MONASH University

Therapeutic Optimisation in Patients with Inflammatory Bowel Disease

by

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THESIS

Submitted to the Faculty of Medicine

Monash University, Melbourne, Australia

as requirement for the degree of

Doctor of Medicine

2016

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Abstract

Unanswered questions in optimising therapy of IBD:

Three closely interrelated areas in assessment of and improvement in therapy in IBD are the subject of the proposed thesis:

- *Need for thiopurine optimisation:* There is an unmet need to develop and improve on conventional immunomodulators to treat IBD. Thioguanine, a non-conventional thiopurine, showed promise in early studies, but interest was tempered due to an association with nodular regenerative hyperplasia of the liver. Long term studies assessing clinical outcome and adverse effects are lacking. Evidence has demonstrated that combining thiopurines with infliximab is more efficacious than using either agent alone. The use of combination therapy with adalimumab has been conflicting, which may be due to the way thiopurines are prescribed in studies, (conventional weight-based) rather than by determining active metabolites.
- *Therapeutic drug monitoring with biologic agents:* Infliximab (IFX) and adalimumab (ADA) have revolutionised the modern management of IBD. Primary and secondary loss of response occur in a proportion. Mechanisms include low drug levels and immunogenicity, leading to increased drug clearance. Therapeutic drug monitoring (TDM) is of benefit in IBD but questions remain: the optimal drug level with IFX and ADA for clinical remission and mucosal healing; methodological issues such as reproducibility of drug levels over time, the correct sampling time of TDM with ADA and differences between the various assays used.
- *Micronutrient optimisation in IBD:* Patients with Crohn's disease are at risk of vitamin B12 deficiency. Holotranscobalamin II is a new method of assessing B12 deficiency that is superior to conventional serum B12. Holotranscobalamin II has not been tested in Crohn's disease. Further, little data exist regarding risk factors, in particular the burden of ileal disease assessed using magnetic resonance imaging.

Aims of the thesis

1. *Optimisation of thiopurines in IBD:* Following a review article, we performed a study comparing ADA monotherapy to combination therapy, exploring the relationship between thiopurine dose intesity via metabolite testing to response at induction and maintenance. A retrospective analysis of the long-term follow-up data on the efficacy and safety of thioguanine was conducted.
2. *Issues in TDM with biologic therapy:* A review article is followed by a study examining cut-off IFX and ADA levels, relating these disease activity using a variety of endpoints. An inter-kit comparison across ELISA TDMs assessed the relative performance for IFX and ADA. A prospective study explored whether ADA TDM can be performed at any point in a treatment cycle, rather than trough, and considered modulating patient and disease factors on levels.
3. *Functional B12 deficiency in Crohn's disease:* A study using holotranscobalamin II to determine prevalence of functional vitamin B12 deficiency in Crohn's disease was conducted which sought to identify relevant patient and disease risk factors.

This work has identified a wide range of results relevant to the optimisation of therapy in IBD.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: .. 

Print Name: ..... MARK WARD

Date: ..... 17TH AUGUST 2016

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Publications during enrolment

Ward MG, Irving PM, Sparrow MP. How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease? World J Gastroenterol 2015; 21(40):11331-11342

Ward MG and Irving PM. Role of Therapeutic Drug Monitoring for Biologics in IBD. Inflamm Bowel Dis Monit 2013;14(2):44-53

Ward MG, Kariyawasam VC, Mogan SB, Patel KV, Pantelidou M, Sobczynska-Malefora A, Porté F, Griffin N, Anderson SHC, Sanderson JD, Harrington DJ, Irving PM Prevalence and Risk Factors for Functional Vitamin B12 Deficiency in Patients with Crohn's Disease. Inflamm Bowel Dis 2015;21:2839-2847

Ward MG, Patel KV, Kariyawasam VC, Goel R, Warner B, Elliott TR, Blaker PA, Irving PM, Marinaki AM, Sanderson JD. Thioguanine in Inflammatory Bowel Disease: Long-Term Efficacy and Safety. United European Gastroenterol J. 2016;0(0):1-8

Ward MG, Warner B, Unsworth N, Chuah S-W, Brownclarke C, Shieh S, Parkes M, Sanderson JD, Arkir Z, Reynolds J, Gibson PR, Irving PM. Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors. Aliment Pharmacol Ther. 2017;00:1-12

Ward MG, Thwaites PA, Beswick L, Hogg J, Rosella G, Van Langenberg D, Reynolds J, Gibson PR, Sparrow MP. Intra-patient variability in adalimumab drug levels within and between cycles in Crohn's disease. Aliment Pharmacol Ther. 2017;45:1135-1145

Kariyawasam VC, Ward MG, Blaker PA, Patel KV, Goel R, Sanderson JD, Irving PM. Thiopurines dosed to a therapeutic 6-thioguanine level in combination with adalimumab is more effective than sub-therapeutic thiopurine based combination therapy or adalimumab monotherapy during induction and maintenance in patients with long standing Crohn's disease. Inflamm Bowel Dis 2017 *(In Press)*

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes seven original papers published in peer reviewed journals and one submitted for publication. The core theme of the thesis is "**Therapeutic Optimisation in Patients with Inflammatory Bowel Disease**". The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Gastroenterology, Alfred Hospital, Melbourne, Australia and the Department of Gastroenterology, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom under the guidance of Professor Peter R Gibson and Dr Peter M Irving.

