from those of Healthy Controls

Prior literature in the field of metabonomics has seen the identification of specific biochemical profiles that separate IBD patients from healthy controls.³¹¹ Most studies to date comparing CD and UC have been challenging largely due to small patient numbers and ill-defined cohorts¹³. Our study applies lipid, bile acid and polar metabolite (Hilic) profiling (serum, urine and faecal samples) to a cohort of 76 CD patients and 10 UC patients profiled over a 16-month period, and demonstrates that severe UC can be separated distinctly from moderate to severe CD confirming the work of Scoville *et al.* who showed differences between the two disease states. The common core of metabolites shared by UC and CD that differentiate healthy from IBD was found to account for 35% of the metabolites measured, which was similar to the percentage of IBD dysregulated

metabolites identified by Scoville *et al.* (31%).³¹⁸ IBD has been associated with increased serum concentrations of primary bile acids, plasmalogens and lysolipids whilst tricarboxylic acid cycle (TCA) intermediates, sterols, sphingolipids, acylcarnitines and endocannabinoids were found in lower concentrations in the serum of IBD patients.^{319,320} We also find higher concentrations of serum bile acids in the IBD patients. However, in contrast we find lower serum lysophospholipid concentrations in IBD patients and higher levels of sphingolipids, acylcarnitines, sterols and TCA intermediates. Increased serum acylcarnitines have been reported in canine IBD.³²¹ The difference in cohort composition may account for some of these discrepancies since the Scoville study assessed 20 CD and 20 UC patients whereas the current study was intentionally biased towards CD (n=76) with a smaller UC group (n=10).

Based on baseline samples, only faecal and urinary metabolite profiles were significantly different between CD and UC patients. Previous NMR based studies have shown that faecal metabolite profiles were more dysregulated in CD patients compared to UC patients.³¹¹

The 16S rRNA analysis demonstrated that significant gut microbial composition variation occurs between IBD patients and healthy controls. Major differences in the composition of CD and healthy individuals at the family level included enriched *Bacteroidaceae*, *Ruminococcaceae*, *Enterobacteraceae*, unclassified *Clostridiales* and *Rikenellaceae*, combined with higher relative proportions of the family *Lachnospiraceae*, *Lactobacillaceae*, *Escherichia-Shigella* as observed in CD patients. There was also a significant reduction in the relative abundances of the genera *Bacteroides*, *Faecalibacterium*, unclassified *Clostridiales* and *Alistipes* in CD patients compared to healthy controls. *Faecalibacterium prausnitzii* has previously been shown to be reduced in the faecal microbiota of CD patients.^{322,323} *F. prausnitzii* is thought to be an important member of the gut microbiota in healthy individuals, as it has been shown to produce anti-inflammatory metabolites (short chain fatty acids butyrate and formate, and D-lactate) that can be used as an energy source for gut epithelial cells.^{324,325} These results are largely consistent with the findings of a review of 72 metataxonomic studies on CD patients vs non IBD individuals, which highlighted enriched *Enterobacteriaceae* in addition to depleted *F. prausnitzii*, and lower levels of

bacteria with gene expression for butanoate and propanoate synthesis, carbohydrate metabolism and amino acid biosynthesis.³²⁶ The genera *Alistipes* has previously been found to be more abundant in healthy individuals than in those with intestinal disorders and is known to be a butyrate producer.³²⁷ Targeted therapy of CD patients with Vitamin D has been shown to induce an increase in relative abundance of several members of the Firmicutes, including *Alistipes* and *Ruminococaceae*, whereas this shift was not seen in healthy individuals following vitamin D ingestion,³²⁸ indicating potential for selective targeting of the microbiome in IBD patients.

5.5.2 Prognostic Metabolic Phenotypes are Associated with Response to Anti-TNF Therapy

A systematic difference was observed in the metabolic profiles of all three biofluids in moderate to severe CD patients who responded to the therapy over the course of anti-TNF therapy. Biomarkers that may contribute to the anti-TNF therapy response included serum lipids (phosphocholines, ceramide, sphingomyelin and triglycerides), serum and faecal bile acids, histidine and urinary cysteine. The combined profile of faecal lipids significantly differentiated CD anti-TNF responsive patients from non-responders with a ROC curve for prediction of response demonstrating a sensitivity of 0.92 and specificity of 0.61. There are no strong predictors of response using clinical parameters such as FC or CRP. A fall in FC after treatment predicts response to therapy.³²⁹ Notably, the issues remain that the pre-treatment levels of CRP or FC do not act as predictors to anti-TNF therapy. It is the change in pre and post-therapy FC that is associated with response.^{250,330} ROC curves created for serum and faecal bile acid profiles also yielded relatively strong predictive capacity with sensitivity 0.92 and specificity of 0.61 respectively.

This study demonstrates that CD patients with higher levels of deoxycholic acid (secondary bile acids) during anti-TNF therapy were more likely to respond to anti-TNF therapy compared with CD patients with low levels. This was the same for some tertiary bile acids. Bile acids require further conjugation to change their solubility to be eliminated in urine and faeces. CD patients that did not respond to anti-TNF therapy had higher levels of circulating primary unconjugated bile acids (cholic acid and chenodeoxycholic acid, Figure S9), which may explain the

significantly high level of bile acids conjugated to sulfate, taurine and glycine in urine and faeces. Bile acids are an important pathway for inflammatory activation of the gastrointestinal tract.^{320,331} It has been demonstrated that primary bile acids are converted to secondary bile acids by some members of the gut microbiota, which underscores the potential role of the microbiome in the aetiology and progression of CD.

Lipid profiling revealed important biomarkers with ceramide and sphingomyelin being elevated in primary non-responders in serum. Recent studies have demonstrated different inflammatory pathways such as sphingosine-1-phosphate, which is known to be elevated in IBD patients.³¹⁹ Sphingolipid metabolism and its downstream metabolites such as ceramide and sphingomyelin are known to be pro-apoptotic via dephosphorylation of Akt and activation of caspases as well as being important in activation-induced cell death of dendritic cells³³². Ceramide and sphingosine-1-phosphate are both pro-inflammatory metabolites, which promote egress of lymphocytes from lymph nodes. This pathway has been identified as a future target for trials in both UC and CD.³³³ The finding from our study that sphingomyelin and ceramide were increased in non-responders may represent a biomarker for patients whereby a pharmacodynamic reason is the cause for anti-TNF failure.

The bile acid diversity may be affected in our cohort of anti-TNF responders as a result of alterations in microbiome occurring from CD activity and mucosal inflammation as seen between healthy controls and Crohn's patients. Prior literature has shown uncertainty as to whether gut dysbiosis leads to altered metabolism of bile acids or vice-versa.³³⁴ Dysbiosis within the small bowel lumen with a tendency toward decreased bacteria bearing bile salt hydrolase activity may explain the increase in conjugated primary bile acids and a decrease in secondary bile acid pool.³²⁰ The findings have been demonstrated in the existing literature, with germ-free mouse experiments showing a loss of function in bile acid transformation pathway resulting in increases in primary, conjugated and sulfated bile acids.³²⁰

The direct effects of bile acids may be its impact on mucosal barrier function.³³⁴ However, some secondary bile acids may be protective and in fact decrease

luminal inflammation via mechanisms outlined previously in the literature.³³⁵ Possible confounding due to the effects of antibiotics on numerous taxa was taken into account, as all patients did not have antibiotics within the last six months of their initial visit. Furthermore, there were numerous unknown bile acids which have not been identified in metabolic libraries which are undergoing further analysis. They have been identified as bile acids based on their spectral mass.

The profiling of polar metabolites showed low urinary cysteine as well as low faecal and serum histidine in non-responders to anti-TNF compared with responders, which may indicate that this pathway is of importance in anti-TNF response. There is emerging evidence that mucosal microbiome is involved in mammalian histamine production which alters mucosal pro-inflammatory cytokines. *Lactobacillus reuteri* may ameliorate mucosal inflammation through its impact on luminal histamine signalling via diacylglycerol kinase.³³⁶ In patients who did not respond to anti-TNF therapy, histidine metabolism might be disturbed as it is a precursor of histamine produced by mast cells that play a central role in the inflammatory response. Histidine is negatively correlated with CRP in CD with decreased histidine levels being associated with risk of relapse in patients with UC.³³⁷ In a murine *in vivo* model, histidine was shown to ameliorate colitis activity in DSS-colitic mice by suppressing the production of pro-inflammatory cytokines, TNF- α and IL-6 and inhibiting NF- κ B by macrophages.³³⁸

Furthermore, concurrent medications may affect the metabolic output of patient samples. However, when the peaks associated with immunomodulatory use were analysed, they were not found to significantly impact the spectral regions associated with bile acid, lipid and polar metabolites. Other drugs such as paracetamol were also similarly removed from the spectral analysis based on non-significance.