In the case of **Chapters 2-9** my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash

					student Y/N*
2	How should immunomodulators be optimised when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease?	Published	80%; Concept and design, preparation of manuscript.	1) Irving PM 5%; Review and revision of final manuscript 2) Sparrow MP 15%; Design and preparation of manuscript	No
3	Metabolite-adjusted thiopurine dosing in combination with adalimumab is superior to adalimumab monotherapy during induction and maintenance in Crohn's disease	Accepted for publication	40% (joint first author); concept and design, data acquisition and analysis, drafting manuscript	1) Kariyawasam VC 40% 2) Blaker PA, Patel KV, Goel R, Sanderson JD, Irving PM remaining 20%; data acquisition and revised manuscript	No
4	Thioguanine in inflammatory bowel disease: Long-term efficacy and safety	Published	70%; concept and design, data acquisition and analysis, drafting manuscript	1) Patel KV 5%; data acquisition, analysis, revised manuscript 2) Kariyawasam VC 2.5%; data acquisition, revised manuscript 3) Goel R, Warner B, Elliott TR, Blaker PA 2% (each); data acquisition, revised manuscript 4) Irving PM 2.5%; data acquisition, revised manuscript 5) Marinaki 2%; laboratory analysis, revised manuscript 6) Sanderson JDS 10%; study design, analysis, revised manuscript	No
5	Role of therapeutic drug monitoring for biologics in inflammatory bowel disease	Published	80%; Concept and design, preparation of manuscript.	1) Irving PM 20%; Design and manuscript revision	No
6	Inter-kit comparison of ELISAs for therapeutic drug monitoring of infliximab and adalimumab in Crohn's disease	Ready for submission	70%; concept and design, data acquisition and analysis, drafting manuscript	1) Unsworth, N 5%; laboratory analysis, drafted laboratory methods and manuscript 2) Warner, B, Chuah, S-S, Shieh, S 2.5%(each); data acquisition, revised manuscript 3) Sanderson, JD 2.5%% revised manuscript 4) Parkes, M and Irving, PM 5%; design and revised manuscript 5) Arkir, Z 5%; laboratory analysis, revised manuscript	No
7	Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors	Published	70%; concept and design, data analysis, drafting manuscript	1) Unsworth, N and Arkir, Z 2%(each); laboratory analysis, revision manuscript 2) Warner, B, Chuah, S-S, Shieh S, Brownclarke C, 2%(each); data acquisition, revised manuscript	No

				3) Sanderson, JD and Parkes, M 2.5%(each); design and revised manuscript 4) Irving, PM and Gibson PR 5% (each); design and revised manuscript 5) Reynolds, J; 3%; some statistical analysis and assistance, revised manuscript	
8	Intra-patient Variability in Adalimumab Drug Levels Within and Across Cycles in Crohn's Disease	Published	70%; concept and design, data acquisition and analysis, drafting manuscript	1) Thwaites, PA, Beswick, L, Hogg, J 2.5%(each); data acquisition, revised manuscript 2) Rosella, G 2%; laboratory analysis, revised manuscript 3) Van Langenberg D 2.5%; study design, revised manuscript 4) Reynolds, J; 5%, statistical analysis, revised manuscript 5) Gibson, PR, Sparrow, MP 6.5% (each); study design, data interpretation, revised manuscript	No
9	Prevalence and Risk Factors for Functional Vitamin B12 Deficiency in Patients with Crohn's Disease	Published	70%; concept and design, data analysis, drafting manuscript	1) Kariyawasm VC 2.5%; data acquisition, statistical analysis, revised manuscript 2) Mogan SB, Patel KV , Pantelidou M 2% (each); data acquisition, revised manuscript 3) Sobczynska-Malefora A 2.5%; laboratory analysis, data analysis, revised manuscript 4) Griffin N, Porte F 2% (each); interpretation of radiology, revised manuscript 5) Anderson SHC, Sanderson JDS 2.5% (each); revised manuscript 6) Harrington DJ 5%; laboratory analysis, data analysis, revised manuscript 7) Irving PM 5% ; study design, data interpretation, revised manuscript	No

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date: 2nd June 2017**

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Acknowledgements

This thesis is the culmination of four years of hard work dating back to the first day I stepped into the Department of Gastroenterology at Guy's and St. Thomas' Hospital in London, United Kingdom. My initial intention was to conduct a clinical fellowship in Inflammatory Bowel Disease over a period of 12 months. Within weeks, under the mentorship of Dr Jeremy Sanderson and Dr Peter Irving, I was inspired to undertake studies into the use of thiopurines, both conventional, and in in the case of Thioguanine, unconventional. Dr Viraj Kariyawasm, from Sydney, joined me within months and a productive collaborative partnership ensued. Life-long friendships were forged and I will always look back fondly on the two years I spent there.

I am deeply indebted to Peter, an innovator in the field of IBD. Despite his busy schedule he has always been engaging and supportive, and was a major driver in the work produced in this thesis. Peter 'planted the seed' inspiring me to conduct research, and contributed significantly to much of the study creation, design and implementation herein. Under his guidance my early writing skills developed considerably, which is reflected in the quality of the publications and manuscripts contained in this thesis.

Returning to Australia, Professor Peter Gibson, Director of the Department of Gastroenterology at the Alfred Hospital, persuaded me to convert and build on the research from London in order to submit this thesis. This was not an easy process in view of changes to the Doctor of Medicine degree and without him as my advocate it would be unlikely that this thesis would have ever seen the light of day. Peter is the ideal mentor and role model; a 'thinker', prolific academically, highly personable and widely respected; he displays qualities that have inspired me to pursue a career in IBD and cultivated my budding interest in research. I am deeply indebted to him; his insights, guidance and support have significantly contributed to the work in this thesis.

Finally, to my loving wife Bonnie, who I am indebted to above all others. Her patience, care and support has inspired me to follow through on this difficult journey. Without her I would not have been able to undertake and complete this research. Accordingly, this thesis is dedicated to her.

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SECTION 1.

CHAPTER 1:

Introduction

CHAPTER 1 – INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), collectively inflammatory bowel diseases (IBD), are chronic inflammatory conditions driven by an exaggerated immune response towards the gastrointestinal tract, in response to antigenic stimulation from the gut microbiota, within a genetically susceptible host. Most face a relapsing and remitting disease course; in an inception cohort of 237 CD patients from South-Eastern Norway 53, 85 and 90% had a disease relapse at 1, 5 and 10 years, respectively.[1] Hospitalisation and surgery are common sequelae. In a population-based cohort of CD from Olmstead County, the cumulative probability of intestinal resection within 10 years of diagnosis was 47.6%; further, 30.8% underwent a second operation within five years of the first.[2] Similar rates have been reported by others in Norway[1] and Canada.[3] Perianal Crohn's disease, a cause of significant morbidity, is seen in up to a third of patients and often necessitates repeat surgical intervention to control distressing and disabling symptoms.[4] Colectomy rates approximate 10% of patients with UC, with a high proportion occurring during the index hospitalisation.[5] Hospitalisation due to active disease, or arising as a complication of medical therapy, are frequent, and, in particularly in the case of CD, are associated with an increased probability in the need for surgery.[6,7] Multiple studies have shown that IBD negatively impacts quality of life[8,9] (QoL) and that QoL is inversely correlated with disease severity.[10] Higher levels of unemployment, work absenteeism and earlier retirement have been reported.[11] Further, these domains are improved in patients who are in clinical remission. Taken together, inflammatory bowel diseases confer significant morbidity. Whether IBD as a whole affects survival is debated; studies have yielded inconsistent results.[12-14] However, high risk patients, particularly with CD, appear to have an increased mortality rate compared to that of the general population.[15,16]

In recent decades there have been significant changes in the epidemiology, management and in patient expectations. The incidence and prevalence in Asia,[17] as in parts of eastern Europe,[18] is increasing towards those seen in Westernised countries. Recent data have demonstrated that Australia has one of

the highest incidences of IBD in the world, being 11.6-17.4 per 100 000 for CD and 7.5-11.2 per 10 000 for UC.[19] These are similar to the incidence reported in Canterbury, New Zealand.[20] Age of onset is occurring earlier,[17] exposing patients to a longer duration of disease and disability.

In parallel, our therapeutic armamentarium has expanded over the last 30 years. We have learned and come to accept that 5-aminosalicylates are of limited to no benefit in CD[21] and that corticosteroid therapy has no role in maintaining remission.[22] A shift in the positioning of immunomodulators (thiopurines and methotrexate), used for decades as monotherapy in both UC and CD, has occurred since the introduction of monoclonal antibodies directed against the pro-inflammatory cytokine, tumour necrosis factor alpha (TNF) - infliximab (IFX) and adalimumab (ADA) - some 10-15 years ago.[23-27] With these new therapies has come changes in management. The time-honoured reactive strategy of aiming for clinical remission with step-wise treatment intensification has shifted to a more aggressive and personalised treat-to-target paradigm.[28] The poor correlation between clinical disease indices and degree of intestinal inflammation, particularly in the case of CD[29] has given way to normalisation of biomarkers (C-reactive protein and faecal calprotectin) and healing the mucosa as the ultimate goal. Identification of high risk patients has led to the adoption of 'top down' or accelerated step-up therapy in order to minimise progression of disease and its associated tissue damage, and improve patient outcomes.[30] New patient-centred models of care have expanded the binary gastroenterologist-patient relationship to include health care providers from a wide array of disciplines, such as IBD-centric surgeons and radiologists, and dedicated IBD-pharmacists, dieticians and clinical nurse specialists.[31,32] Delivery of care has gone beyond the consulting suite to include dedicated multidisciplinary teams,[31] telemedicine[33] and virtual clinics.[34]

The changing landscape has brought optimisation of therapy into the spotlight. In this regard, numerous advances in the fields of pharmacogenetics, pharmacokinetics and pharmacodynamics have added to our understanding. The discovery of a trimodal pharmacogenetics variation in thiopurine methyltransferase (TPMT), a key enzyme in the thiopurine pathway, whereby 10% of the population

have intermediate activity and 0.3% extremely low activity, has, accordingly, led to dose reduction (or avoiding using the drug altogether), which reduces the risk of potentially life-threatening myelosuppression.[35] Dosing thiopurines to a target concentration of 6-thioguanine nucleotide (TGN) > 235-260 pmol/8x10^8 red blood cell (RBC) is associated with improved rates of clinical remission with an odd's ratio (OR) of 3.15; 95% confidence interval (CI): 2.41-4.11).[36] Further, thiopurine metabolite testing (which includes assessment of the methylated metabolite concentration) identifies common profiles that can guide further dose adjustment.[37] Despite the significant benefits seen with anti-TNF therapy, approximately 10% of patients fail to respond, and of those who do, between 20-40% go on to develop secondary loss of response by 12 months.[38] Early standard-of-care involved episodic administration of anti-TNF therapy, which we subsequently learned was associated with the development of immunogenicity, which, in turn, was associated with a shorter duration of response and an increased risk of infusion reactions.[39] More recently, with the introduction of therapeutic drug monitoring of IFX and ADA, significant advances have been made into the understanding of the complex pharmacokinetic-pharmacodynamic relationship of these therapies which has, in turn, provided strategies to improve outcomes.[40] Nutritional optimisation, by addressing macro and micro-nutrient deficiencies commonly seen in patients with IBD, is of significant importance and frequently overlooked when the focus is on medical management.[41]

During my short career in gastroenterology, there has been a major shift in attitudes to therapeutic optimisation. Such a relatively rapid evolution has been a major stimulus to the work described in the present thesis. All of the work has addressed the core issue of how different therapeutic approaches can be optimised in the individual patient – from the repletion of vitamin B12 first recognised and applied as parenteral therapy in 1952,[42] to the use of thiopurines first reported in 1980,[43] to reactive[44,45] and, more recently, proactive application of therapeutic drug monitoring of biologic therapy, namely anti-TNF.[46,47]

Immunomodulators, namely thiopurines and methotrexate, have been used for decades in the management of CD and, to a lesser extent, UC. The conventional thiopurines, azathioprine (AZA) and mercaptopurine (MP), have been shown to be effective as steroid-sparing agents and for maintenance of clinical remission in numerous trials and confirmed in Cochrane reviews. [48,49] Despite this benefit, there are high rates of drug withdrawal due to intolerance. Serious adverse effects, including pancreatitis,[50] lymphoma[51] and non-melanoma skin cancers,[52] have been recognised and well characterised. Further, a substantial proportion of patients fail to respond and are escalated to more intensive therapy. Whether thiopurine monotherapy is superior to placebo in early CD has been questioned in light of two recent studies[53,54] although patient selection and methodology may explain the relative lack of efficacy reported.[55] Since the arrival of anti-TNF therapy, which has demonstrated improved efficacy both for induction and during maintenance, coupled with an attractive safety profile, the optimal role of thiopurines and methotrexate has been debated. In the landmark SONIC study, treatment naïve patients with moderate-to-severe CD randomised to combination therapy with IFX and AZA achieved significantly higher rates of corticosteroid-free clinical remission and mucosal healing compared with either agent alone. A similar benefit was observed in patients with UC.[56] The situation with ADA is less clear, in part because of a lack of randomised controlled trials designed to address this question. These, and other data,[57] have resulted in combination therapy emerging as the recommended treatment strategy for the majority of patients with moderate-to-severe disease. Once this decision has been made, questions arise as to how best to optimise the immunomodulator, considering the risk-benefit profile within for each individual patient.