Biomarkers including a panel of faecal, urine and serum bile acids along with cysteine have each shown an AUC of > 0.7 which represents a novel panel of metabonomic-derived biomarkers predicting response to anti-TNF therapy in CD.

5.5.3 Metataxonomic

Anti-TNF therapy did not significantly alter the bacterial richness of the gut microbiota in IBD patients. In fact, it appeared as though there was relative

stability in the microbial taxa. During anti-TNF treatment, *Bacteroidia* decreased when one-time point post-treatment was analysed (at baseline and one sample taken at 5–7 months). There were no significant differences when samples were classified according to response status by using pairwise comparisons likely owing to the small sample size. Stratification of IBD patients into responders vs. non-responders to anti-TNF therapy using the response index showed trends in several bacterial taxa which did not reach statistical significance.

5.6 Limitations, Current and Future Work

Although this constitutes the largest longitudinal cohort study of IBD patients using metabonomic and metataxonomic profiling to date, the relatively small numbers of responders and non-responders from this cohort may explain the lack of statistical significance demonstrated by some assays and the 16S rRNA gene sequencing datasets. However, the small number of samples by responder class is overcome by the well-defined phenotype of Crohn's patients who have moderate to severe luminal disease rather than a heterogeneous IBD cohort as with many of the previous published studies. Prior studies in the field have only recruited approximately 20 patients and similar numbers of controls in order to differentiate IBD from healthy or CD from UC and mostly have not used Benjamin-Hochberg FDR.^{311,339} Furthermore, the longitudinal approach employed in this study allowed us to demonstrate alterations in metabolic and microbiota profile, which has not been demonstrated or co-analysed in previous studies. Our study also endeavoured to be rigorous in defining patients who clearly responded to anti-TNF therapy versus those who did not respond based on objective, in addition to subjective, markers of inflammation. Our study has provided possible mechanistic insights into causes of nonresponse to anti-TNF therapy which require further elaboration with in-vivo models. The response index, albeit non-validated, is an objective measure of response to anti-TNF therapy and allows for separation between responder and non-responder groups when "partial" responders are removed. The response index is therefore of potential benefit as in contrast, the CDAI contains mainly subjective measures of patient response and may not accurately identify primary nonresponse.

Metabonomics and metataxonomics are less invasive techniques than the only current predictive biomarker of long-term response to anti-TNF therapy through

endoscopy demonstrating mucosal healing.¹³ Furthermore, endoscopy requires full bowel preparation, which may alter adherent and non-adherent microbiota and the host's metabonome. Currently, the analysis of samples is expensive and require significant expertise, but with greater knowledge about which taxa or assays are important, targeted assays can be developed, allowing for faster and cheaper analysis to be performed.

5.7 Conclusion

In summary, we showed that metabonomic profiling can be used to discover predictive biomarkers for anti-TNF therapy response in patients with Crohn's disease. These data demonstrate that anti-TNF therapy response may be predicted by a patient's metabonomic and microbiota profile, with important pathways of inflammation such as bile acids, PC, ceramide, sphingomyelin, triglyceride and amino acids, which all represent predictive biomarkers of response to anti-TNF therapy in Crohn's disease. These findings outline a promising prognostic test for anti-TNF therapy response and require validation in independent patient cohorts in IBD and other diseases that adopt anti-TNF therapy such as rheumatoid arthritis.

5.8 Supplementary Information

Metabonomics and the gut microbiome predict response to anti-TNF therapy in inflammatory bowel diseases.

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Abbreviations; Bile acid, BA; Crohn's disease, CD; inflammatory bowel disease, IBD; C-reactive protein, CRP; faecal calprotectin, FC; Healthy control, HC; Orthogonal partial least squares discriminant analysis, OPLS-DA; Principal component analysis, PCA; Ulcerative colitis, UC.

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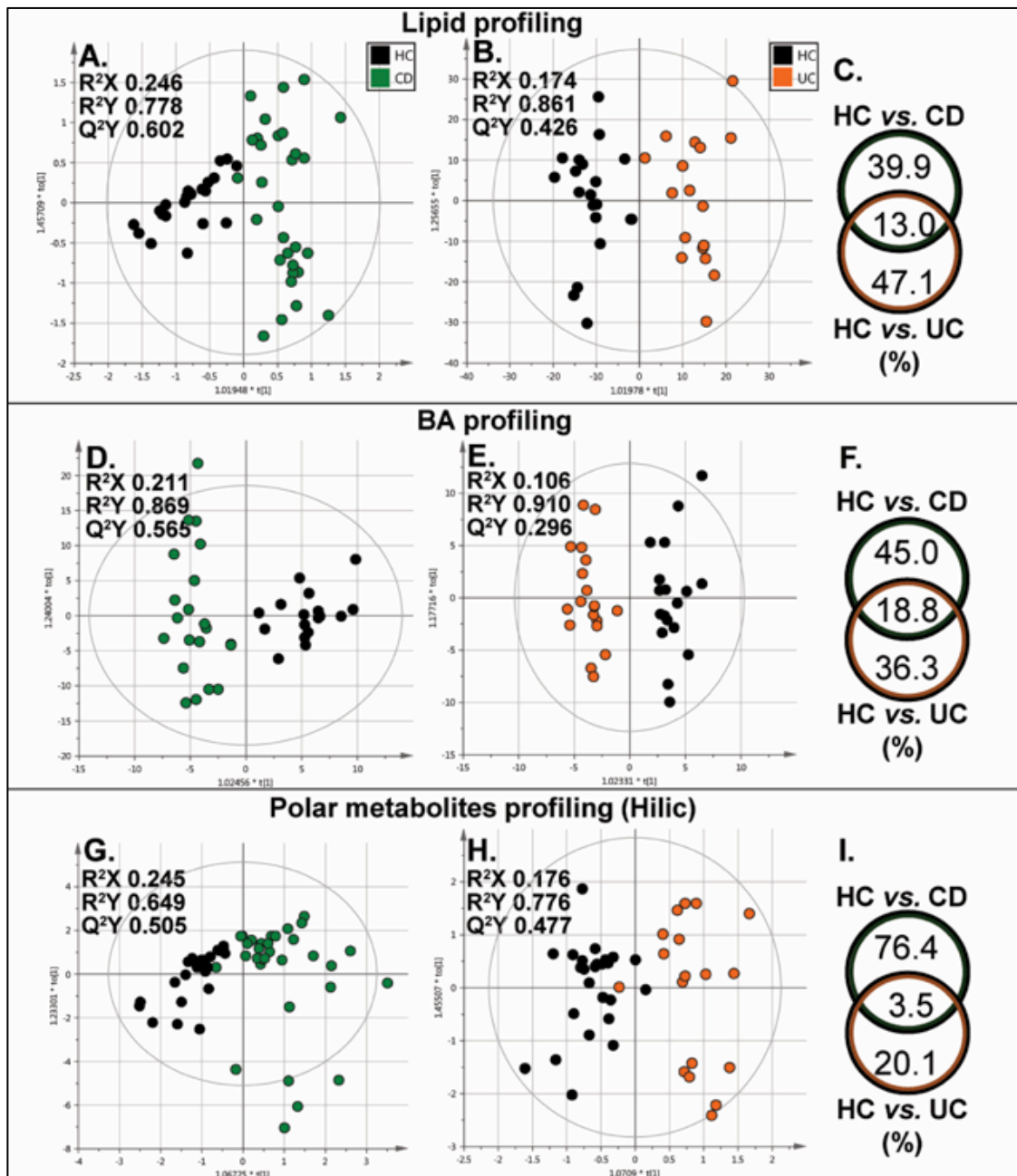


Figure S1. OPLS-DA scores plots of faecal UPLC-MS data. Healthy participants were compared to CD (A, D and G) and to UC patients (B, E and H) at day0. Three assays were implemented, lipid profiling (A, B and C), BA profiling (D, E and F) and polar metabolite profiling (G, H and I). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 2168; 405 and 2346 features detected by respectively lipid; BA and Hilic profiling (C, F and I).