Therefore, the first major aim of this thesis was to perform a review of the literature to address how to optimise immunomodulators when used in combination therapy with anti-TNF agents in the management of IBD.

The resulting publication (Ward MG, 2015) and Chapter 2 of this thesis, begins with an overview of the available literature reporting the efficacy of combination therapy with thiopurines and MTX when used with IFX or ADA in CD and UC. Whilst the evidence appears robust for the combination of IFX and thiopurines in treatment naïve patients with IBD, whether this benefit extends to the use of MTX, or indeed ADA, is less clear, as reported in the COMMIT study[58] and a meta-analysis of randomised controlled trials, respectively.[59] Following is a balanced assessment of the other side of the pendulum, namely infections and malignancy. Despite theoretically conferring an increased overall risk of immunosuppression, data from registration trials and large retrospective observational cohorts is largely reassuring. The association between thiopurine monotherapy and an increased risk of non-melanoma skin cancer[52] and lymphoma[51] has been reported extensively. Whether these risks are increased further when anti-TNF is added is limited by a lack of high quality data, largely due to confounding by previous thiopurine exposure. On balance, both adverse outcomes are probably increased in combination therapy above and beyond that seen with either thiopurine or anti-TNF monotherapy, however results are conflicting.

Unanswered questions regarding optimisation of immunomodulators when used in combination therapy are then addressed under the broad sections of which drug (thiopurine or MTX), when should immunomodulators be started (when used in combination) what dose (are lower doses as efficacious and safer) and can immunomodulators be stopped (after a period of combination therapy). This integrative discussion pays special attention to the observation that immunomodulators confer benefit above and beyond their mode of action on disease itself, by favourably influencing the pharmacokinetics of anti-TNF therapy in turn increasing drug levels and reducing anti-drug antibody formation.[60-65] Finally, directions for future research are proposed, including the need for prospective studies which examine the clinical and pharmacokinetic outcomes of combination therapy according

to different immunomodulatory regimens which report levels of both anti-TNFs and immunomodulators.

As outlined above, and discussed in depth in the review article, whilst the benefit of combination therapy with IFX in treatment naïve patients appears clear, the situation with ADA is less compelling. Compared with IFX, a lack of data, particularly prospective randomised controlled studies, is acknowledged. Presented recently in abstract form, a 'SONIC-like' study randomising treatment naïve CD patients to ADA monotherapy or combination therapy with a thiopurine found no difference in clinical remission at week 26 between the two treatment arms, however higher ADA drug levels and an improvement in endoscopic activity was observed in the combination therapy cohort.[66] In rheumatoid arthritis, a clear superiority of combination therapy with an immunomodulator (MTX) and ADA over ADA or MTX alone has been reported.[67]

Data from observational studies has generally found no benefit of combination therapy over ADA monotherapy[57] and has led some, given the observation that ADA is relatively less immunogenic than IFX, to treat patients with ADA monotherapy thereby avoiding potential toxicity associated with immunomodulators. A related, but more commonly encountered scenario, is whether to continue immunomodulators in patients failing these therapies that subsequently step-up to anti-TNF. A meta-analysis of 11 randomised placebo-controlled studies in CD excluding treatment naïve patients found combination therapy was no more effective than monotherapy in inducing six-month remission (OR 1.02; 95% CI: 0.80-1.31) or response (OR 1.08; 95% CI: 0.79-1.48).[59] A benefit of combination therapy with IFX was seen when a sensitivity analysis which included data from ACCENT 2[68] was performed (OR 1.79; 95% CI: 1.06 – 3.01). Considering ADA, combination therapy was no more beneficial than ADA monotherapy (OR 0.88; 95% CI: 0.58-1.35).

Recent data with IFX has implied a relationship between the intensity of concomitant immunomodulation and outcomes in CD. A post-hoc analysis of SONIC identified that patients with an increase of 7 femtolitres in the mean corpuscular volume, used as a surrogate for therapeutic TGNs (as these were not measured directly) were more likely to maintain therapeutic trough IFX levels at week 30 ($p = 0.003$) and were more likely to achieve mucosal healing ($p = 0.017$).[69] Yarur and colleagues found higher IFX drug levels in patients treated in combination with a thiopurine, a positive correlation between TGNs and IFX drug levels, and identified a threshold of 125 pmol/$8x10^8$RBC which best predicted 'higher' IFX drug levels.[64] To date, no studies have been published that examine this relationship in patients with CD treated with combination therapy with ADA.

Accordingly, the second aim of this thesis was to perform a retrospective study comparing clinical outcomes of patients with CD, after induction and during maintenance, treated with ADA monotherapy compared to combination therapy, stratified by TGNs.

The resulting study, submitted for publication (Kariyawasam VC and Ward MG, joint first authors, 2016) serves as Chapter Three of this thesis. We retrospectively studied consecutive patients with moderate-to-severe CD who commenced ADA at a single institution between 2006 and 2013. Response after induction (week 12) was assessed by physician global assessment after considering prospectively collected clinical indices (Harvey-Bradshaw Index[70]) and the results of biomarkers (C-reactive protein (CRP) and faecal calprotectin) and imaging or endoscopy, and were classified as complete, partial or non-response. Outcomes on maintenance therapy were considered in 6-monthly semesters and defined as either a flare semester (active disease resulting in treatment modification), failure semester (ADA withdrawal to lack/loss of response despite treatment intensification or the development of adverse effects) or remission semester (absence of flare or failure). Concomitant immunomodulation (CIM)

during induction was defined as a stable dose of immunomodulator ≥ 3 months prior to commencing ADA and continued for ≥ 6 months, and during maintenance, as ≥ 3 months of a 6-month semester. Patients treated with thiopurines were stratified according to TGNs with > 235 pmol/8x10^8RBC considered therapeutic. Response to induction was significantly higher in the CIM group compared to ADA monotherapy (83 vs 61%, p = 0.02) and primary non-response lower (12 vs 30%, p = 0.02). Further, patients with therapeutic TGNs compared to sub-therapeutic TGN and ADA monotherapy had higher response rates (p = 0.011). Therapeutic TGNs (OR 4.32, 95% CI: 1.41-13.29, p = 0.01) and albumin level (OR 1.09, 95% CI: 1.01-1.18, p = 0.03) were independent predictors of response to induction on multivariate analysis. The maintenance analysis included 280 semesters in 91 patients. Similar benefits with CIM (81 vs non-CIM 60%, p < 0.0001) and therapeutic TGNs (86 vs sub-therapeutic TGN 58%, p = 0.004) were observed. Ileal disease location (OR 0.21, 95% CI: 0.08-0.57, p = 0.002) and therapeutic TGNs (OR 3.71, 95% CI: 1.87-7.34, p < 0.0001) were independent predictors of remission semesters. Time to ADA failure was significantly longer in the CIM group compared with ADA monotherapy (68.5 vs 35.7 months, p = 0.009 $_{\text{log rank}}$) and therapeutic TGN ≥ 3 months prior to ADA (HR 0.37, 95% CI: 0.15–0.89, p = 0.026) was an independent predictor of time to failure using Cox regression analysis.

There were several findings of clinical relevance. First, combination therapy with an immunomodulator resulted in a higher response at induction compared to ADA monotherapy. Second, during maintenance, combination therapy was associated with a decrease in the proportion of flare semesters. Third, sub-therapeutic TGNs at induction and during maintenance were associated with worse outcomes and an increased risk of ADA failure compared to therapeutic TGNs. Fourth, the attainment of therapeutic TGNs at the same time as starting ADA was important. Taken together, this study supports the use of combination therapy over ADA monotherapy in the management of CD. Further, for the first time, we found that the intensity of thiopurine therapy, by dosing to a TGN > 235 (rather than their use *per se*) was of relevance. Although drug levels and anti-drug antibodies were not measured, these findings infer that the benefit of optimised thiopurines may due to an improvement in the pharmacokinetics of ADA.

Thioguanine (TG) is a non-conventional thiopurine that has been studied in haematological malignancies[71] and in small uncontrolled studies in both CD and UC. By being directly converted to the therapeutically active 6-thioguanine nucleotides, TG bypasses numerous intermediate metabolites involved in the conventional thiopurine pathway.[72] This can circumvent potential toxicity and adverse effects seen with AZA and MP, including pancreatitis, leading to improved rates of tolerance. Pilot studies of TG in IBD demonstrated similar response rates to those for conventional thiopurines.[73,74] However, interest in the drug was tempered by an association with nodular regenerative hyperplasia (NRH) of the liver, seen in up to 76% of treated patients.[75,76] NRH is a recognised, albeit uncommon, complication of conventional thiopurines, IBD itself and other chronic inflammatory conditions.[77] Subsequent studies using lower dose TG (< 40 mg oral daily) have reported little to no NRH,[78,79] suggesting that low dose TG may in fact be safe.

Therefore, the third aim of this thesis was to examine the long term safety and efficacy of TG in a cohort of IBD patients intolerant of, or refractory to, conventional immunomodulators.

This study was performed, and the resulting publication (Ward MG et al. 2016) comprises Chapter Four of this thesis. The study, which involved 54 IBD patients and reported 126 patient-years of follow-up (the largest to date in the literature), found serious adverse events occurred in four patients. Two elderly patients developed solid organ malignancy (breast and gastric) after previous treatment with conventional thiopurines and co-therapy with anti-TNF agents. One patient was admitted to hospital with neutropenic sepsis which responded to antibiotics and another developed a portal hypertensive syndrome with jaundice and ascites; both patients recovered after TG was withdrawn. Pancreatitis did not recur, despite 35% of patients developing this when treated with AZA as first-line

therapy. TG was well tolerated in this difficult to treat cohort; 16/54 (30%) ceased therapy due to side effects or biochemical abnormalities, despite high rates of intolerance to AZA, MP or MTX previously. Finally, no cases of NRH were observed using a dedicated safety monitoring programme which included screening with liver biopsy and/or dedicated liver imaging. TG was efficacious, with clinical response observed in 59 and 43% of patients at 6 and 12 months, respectively. 33% of patients continued TG through during follow-up with median duration of therapy of 32 months (range 12-132).

In this retrospective study, the efficacy of and tolerance to TG in the patients studied who were previously intolerant or refractory to conventional thiopurines were similar to those usually seen with conventional immunomodulators. It is an acceptable alternative immunomodulator when failure to these therapies has occurred. The complication that has worried physicians, NRH, was not observed using low dose TG.

IFX, and successively ADA, monoclonal antibodies directed against TNFα have revolutionised the modern management of IBD. This class of therapy is efficacious for both induction and maintenance of moderate-to-severe luminal and perianal CD and moderate-to-severe ulcerative colitis. Subsequently, IFX was found to be non-inferior to cyclosporine as rescue therapy in patients with acute severe ulcerative colitis failing corticosteroids[80] and, due to its relative ease of administration and safety profile, has become the first choice in many units around the world. A small proportion of patients do not respond to these drugs (primary non-responders) and, depending on the definition employed, 20-40% of initial responders go onto lose response by 12 months, with a further 10% annually thereafter.[38] This is a key issue in IBD as, unlike other chronic autoimmune conditions, few alternative effective therapies exist. Factors associated with, but not limited to, primary non-response and secondary loss of response include episodic therapy, development of anti-drug antibodies leading

to immunogenicity and consequently increased drug clearance, the degree of inflammatory burden and patient factors such as previous drug treatment history, serum albumin and body mass index.[40] Therapeutic drug monitoring (TDM) of anti-TNF therapy, specifically measuring drug levels and, to a lesser extent, anti-drug antibodies of both IFX and ADA has gone some way to improving our understanding of the pharmacokinetic-pharmacodynamic relationship of these therapies. This has led to strategies for optimising response to anti-TNF agents in order to avoid drug failure. Despite a large body of evidence demonstrating an inverse relationship between drug levels and outcomes,[46,81-84] many unanswered questions remain.