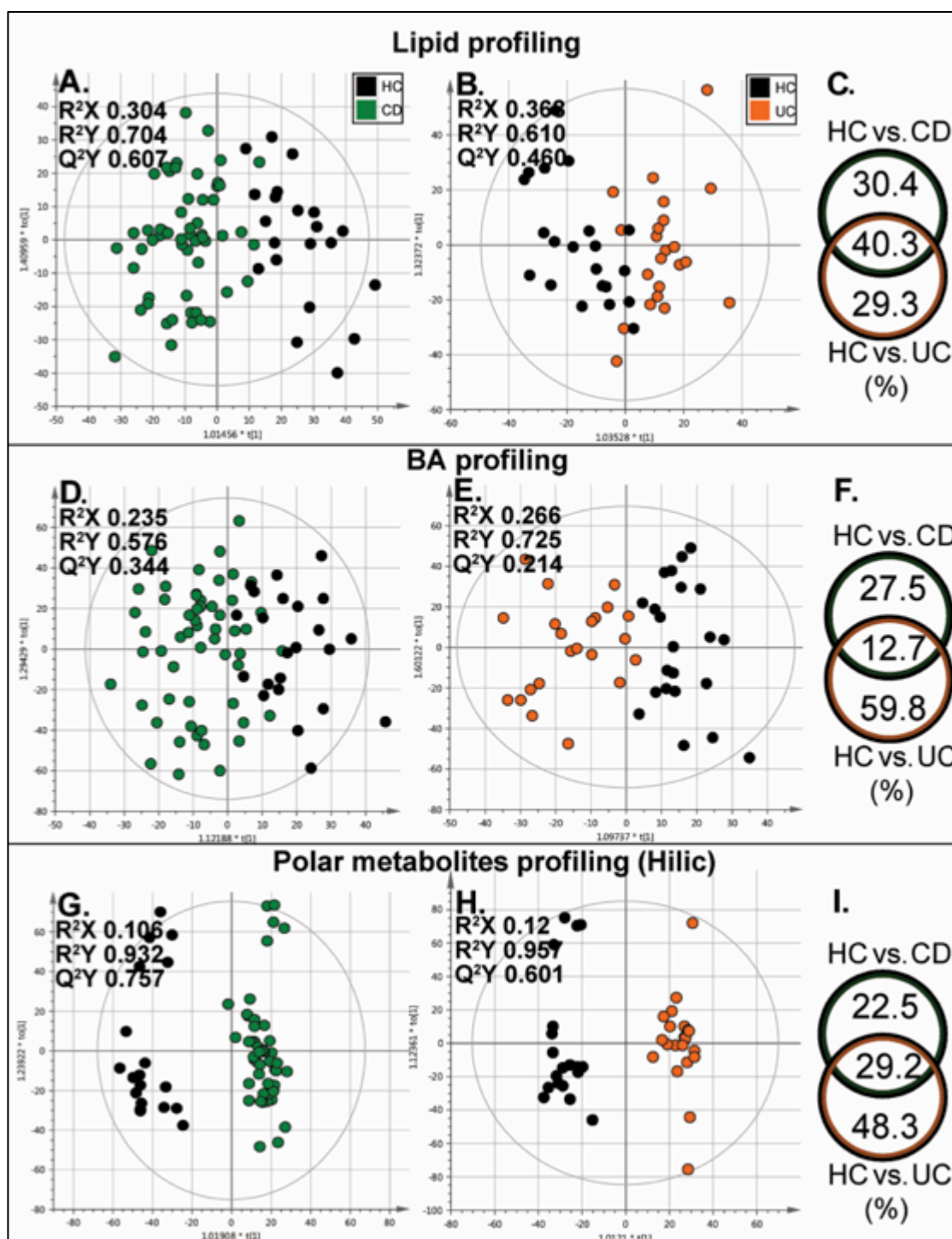


Figure S2. OPLS-DA scores plots of serum UPLC-MS data. Healthy participants were compared to CD (A, D and G) and to UC patients (B, E and H) at day0 (before treatment). Three assays were implemented, lipid profiling (A, B and C), BA profiling (D, E and F) and polar metabolite profiling (G, H and I). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 1278; 387 and 2288 features detected by respectively lipid; BA and Hilic profiling (C, F and I).

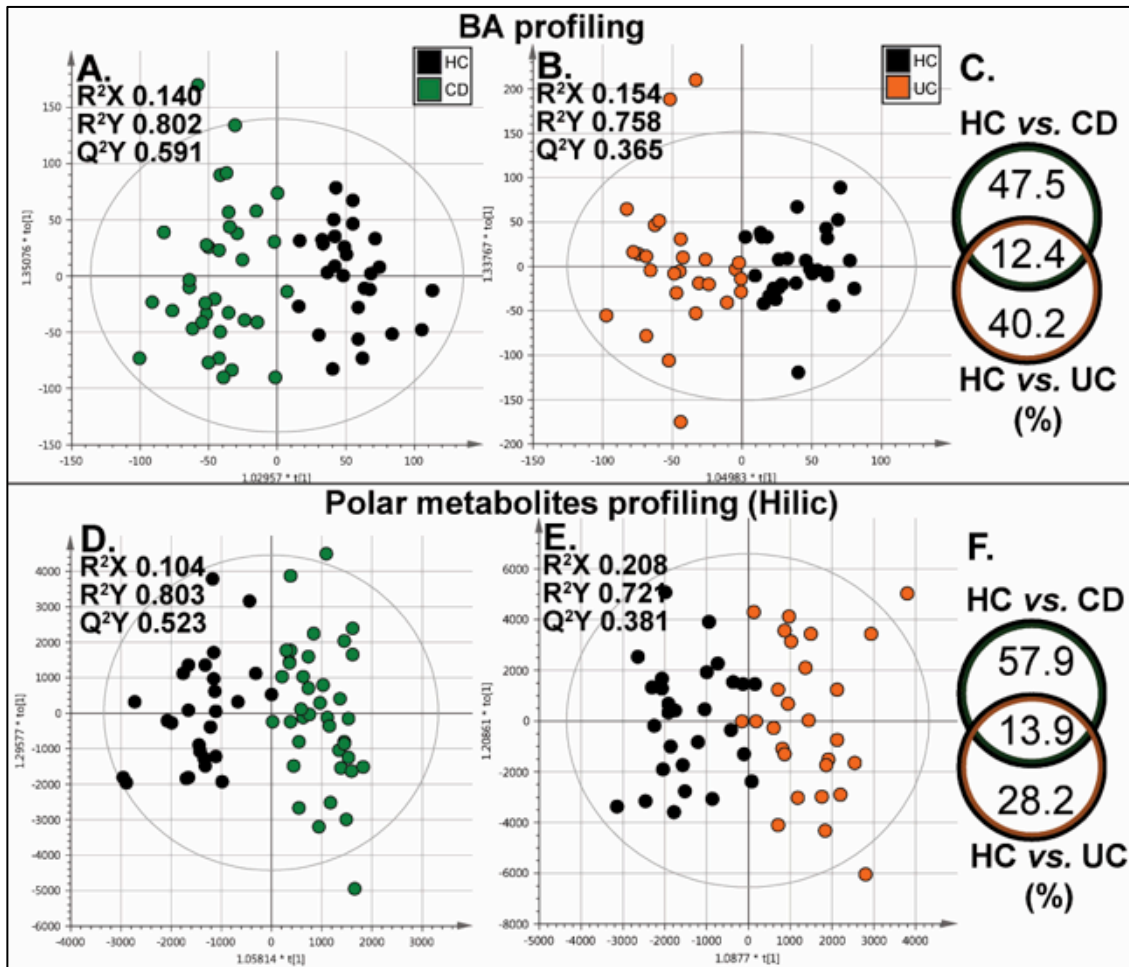


Figure S3. OPLS-DA scores plots of urine UPLC MS data. Healthy participants were compared to CD (A and D) and to UC patients (B and E) at day0 (before treatment). Two assays were implemented, BA (A, B and C) and polar metabolite profiling (D, E and F). The Venn diagrams highlight the percentage of significant features associated with each group (HC, CD and UC) out of a total of 518 and 2790 features detected by respectively BA and Hilic profiling (C and F).

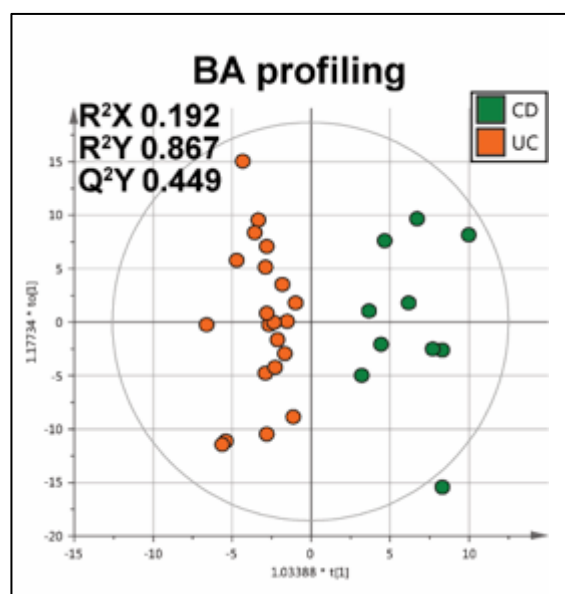


Figure S4. OPLS-DA scores plots of faecal UPLC-MS data comparing CD vs. UC on BA profiling. Data analysed at day 0 (before treatment).