In view of these issues, the fourth aim of this thesis was to review the utility of therapeutic drug monitoring for anti-TNF therapy in IBD.

In this published paper (Ward MG 2013) which comprises Chapter Five of this thesis, an overview of the benefits and rates of primary and non-response to IFX and ADA in CD and UC is presented. Methodology of commonly used TDM platforms follows, with a focus on enzyme-linked immunosorbent assays (ELISAs), given their widespread application. The inverse relationship between IFX and ADA drug levels and clinical outcomes is reviewed and significant data are cited. TDM in the clinical context is described, drawing on evidence from pivotal studies which demonstrate that the optimal strategy in patients with sub-therapeutic IFX is dose intensification (rather than within-class switching to ADA), and that, conversely, in the situation of detectable anti-drug antibodies, patients should be switched within-class (as opposed to increasing the dose of IFX).[45] The complexity surrounding the significance of anti-drug antibodies is addressed in brief (transient versus sustained[85], differing assay methodology and reportable units).

Despite a large body of literature supporting the utility of TDM in IBD, several key areas are yet to be addressed. These include: (but are not limited to) the relative performance and agreement between ELISA assays, the lack of data supporting an association between ADA drug levels and outcomes, what drug levels thresholds should be targeted for different indices of disease activity, and finally, considering ADA, whether TDM should be peformed at trough or at any point within a treatment cycle. These areas of debate are considered in the forthcoming Chapters of this thesis.

Methodology for performing TDM can be broadly classified into three different platforms: ELISA, radio-immunoassay (RAI) and homogeneous mobility shift assay (HMSA). To date, the majority of data has come from studies employing ELISA. This is explained by its widespread uptake compared to other platforms due to factors such as a relatively lower cost, increased access and availability in many countries, and simpler materials and methods required within the laboratory. Considering ELISAs, a large number of commercially available and academic 'in-house' kits are in routine use. Despite being designed on the same principle, significant inter-kit differences exist, such as the detector moiety[86-89] (for both drug and anti-drug antibodies) which can influence the sensitivity and specificity of the assay. Surprisingly, there is little data comparing the relative performance of commonly available ELISAs.[90-92] This is of relevance clinically, as samples reported as therapeutic with one assay may be different on other assays, which could, in theory, translate to different management strategies should patients be misclassified as having therapeutic or sub-therapeutic drug levels.

Therefore, the fifth major aim of this thesis was to perform an inter-kit comparison of ELISAs commonly used for TDM in CD to evaluate the relative performance of each assay, and to examine these differences qualitatively in regard to misclassification rate of therapeutic compared to sub-therapeutic drug levels comparing to a reference assay.

This study was performed (Ward MG, pending submission 2016) and comprises Chapter Six of this thesis. In this round-robin analysis performed in a single laboratory, serum samples from patients with CD were compared using Lisa-Tracker (LT) Premium (Theradiag, France), IDK*monitor*® (IM) (Immundiagnostik, Germany), Promonitor (PRO) (Progenika Biopharma, Spain) and RIDASCREEN (RS) (R-Biopharm AG / KU Leuven) ELISAs. Drug levels (reported in µg/mL) from approximately 100 IFX samples were measured on all kits and drug levels from 99 ADA samples on LT, PRO and IM. Drug level assays measure free IFX or ADA, as appropriate, but differ in microtiter plate coating and secondary detection reagents. Anti-drug antibodies were evaluated for IFX and ADA on LT, PRO and IM and are reported in ng/mL (LT) and AU/mL (IM and PRO). LT and PRO utilises a specific bridging ELISA to quantitatively measure free anti-drug antibodies, whereas IM utilises a dissociation step to enable detection of total anti-drug antibody generating semi-quantitative results. Statistical analyses included method comparisons by means of difference plots and Passing Bablok analysis, correlation, and agreement and reliability by intra-class coefficients and Bland-Altman proportion plots. Using LT as the reference assay (given it is in use at our institution) we compared drug levels from other assays to proposed therapeutic cut-offs (in this case < 2 for IFX and < 4.9 µg/mL for ADA) to qualitatively determine the proportion of patients who would be misclassified (therapeutic vs sub-therapeutic).

This study demonstrated that significant variation in drug levels existed between most assays. IFX drug levels with RIDA were positive biased against those with LT (2.7), IM (3.1) and PRO (2.0) and the degree of bias between RIDA and LT was concentration dependant (as illustrated in the Passing Bablok plots), whereas bias against PRO and IM was variable. The latter is important, as the application of concentration dependant bias allows generalisability between assays if a corrective factor is known. The situation of variable bias is more troubling, this means that the results obtained on RIDA, PRO and IM cannot be directly compared with precision. Bias between assays measuring

ADA was consistent. As might be expected, the IM assay detected anti-drug antibodies more frequently due to its ability to measure total (free and bound) anti-drug antibody, but the significance of these requires further study. An important clinical consequence of the variation in drug level assessment was the observed mis-classification rate of > 6% of IFX samples and > 19% of ADA samples. This means that patients who undergo drug levels assessment obtained on PRO and IM may return results that would be incorrectly classified as therapeutic or sub-therapeutic when compared to the reference assay, in this study LT. This has clinical implications as management decisions made on the results on drug level status are significantly different (dose intensification vs within or out-of class switching).