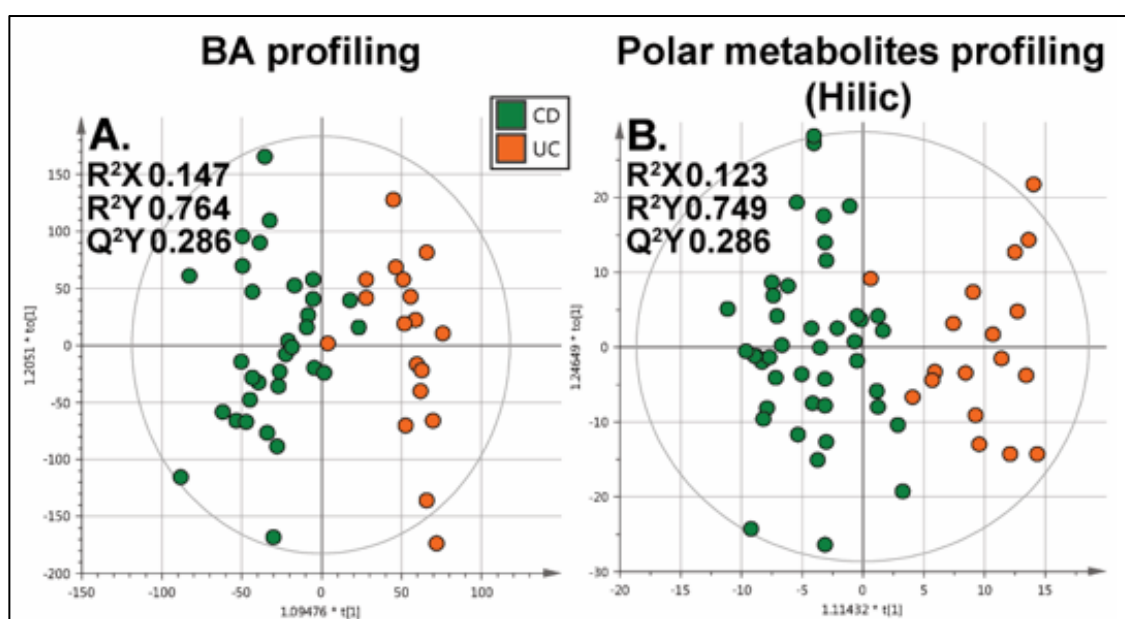


Figure S5. OPLS-DA scores plots of urine UPLC-MS data comparing CD vs. UC. BA profiling (A) and polar metabolite profiling (Hilic, B) of urine. Data analysed at day 0 (before treatment).

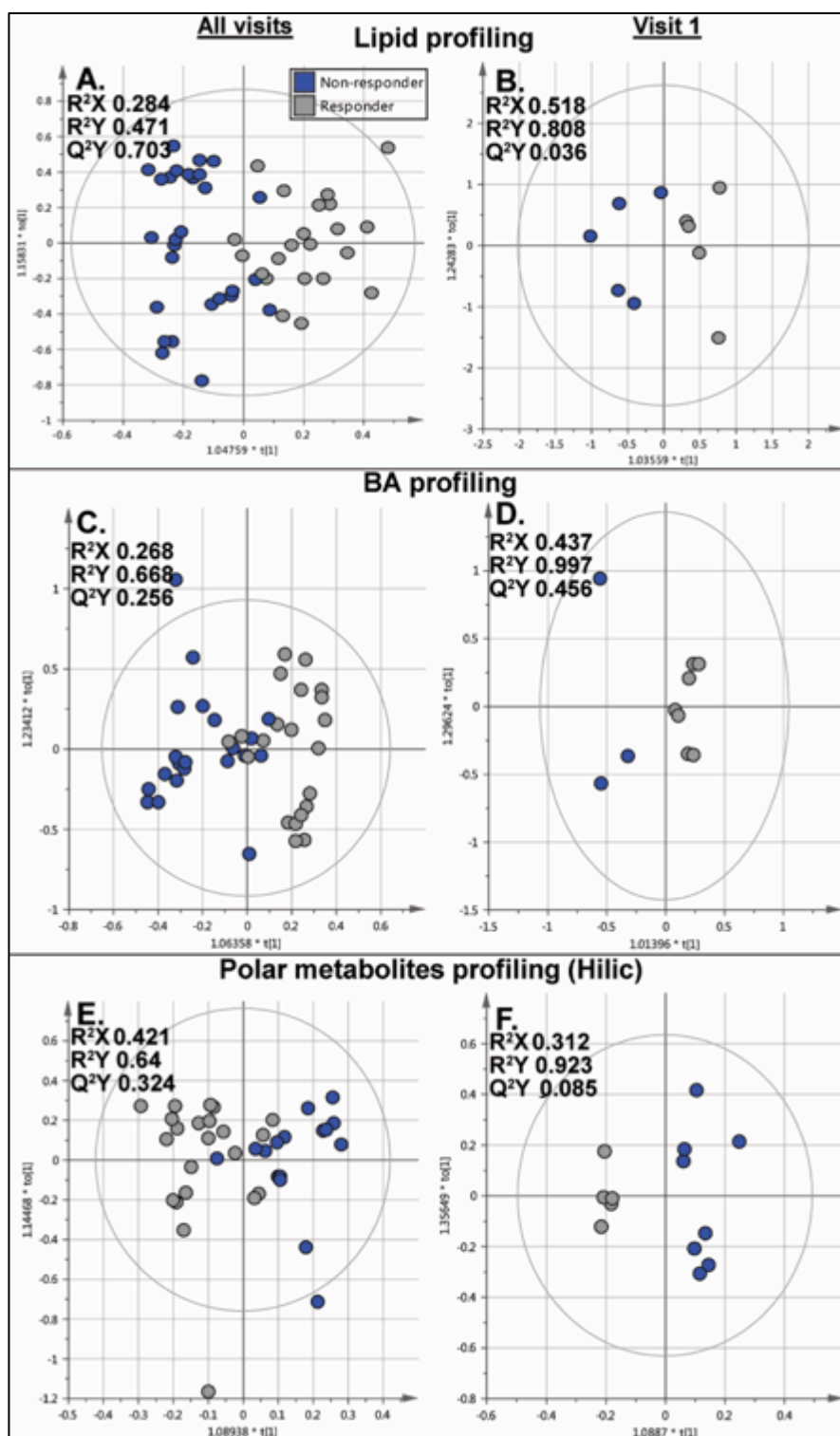


Figure S6. OPLS-DA scores plots of faecal samples for the prediction of the response of CD patients to anti-TNF therapy using the three UPLC-MS profiling methods. Three assays were implemented, lipid profiling (A and B), BA profiling (C and D) and polar metabolite profiling (Hilic, E and F). Models were built with all visits and visit 1 (baseline prior to anti-TNF therapy delivery).

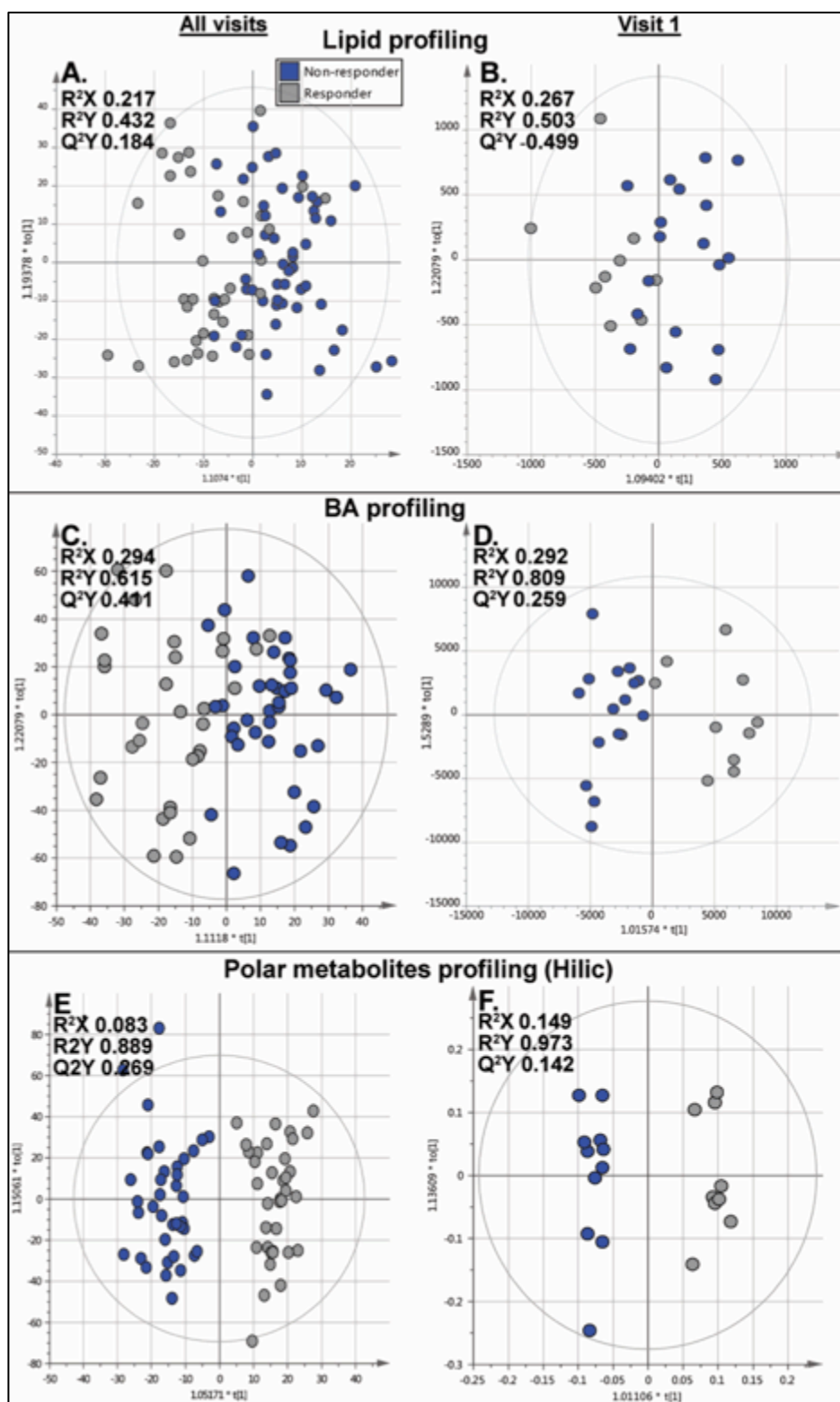


Figure S7. OPLS-DA scores plots of serum samples for the prediction of the response of CD patients to anti-TNF therapy using the three UPLC-MS profiling methods. Three assays were implemented, lipid profiling (A and B), BA profiling (C and D) and polar metabolite profiling (Hilic, E and F). Models were built with all visits and visit 1 (before anti-TNF treatment).

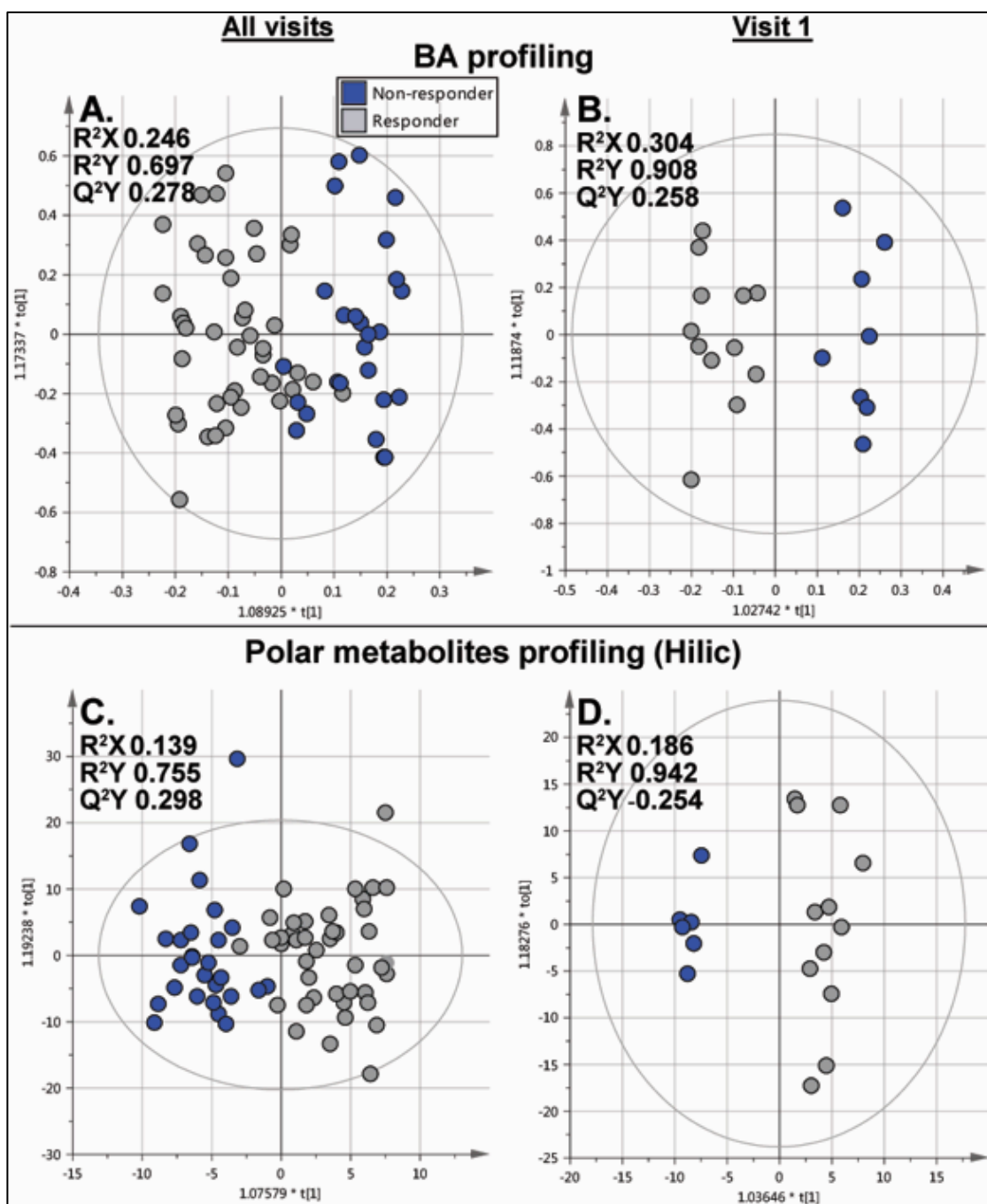


Figure S8. OPLS-DA scores plots of urine samples for the prediction of the response of CD patients to anti-TNF therapy using the two UPLC-MS profiling methods. Two assays were implemented, BA profiling (A and B) and polar metabolite profiling (Hilic, C and D). Models were built with all visits and visit 1 (before anti-TNF treatment).

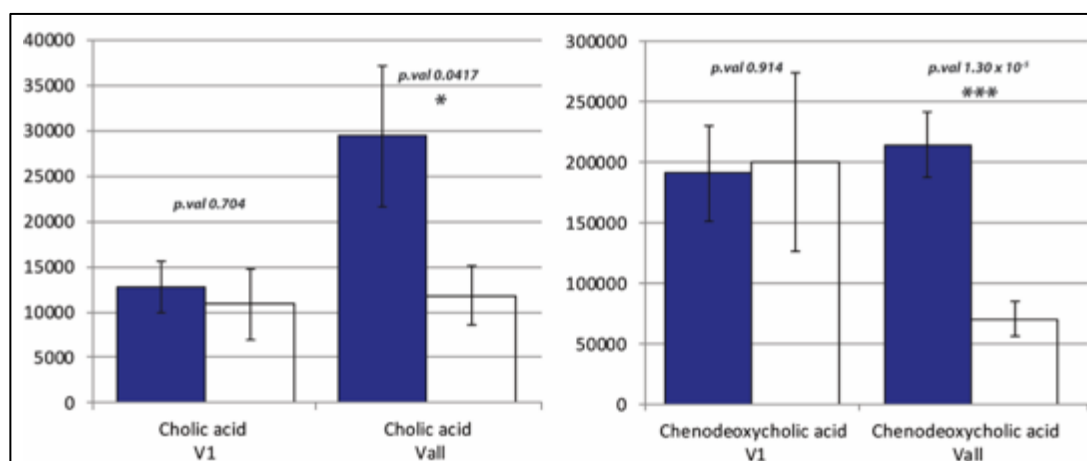


Figure S9. Levels of primary BAs in serum of responder and non-responder patients. Cholic acid and chenodeoxycholic acid UPLC- MS data were analysed comparing visit 1 (V1) and all visits (Vall). Two-tailed Student's *t*-test **p*<0.05*, ***p*<0.01 and ****p*<0.001.

Table S1. Pairwise OPLS-DA models of the three classes (HC, CD and UC) for the three UPLC-MS profiling methods and the three biofluids. OPLS-DA models are built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples. OPLS-DA parameters (*R*²*X*, *R*²*Y*, *Q*²*Y* and *p*-value from CV ANOVA) demonstrate the robustness of each model. Data analysed at day 0 (before treatment).