Over the last decade there has been a steady increase in the number of studies investigating the relationship between drug levels, anti-drug antibodies and outcomes in both CD and UC. Preliminary data came from the era of episodically administered IFX, where an association between longer duration of response and detectable drug levels, and a link between anti-drug antibodies and loss of response and infusion reactions was observed.[39] Concomitant immunomodulation was shown to decrease immunogenicity but post-hoc analyses failed to show that this translated into improved clinical outcomes.[57] SONIC[61] and SUCCESS[56], prospective studies that randomised treatment naïve patients to IFX or thiopurine monotherapy or combination therapy, demonstrated improved outcomes in the combination arms, and, in the case of SONIC, this correlated with an increase in IFX trough levels. Subsequently a large body of data has supported an inverse relationship between IFX drug levels and outcomes, and a therapeutic threshold of 2-3 μg/mL has been identified that best predicts clinical remission.[93] The situation with ADA is less clear, some authors have found a similar association,[89,94,95] whereas others have not.[96] Recent data has shown that higher target thresholds are needed to achieve a 'deeper' level of disease control,[97,98] of relevance as we move beyond symptom control to the goal of healing the mucosa.[28] The relationship between anti-drug antibodies and outcomes is complex, due to methodological differences in assay design and a lack of standardisation

in the units in which they are reported.[40] Meta-analyses have shown a negative association between anti-drug antibodies and outcomes and an increased rate of infusion reactions.[99,100] The identification and influence of patient and disease factors (including, but not limited to: albumin, weight, inflammatory burden, anti-drug antibodies and concomitant immunomodulation)[101,102] is of relevance to gain insights mechanistically into the observed inter-patient variation in anti-TNF pharmacokinetics and pharmacodynamics. Recently, data pertaining to the relationship between timing and intensity of immunomodulators when used in combination with anti-TNF, and in turn, drug levels and anti-drug antibodies has led to several conclusions of clinical significance. Firstly, immunogenicity seems to occur early, within the first 12 months of anti-TNF treatment.[62] Secondly, a correlation between IFX drug levels and TGNs and the identification of a TGN threshold of 125 pmol/8x10^8 RBC that augmented IFX levels, raises the possibility that thiopurines dose reduction by approximately 50% of that required when used alone may be sufficient in patients treated with IFX.[64]

Therefore, the sixth major aim of this thesis was to perform a cross-sectional study in a well characterised cohort of patients with CD treated with maintenance IFX and ADA in order to address the following areas of interest: (1) examine the association of drug levels with the achievement of targets from clinical to deep remission comparing ADA to IFX, so that cut-off concentrations that might predict these end-points can be identified, (2) investigate patient and disease factors that might modulate drug levels, and (3) address the association between TGNs and IFX and ADA drug levels.

This study comprises Chapter Seven of this thesis (Ward MG, submitted 2016). Therapeutic drug monitoring using the Lisa-Tracker ELISA was performed in 191 patients with CD (IFX = 96, ADA = 95) and drug levels were compared across three endpoints (clinical remission; Harvey-Bradshaw index ≤ 4, biochemical remission; C-reactive protein < 5 mg/L, and, as a surrogate of mucosal healing; faecal calprotectin < 59 μg/g). IFX drug levels were collected at trough, and ADA at any

point in a treatment cycle. Patients were dosed at 5mg/kg 8-weekly, 5mg/kg 6-weekly and 10mg/kg 8-weekly (IFX) or 40mg fortnightly, weekly or every 10 days (ADA). In patients treated with thiopurines, correlation with TGNs and according to cut-offs of <125, 125-235 and >235 were explored.

There were several key findings of clinical interest. First, significant differences in IFX drug levels were observed between patients with active disease compared to remission, permitting the identification of target thresholds on ROC analysis, further, the target threshold was higher when 'deeper' levels of remission were considered (>1.5, >3.4 and >5.7 μg/mL for clinical and biochemical remission and mucosal healing, respectively). Second, no such relationship was observed with ADA. Third, higher doses of IFX or ADA, and in the case of IFX, elevated CRP and mucosal inflammation and BMI, and for ADA, weight and albumin, significantly influenced drug levels, accounting for 23-31% of the variation in drug levels. Finally, TGNs did not correlate with drug levels and TGNs were similar between TGN cut-offs.

This study, comparing IFX and ADA drug levels in a large number of patients using identical methodology, adds to the literature supporting a relationship between IFX drug levels and disease activity but raises questions about the pharmacokinetic-pharmacodynamic relationship with ADA. This lack of association may, in part, be explained by the relatively low number of ADA drug levels sampled at trough (21%) before they theoretically reach a nadir, and a signal that this may be of relevance was the observed trend between lower ADA drug levels with increasing days between last dose and drug level sampling on multivariate regression analysis ($\beta = -0.135$, $p = 0.065$). The identification of modulating patient and disease factors which influenced drug levels and the finding that drug levels were similar across a range of TGNs, of relevance in the optimisation of patients treated with anti-TNF therapy, should act as a stimulus for future work.

The literature presented thus far supports the premise that TDM is an important tool in the optimisation of patients with IBD treated with anti-TNF therapy. Considering IFX, TDM is routinely performed at trough, defined as just prior to the next scheduled dose, when drug levels are at their lowest.[103] There are clear difference in the pharmacokinetics between intravenous and subcutaneous administered monoclonal antibodies; for IFX, high peak and low trough concentrations are observed compared to ADA, which displays a more uniform concentration-time profiles at steady state.[104] This, and limited data, has led some experts to propose that ADA TDM can performed at any time point in a treatment cycle.[97,103,105,106] This is relevant clinically as patients treated with ADA, as opposed to IFX, administer the drug at home and undertaking TDM at trough can therefore necessitate a return for sampling. There is a paucity of data examining patient and disease factors that influence ADA pharmacokinetics.[107] Clearance is generally linear, exhibiting dose-proportional behavior, and is influenced by body weight, inflammatory burden and the presence of circulating antibodies-to-ADA. No difference in the bioavailability of ADA between delivery device (pen vs syringe) has been observed, although data in IBD is lacking.[108]

Accordingly, the seventh major aim of this thesis was to perform a prospective pilot study addressing the hypothesis that there are minimal variations of ADA drug levels between and within a cycle, by assessing and comparing intra-individual ADA drug levels at multiple time-points during and between fortnightly dosing regimens amongst patients with CD, and to examine potential modulating factors thereof.