		Serum			Faeces			Urine	
		Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	BA
HC vs. CD	<i>R</i> ² <i>X</i>	0.106	0.304	0.235	0.245	0.246	0.211	0.104	0.140
	<i>R</i> ² <i>Y</i>	0.932	0.704	0.576	0.649	0.778	0.869	0.803	0.802
	<i>Q</i> ² <i>Y</i>	0.757	0.607	0.344	0.505	0.602	0.565	0.523	0.591
	<i>p</i> -val	<u>1.08x10⁻¹⁸</u>	<u>5.5x10⁻¹⁴</u>	<u>2.8x10⁻⁶</u>	<u>8.2x10⁻⁸</u>	<u>6.1x10⁻⁹</u>	<u>1.1 x10⁻⁵</u>	<u>2.6x10⁻⁹</u>	<u>1.6x10⁻¹⁰</u>
HC vs. UC	<i>R</i> ² <i>X</i>	0.120	0.368	0.266	0.176	0.174	0.106	0.208	0.154
	<i>R</i> ² <i>Y</i>	0.957	0.610	0.725	0.776	0.861	0.910	0.721	0.758
	<i>Q</i> ² <i>Y</i>	0.601	0.460	0.214	0.477	0.426	0.296	0.381	0.365
	<i>p</i> -val	<u>1.2x10⁻⁶</u>	<u>6.1x10⁻⁵</u>	<u>0.0015</u>	<u>3.3x10⁻⁵</u>	<u>0.0011</u>	<u>0.021</u>	<u>6.4x10⁻⁵</u>	<u>9.5x10⁻⁵</u>
CD vs. UC	<i>R</i> ² <i>X</i>	0.0938	0.558	0.245	0.281	0.183	0.192	0.123	0.147
	<i>R</i> ² <i>Y</i>	0.839	0.291	0.727	0.574	0.732	0.867	0.749	0.764
	<i>Q</i> ² <i>Y</i>	0.123	0.0962	-0.022	-0.107	0.138	0.449	0.188	0.286
	<i>p</i> -val	0.07	0.084	-	-	0.19	<u>0.0029</u>	<u>0.018</u>	<u>0.0038</u>

Table S2. Pairwise OPLS-DA models between visits of CD patients for each of the three UPLC-MS profiling methods and the three biofluids. OPLS-DA models are built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples from CD. OPLS-DA parameters (R^2X , R^2Y , Q^2Y and p-value from CV ANOVA) demonstrate the robustness of each model. Data were analysed comparing visit 1 (V1) to visit 2 (V2), V1 to visit 3 (V3) and V1 to visit 4-5-6 (V4-5-6).

		Serum			Faeces			Urine	
		Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	BA
V1 vs. V2	R^2X	0.055	0.247	0.3	0.436	0.245	0.234	0.152	0.138
	R^2Y	0.883	0.294	0.241	0.278	0.46	0.542	0.427	0.482
	Q^2Y	-0.424	-0.451	-0.281	-0.203	-0.25	-0.0286	-0.221	-0.13
	p-val	-	-	-	-	-	-	-	-
V1 vs. V3	R^2X	0.068	0.268	0.263	0.207	0.131	0.241	0.157	0.147
	R^2Y	0.892	0.356	0.356	0.529	0.62	0.654	0.462	0.523
	Q^2Y	-0.102	-0.16	-0.388	-0.098	-1.06	-0.170	-0.308	-0.513
	p-val	-	-	-	-	-	-	-	-
V1 vs. V4-5-6	R^2X	0.067	0.153	0.141	0.213	0.229	0.289	0.146	0.137
	R^2Y	0.82	0.376	0.471	0.488	0.47	0.617	0.461	0.496
	Q^2Y	0.028	-0.133	-0.0687	-0.18	-0.242	-0.269	-0.159	-0.159
	p-val	-	-	-	-	-	-	-	-

Table S3. Pairwise OPLS-DA models of CD anti-TNF treatment between responders and non-responders. OPLS-DA models were built with data from polar metabolites (Hilic), lipids and BA profiling and were applied to analyse serum, faeces and urine samples from CD. OPLS-DA parameters (R^2X , R^2Y , Q^2Y and p-value from CV ANOVA) demonstrate the robustness of each model. Data were analysed on all visits, visit 1 (V1, before anti-TNF treatment) and V1 with only one point per patients.

		Serum			Faeces			Urine	
		Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	Lipids	BA	Polar metabolites (Hilic)	BA
Resp. vs. Non Resp. all visits	R^2X	0.0835	0.217	0.294	0.421	0.284	0.268	0.139	0.246
	R^2Y	0.889	0.432	0.615	0.640	0.703	0.668	0.755	0.697
	Q^2Y	0.269	0.184	0.411	0.324	0.471	0.256	0.298	0.278
	p-val	<u>2.11×10^{-4}</u>	<u>0.0014</u>	<u>4.9×10^{-7}</u>	<u>0.0099</u>	<u>6.8×10^{-6}</u>	<u>0.024</u>	<u>1.3×10^{-4}</u>	<u>3.5×10^{-4}</u>
Resp. vs. Non-Resp. all visits one point per patients	R^2X	0.0958	0.307	0.364	0.256	0.323	0.337	0.169	0.195
	R^2Y	0.940	0.334	0.674	0.92	0.877	0.839	0.86	0.879
	Q^2Y	0.244	0.602	0.289	0.291	0.464	0.207	0.14	0.339
	p-val	<u>0.015</u>	1	<u>0.0208</u>	0.143	<u>0.024</u>	0.52	0.28	<u>0.049</u>
Resp. vs. Non Resp. V1	R^2X	0.149	0.267	0.292	0.312	0.518	0.437	0.186	0.304
	R^2Y	0.973	0.503	0.809	0.923	0.808	0.997	0.942	0.908
	Q^2Y	0.142	-0.499	0.259	-0.085	0.0361	0.456	-0.254	0.258
	p-val	0.650	-	0.160	-	0.900	0.86	-	0.310

Table S4. BA metabolites correlating with the longitudinal response of CD patients to anti-TNF treatment. List of significant BA markers identified to vary between patient that respond and do not respond to the anti-TNF treatment in serum, urine and faeces samples. Two-tailed Student's *t*-test **p*<0.05*, ***p*<0.01 and ****p*<0.001.

Faeces		Serum		Urine	
Markers	Trend in response to anti-TNF compared no response	Markers	Trend in response to anti-TNF compared no response	Markers	Trend in response to anti-TNF compared no response
BA1 Ursocholic acid conjugated to sulfate and taurine (<i>m/z</i> 546.3044 at 6.68 min)	↑ <i>p</i> =0.0072	BA4 Diketo-cholic acid conjugated to glycine (<i>m/z</i> 460.2704 at 3.58 min)	↑ <i>p</i> =0.0244	BA9 Cholic acid isomer conjugated to glycine (<i>m/z</i> 448.3054 at 5.83 min)	↓ <i>p</i> =0.0043
BA2 Unknown BA conjugated to sulfate and taurine (<i>m/z</i> 547.3277 at 4.57 min)	↓ <i>p</i> =0.0376	BA5 Diketo-chenodeoxycholic acid conjugated to glycine (<i>m/z</i> 444.2751 at 5.51 min)	↓ <i>p</i> =0.0282	BA10 Keto-chenodeoxycholic acid isomer conjugated to N-acetyl-glucosamine (<i>m/z</i> 592.347 at 5.67 min)	↑ <i>p</i> =0.047
BA3 Diketo-chenodeoxycholic acid isomer conjugated taurine (<i>m/z</i> 494.3109 at 6.3 min)	↑ <i>p</i> =0.0315	BA6 Deoxycholic acid conjugated to glycine (<i>m/z</i> 448.3055 at 8.6 min)	↑ <i>p</i> =0.0054	BA11 Lithocholic acid conjugated to sulfate (<i>m/z</i> 455.246 at 9.23 min)	↓ <i>p</i> =0.0454
		BA7 3 Dehydrocholic acid (<i>m/z</i> 405.2653 at 7.94 min)	↑ <i>p</i> =9.9x10 ⁻⁴	BA12 Lithocholic acid isomer conjugated to sulfate and glycine (<i>m/z</i> 512.2671 at 7.09 min)	↓ <i>p</i> =0.0345
		BA8 Unsaturated-chenodeoxycholic acid isomer conjugated to glycine (<i>m/z</i> 446.2903 at 4.26 min)	↑ <i>p</i> =0.0154	BA13 Chenodeoxycholic acid isomer conjugated to sulfate and glycine (<i>m/z</i> 528.2911 at 5.91 min)	↓ <i>p</i> =0.0138

Table S5. Summary metabolites and pathways variations observed between HC vs. IBD and CD vs. UC. Two-tailed Student's *t*-test.

		<i>m/z</i> _RT (min)	IBD	HC	p.val	CD	UC	p.val
Primary BAs	Cholic acid	407.28_9.18	43210 (± 45562)	26993 (± 26263)	1.43E-02	46948 (± 47124)	20906 (± 25653)	2.09E-04
	Chenodeoxy cholic acid	391.28_10.33						
	Tauro-chenodeoxy cholic acid	448.30_8.17						
	Glyco-Chenodeoxy cholic acid	498.28_5.87						
	Tauro-cholic acid	514.30_4.35						
	Glyco-cholic acid	466.30_6.26						
Secondary BAs	Lithocholic acid	375.28_10.88	50531.96 (± 41247)	25849.05 (± 24093)	1.25E-04	51118.47 (± 42603)	47032.48 (± 32373)	5.97E-01
	Tauro-lithocholic acid	466.29_10.73						
	Deoxycholic acid	391.28_10.44						
	Taurodeoxycholic acid	498.27_6.32						
	Glyco-lithocholic acid	432.31_10.14						
	Glycodeoxycholic acid	448.30_8.17						
Lysophos-pholipids	LPC 14:0	468.30_0.887	21836.76 (± 6619)	23369.07 (± 2881.5)	0.0556	22344.43 (± 6310.5)	19370.94 (± 7574.5)	0.0352
	LPC 16:0	496.34_1.151						
	LPC 18:2	520.34_0.989						
	LPC 18:1	522.35_1.204						
	LPC 18:0	524.37_1.455						
	LPC 15:0	482.32_1.008						
	LPC 16:1	494.32_0.932						
	LPC 17:0	510.35_1.334						
	LPC 17:1	508.37_1.395						
	LPC 18:3	518.32_0.856						
	LPC 20:0	552.40_2.252						
	LPC 20:1	550.39_1.637						
	LPC 20:2	548.37_1.28						
	LPC 20:3	546.35_1.073						
	LPC 20:4	544.34_0.954						
Sphingolipids	SM(d18:1_12:0)	647.51_3.185	21563.14 (± 5703.63)	30103.05 (± 7128.43)	2.22E-05	21586.43 (± 5715.82)	21450.01 (± 5725.44)	0.8983
	SM(d18:1_14:0) or SM(d16:1_16:0)	675.54_4.072						
	SM(d18:2_14:0)	673.5304_3.305						
	SM(d18:1_15:0)	689.56_4.633						
	SM(d18:1_16:0)	703.57_5.261						
	SM(d18:2_16:0)	701.56_4.231						
	SM(d18:1_17:0)	717.59_6.098						
	SM(d18:1_18:0)	731.60_7.015						
	SM(d18:1_20:0)	759.63_9.353						
	SM(d18:1_21:0)	773.65_10.788						
	SM(d18:1_22:0)	787.66_12.289						
	SM(d18:1_22:1)	785.65_9.287						

		<i>m/z</i> _RT (min)	IBD	HC	p.val	CD	UC	p.val
	SM(d18:2_22:0)	785.65_9.726						
	SM(d18:1_23:0)	801.68_12.996						
	SM(d18:1_23:1) or SM(d18:2_23:0)	799.67_11.212						
	SM(d18:1_23:1) or SM(d18:2_23:0)	799.67_10.65						
	SM(d18:1_24:1)	813.68_12.144						
	SM(d18:2_24:1)	811.66_9.639						
	SM(d18:1_25:0)	829.71_12.843						
	SM(d19:1_24:1)	827.70_12.967						
Sterols	Cholesterol	369.35_5.385	845.98 (± 811.97)	169.98 (± 59.88)	1.23E-24	751.72 (± 414.71)	1303.77 (± 1685.47)	0.0623
	Stigmasterol	395.27_0.932						
	Desmosterol	367.24_0.864						
Carnitines	Acetyl-L-Carnitine	204.16_4.99	580803.19 (± 299251)	543079.86 (± 157946)	0.3557	560669.31 (± 253073)	702300.73 (± 475660)	0.1336
	Decanoyl-L-Carnitine	316.24_3.97						
	Hexanoyl-L-Carnitine	260.18_4.29						
	Isobutyryl-L-Carnitine or Butyryl-L-Carnitine	232.15_4.56						
	Isovaleryl-L-Carnitine or 2-Methylbutyryl-L-Carnitine	246.16_4.41						
	Lauroyl-L-Carnitine	344.27_3.86						
	L-Carnitine	162.11_5.27						
	Myristoyl-L-Carnitine	372.30_3.78						
	Octanoyl-L-Carnitine	288.21_4.1						
	Palmitoyl-L-Carnitine	400.34_3.72						
	Propionyl-L-Carnitine	218.13_4.74						
	Stearoyl-L-Carnitine	428.37_3.66						
TCA intermediates	Citrate	104.10_3.86	32473.69 (± 15451)	20186.02 (± 6853.2)	2.63E-08	33361.62 (± 15930.5)	27115.51 (± 10695.3)	0.0106
	Oxaloacetic	268.96_1.8						
	Fumarate	115.03_1.44						

Chapter 6: Summary and Future Directions

6.1 Summary of Findings

In this thesis we have designed and conducted a series of clinical and scientific studies that have sought to find predictors primary nonresponse and loss of response to anti-TNF therapy. Two common *real world* clinical scenarios, namely altered body composition and stricturing disease which are known to be associated with variable response to anti-TNF therapy were used to explore clinical predictors of response to therapy. This thesis also explores *experimental* non-invasive biomarkers of response to anti-TNF therapy via metabolic profiling.

6.1.1 Body Composition

Although BMI has been associated with variable response to anti-TNF therapy it has been unclear as to which body composition parameters are associated with nonresponse to therapy. BMI measurement itself is a crude analysis of a patient's body composition but is broadly comprised of fat mass and muscle mass both of which have been linked to poor outcomes in Crohn's disease. We hypothesized that response to anti-TNF therapy in Crohn's disease may be associated with fat mass and muscle mass. We first sought to explore whether there was any association between specific body composition parameters and response to anti-TNF therapy among patients with Crohn's using body composition parameters derived from CT imaging prior to anti-TNF administration. In this cohort study we demonstrated that myopenia, which was found in patients in the lowest quartile of muscle mass, was associated with increased rates of primary non-response to anti-TNF therapy.²³⁰ We inferred that the mechanism behind primary non-response among patients with myopenia who have high fat free mass may be due to lower drug levels as a consequence of a higher volume of distribution. However, the accumulation of anti-TNF within muscle tissue may not only be due to the muscle's distributive capacity but also the fact that TNF alpha has been shown to be expressed by human muscle³⁴⁰.

6.1.2 Anastomotic Crohn's Disease

Having demonstrated a clinical predictor of primary nonresponse among patients with altered body composition we sought to establish clinical predictors of

response to anti-TNF therapy in a separate cohort. Recurrence of Crohn's disease with the development of anastomotic strictures following resectional surgery is common. We sought to evaluate the need for balloon dilatation or re-operation in patients with known anastomotic strictures who were treated with anti-TNF therapy. In our anastomotic Crohn's disease study, we found that anti-TNF initiation after balloon dilatation was associated with lower rates of repeat dilatations. We also discovered that endoscopic disease activity, based on the Rutgeerts score at initial dilatation was associated with subsequent need for resection of the anastomosis. Taken together, these findings provide evidence to support current expert consensus guidelines which suggest a "treat-to-target" approach to the management of IBD.¹⁰

6.1.3 Endoscopy and Histology as Predictors of Response to Anti-TNF Therapy

Having demonstrated the value of endoscopic predictors of response to anti-TNF therapy in the setting of stricturing Crohn's disease we sought to investigate predictors of response to anti-TNF therapy in non-stricturing luminal Crohn's disease. In particular we explored whether there was any association between endoscopic and histologic disease activity and subsequent outcome in a longitudinal cohort study of anti-TNF treated patients with Crohn's disease. We found that the number of segments of disease involved as well as histologic involvement were independently associated with response to anti-TNF therapy. In particular, the involvement of greater than 5 bowel segments and presence of granulomas was associated with primary non-response whereas isolated ileal disease involvement was associated with lower rates of PNR. The presence of lymphoid aggregates was associated with secondary loss of response to anti-TNF therapy. These data confirm the value of mucosal healing as a therapeutic target and demonstrate the value of histologic disease activity as a further marker of depth of remission and predictor of response to anti-TNF therapy.

6.1.4 Metabolic Profiling

In our prospective translational study, we sought to find non-invasive predictors of response to anti-TNF therapy by undertaking metabolic profiling. Previous studies have focussed on correlating metabolic profiles with distinguishing active disease from remission. We sought to evaluate whether there was any

association between metabolic biomarkers and treatment response in a cohort of anti-TNF treated CD patients who were followed longitudinally over 12 months with serum, urine and faeces collected for metabolite profiling. We developed a panel of metabolic biomarkers comprised of bile acid, polar metabolites and fatty acids which when measured using LC-MS which were to be found predictive of primary nonresponse. All three biomarkers were predictive of response to anti-TNF therapy, of which bile acid profiling demonstrated the strongest association with anti-TNF response (AUROC of 0.80). Although it is unclear as to why an increase in primary bile acids was strongly associated with primary non response primary bile acids have been shown to be colitogenic in mouse models.³⁴¹ Moreover the small intestinal microbiome plays a role in the deconjugation of secondary bile acids. Dysbiosis that occurs in Crohn's disease may result in a lack of functional microbiota to deconjugate secondary bile acids, thus creating an increased pool of primary bile acids which flow through into the colon creating an environment which favours inflammatory activity.

6.2 Future Directions

This thesis has demonstrated clinical and experimental predictors and biomarkers of response to anti-TNF therapy.

We inferred from our body composition study that myopenia was associated with primary nonresponse due to possible pharmacokinetic alterations via an impact on volume of distribution. Further work is required to quantify to extent body composition parameters correlate with TNF alpha muscle expression and anti-TNF levels. We intend to further investigate whether myopenia and visceral obesity are predictive of primary nonresponse to anti-TNF therapy in a prospective longitudinal cohort study. At baseline, patients will undertake a dietary questionnaire, anthropometry and physiotherapy assessment with whole body densitometry. These patients will then be followed up with objective markers of inflammation at 4 weekly intervals, in order to determine the patient's response to anti-TNF therapy. At week 24, patients will undergo further whole body DEXA evaluation to determine changes in their body composition parameters.

Our metabolic profiling work has demonstrated that interaction between the host and microbiome in Crohn's disease may affect response to anti-TNF therapy. We

would now like to validate our metabolic predictors of response to anti-TNF therapy in a larger cohort. A validation cohort is currently being assembled in the United Kingdom.

Our 16S RNA sequencing data did not identify any biomarkers associated with response which may have been due to the small sample size of responders and nonresponders. Expanding the cohort may identify important microbiome data which could then be investigated to find any potential correlations with the metabolic pathway alterations identified in our data.

Further studies performed in vivo with murine models will be helpful to delineate whether the primary bile acids predictive of anti-TNF non-response result in increased inflammatory activity via flow cytometry. A possible treatment modality for augmenting response to anti-TNF therapy may include either sequestration of primary bile acids or conversion to secondary bile acids to re-enter the enterohepatic circulation. Future studies should also explore the farnesoid X receptor which acts as part of the feedback loop which increases the production of primary bile acids.³³⁴ Via blockade of the farnesoid X receptor, it may be possible to decrease the overall pool of bile acids which may lead to decreases in the primary bile acid pool and therefore mitigate its impact on luminal inflammatory activity.

Primary nonresponse to anti-TNF therapy is common in Crohn's disease and represents a difficult clinical problem. Numerous pharmacodynamic and pharmacokinetic factors are involved. This thesis has identified several clinical and scientific biomarkers that are associated with anti-TNF therapy response in Crohn's disease. The integration of clinical predictors, microbiota and metabolomics using a systems biology approach is the next step to establish a predictive model which will help facilitate a personalised medicine approach to anti-TNF therapy.

Appendix I: Abbreviations and Definitions

AGA	American Gastroenterology Association
ASCA	Anti-saccharomyces cerevisiae antibody
ATA	Antibodies to adalimumab
AUC	Area under curve
BA	Bile acids
BIA	Bioelectrical impedance analysis
BMD	Bone mineral density
BMI	Body mass index
BRC	Biomedical research centre
CD	Crohn's disease
CDAI	Crohn's disease activity index
CDEIS	Crohn's disease endoscopic index of severity
CE	Capillary electrophoresis
CRP	C-reactive protein
CT	Computed tomography
DXA	Dual energy x-ray absorptiometry
ECCO	European Crohn's and Colitis Organisation
EN	Enteral nutrition
ESR	Erythrocyte sedimentation rate
FC	Faecal calprotectin
FD	Fistulizing disease
FFMI	Fat-free mass index
FN	False negative
FP	False positive
GABA	Gamma-aminobutyric acid
GAIN	Gauging adalimumab efficacy in infliximab non-responders
GWAS	Genome wide association studies
HBI	Harvey-Bradshaw Index
HC	Healthy control
HU	Hounsfield unit
IBD	Inflammatory bowel disease
IBDQ	Inflammatory bowel disease questionnaire
LBM	Lean body mass
LSMI	Lumbar skeletal muscle index
LVFI	Lumbar visceral fat index
MA	Muscle attenuation
MeSH	Medical library subject heading
MSI	Mass spectrometry imaging
NIHR	National Institute for Health Research
NMR	Nuclear magnetic resonance
OR	Odds ratio
PCA	Principal component analysis
PNR	Primary non response
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QC	Quality control
ROC	Receiver operating characteristic
SDA	System dynamics analysis

SMA	Skeletal muscle area
SMI	Skeletal muscle index
STAMP	Statistical analysis of metagenomic profiles
TAXIT	Trough concentration adapted infliximab treatment
TBW	Total body water
TN	True negative
TNF	Tumour necrosis factor
TP	True positive
UC	Ulcerative colitis
VFA	Visceral, subcutaneous fat area

Appendix II: Ethics Approvals

Health Research Authority

NRES Committee London - South East

Bristol Research Ethics Committee Centre
Level 3, Block B
Whitefriars,
Lewins Mead,
Bristol
BS1 2NT

Telephone: 01173421386
Facsimile: 01173420445

09 January 2015

Dr Nik Ding
London North West Healthcare NHS Trust
40 Elgin Mansions
Elgin Avenue
Maida Vale
W9 1JQ

Dear Dr Ding

Title of the Research Database:	The Influence Of Body Composition On Clinical Outcomes in Crohn's
REC reference:	14/LO/2110
IRAS project ID:	166341

Thank you for your letter responding to the Committee's request for further information on the above research database and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 05 January 2015. A list of the members who were present at the meeting is attached.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC manager, Mr Rajat Khullar, nrescommittee.london-southeast@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion of the above research database on the basis described in the application form and supporting documentation

Duration of ethical opinion

The favourable opinion is given for a period of five years from the date of this letter and provided that you comply with the standard conditions of ethical approval for Research Databases set out in the attached document. You are advised to study the conditions carefully. The opinion may be renewed for a further period of up to five years on receipt of a fresh application. It is suggested that the fresh application is made 3-6 months before the 5 years expires, to ensure continuous approval for the research database.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
IRAS Checklist XML [Checklist_10112014]		10 November 2014
IRAS Checklist XML [Checklist_02012015]		02 January 2015
Other [Ethics protocol]		31 October 2014
Other [Ethics protocol]	2	02 January 2015
Other [Data protection policy]	1.0	02 January 2015
Other [REC reply to questions]	1	02 January 2015
REC Application Form [RD_Form_10112014]		10 November 2014
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Research governance

A copy of this letter is being sent to the R&D office responsible for London North West Healthcare NHS Trust.

Under the Research Governance Framework (RGF), there is no requirement for NHS research permission for the establishment of research databases in the NHS. Applications to NHS R&D offices through IRAS are not required as all NHS organisations are expected to have included management review in the process of establishing the database.

Research permission is also not required by collaborators at data collection centres (DCCs) who provide data under the terms of a supply agreement between the organisation and the database. DCCs are not research sites for the purposes of the RGF.

Database managers are advised to provide R&D offices at all DCCs with a copy of the REC application for information, together with a copy of the favourable opinion letter when available. All DCCs should be listed in Part C of the REC application.

NHS researchers undertaking specific research projects using data supplied by a database must apply for permission to R&D offices at all organisations where the research is conducted, whether or not the database has ethical approval.

Site-specific assessment (SSA) is not a requirement for ethical review of research databases.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached standard conditions give detailed guidance on reporting requirements for research databases with a favourable opinion, including:

- Notifying substantial amendments
- Submitting Annual Progress reports

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

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HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

14/LO/2110

Please quote this number on all correspondence
--

Yours sincerely



pp Professor David Caplin
Chair

E-mail: nrescommittee.london-southeast@nhs.net

*Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
Approval conditions*

Copy to: Prof Sue Clark, London North West Healthcare NHS Trust

NRES Committee London - South East

Attendance at Sub-Committee of the REC meeting on 05 January 2015

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Professor David Caplin	Physicist	Yes	
Mr Ron Driver	Statistician	Yes	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Mr Raj Khullar	REC Manager

Health Research Authority

NRES Committee London - South East

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14/LO/2110

Please quote this number on all correspondence
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Yours sincerely



pp Professor David Caplin
Chair

E-mail: nrescommittee.london-southeast@nhs.net

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