The resulting study (Ward MG, submitted for publication 2016) comprises Chapter Eight of this thesis. We prospectively evaluated 111 ADA drug levels in 19 patients with CD maintained on fortnightly ADA sampled at day 4-6, day 7-9 and trough (day 13-14) across two consecutive treatment cycles. Where used, concomitant immunomodulator doses remained stable for at least 12 weeks prior to enrolment. At each visit, indices of disease activity were assessed (Harvey Bradshaw

index ≥ 5 considered active clinical disease, C-reactive protein >3 mg/L active systemic inflammation) and during each fortnight mucosal inflammation was assessed using faecal calprotectin; ≥150 μg/g considered to be active disease. Patient and disease characteristics were collected, including delivery device (pen vs. syringe), smoking status and BMI. Inter and intra-patient variation in ADA drug levels was analysed and linear mixed models were constructed to examine the relationship between covariates and absolute trough levels and achievement of a therapeutic trough level (≥ 4.9 μg/mL).[94]

There were several findings of significance. Firstly, intra-patient drug levels at any point in a cycle reliably predict those in the next, suggesting the results of a single drug level can be interpreted with confidence and do not need to be repeated. Secondly, drug levels remained stable during the first nine days of a treatment cycle, but then declined significantly to trough. Thirdly, a threshold similar to that taken at trough, tested within the first 9 days of a cycle, predicted a therapeutic trough level with high sensitivity but relatively low specificity. Finally, non-temporal factors – syringe rather than pen delivery device (albeit with very small numbers) and current smoking - were independently associated with trough drug levels. Predictive models which incorporated drug levels at either day 4-6 or day 7-9, accounted for 66% and 80% of the variation in trough levels respectively.

These data suggest that drug levels obtained in the first 9 days of a treatment cycle which are above those proposed as therapeutic at trough (≥ 4.9 μg/mL) predict therapeutic trough levels with reasonable accuracy. Although drug levels declined significantly from day 4-6 (-1.06 μg/mL) and day 7-9 to trough (-0.89 μg/mL, each $p<0.001$), these small magnitudes were not necessarily clinically significant. A novel finding, that current smoking negatively influences ADA drug levels, may explain why patients with CD who smoke have worse clinical outcomes.[109] The finding that syringe delivery device significantly increased drug levels should be interpreted with caution, given numbers were small, but warrants further evaluation. Larger replication studies are needed before these recommendations can be

incorporated into everyday clinical practice, however this study adds valuable understanding to the optimisation of TDM with ADA in CD.

Malnutrition is common in patients with IBD; in one study of CD patients attending the outpatient clinic, protein-energy malnutrition was found in 23%.[110] Hypoalbuminaemia is found in 25-80% and 25-50% of hospitalised patients with CD and UC, respectively.[111] Nutritional deficiencies are often under overlooked by the clinician when the primary focus is on medical management of the underlying disease. Predisposing factors can be divided into disease and patient factors. Small bowel inflammation and loss of function after surgery in CD can lead to macronutrient (protein and energy) and micronutrient (vitamins, minerals and trace elements) deficiencies through malabsorption. Iron deficiency commonly occurs via hepcidin-mediated impaired absorption and utilisation of dietary iron and from chronic bleeding through gut losses.[41] Patient factors include anorexia via increased levels of TNF-a, interleukin-1 and other pro-inflammatory cytokines[112], drug-nutrient interactions (corticosteroids-calcium and sulfasalazine-folate) and increased requirements from a hyper-catabolic induced state. Fear of eating due to discomfort from obstructive pain or bloating and worsening of underling diarrhoea are common. Accordingly, optimisation of nutrient deficiencies is a key issue in the management of IBD.

Vitamin B12 (cobalamin) is found almost exclusively in food of animal origin and is important for erythropoiesis, DNA synthesis and metabolism of carbohydrate, fat and protein.[113] It is absorbed almost exclusively in the terminal ileum. Patients with CD are therefore at significant risk of B12 deficiency due to inflammation, stricturing complications or indeed after surgical resection. Studies have observed B12 deficiency in 5.6-38% of patients with CD by measuring serum B12 [114] and identified prior ileal (OR 7.2; 95% CI: 1.97-26.51) or ileocolonic resection (OR 5.81; 95% CI: 2.09-16.12) and the need for ongoing medical therapy (OR 2.59; 95% CI: 1.03-6.47) as independent risk

factors.[115] No data exists on the relationship between the burden of terminal ileal inflammation using magnetic resonance imaging (MRI). Identifying B12 deficiency by measuring serum B12 levels suffers from relatively low specificity. Alternatives include assessment of methylmalonic acid (MMA) or homocysteine which are hampered by cost and limited availability, and specificity, respectively. A relatively new assay that measures the transcobalamin II-cobalamin complex (holoTC) has been shown to be a superior test to serum B12 in the assessment of B12 deficiency[116] but has not been explored in patients with CD.

Therefore, the eighth aim of this thesis was to identify the prevalence of vitamin B12 deficiency in a large retrospective cohort of patients with CD using holoTC testing, supported by MMA, and to identify risk factors, in particular terminal ileal disease burden using MRI. We also sought to compare the performance of holoTC versus serum B12 in paired samples.

This study was performed, and the resulting publication (Ward MG, 2015) comprises Chapter Nine of this thesis. Adult patients with CD were compared to patients with UC (controls); a sub-group of consecutive patients underwent paired testing of serum B12 and holoTC. Risk factors for B12 deficiency, identified *a priori*, included age, gender, smoking status, disease phenotype according to Montreal classification,[117] treatment with concomitant immunomodulation, disease duration and disease activity (according to clinical indices and levels of C-reactive protein). We also investigated the relationship between prior ileal resection length (0, 1-20 and >20cm) by obtaining past operative reports of histology. Ileal disease activity was assessed by ileocolonoscopy or by MRI. A sub-group analysis was conducted amongst CD patients who underwent an MRI within 6 months of B12 assessment assessed for active inflammation (>6mm mural thickening with mural enhancement), length of small bowel involvement, number of skip lesions, pre-stenotic dilatation (>3cm) and strictures (luminal narrowing with pre-stenotic dilatation). HoloTC < 25pmol/L was defined as B12

deficiency, and >50 as replete. Intermediate values underwent MMA analysis; values >280nmol/L confirmed B12 deficiency in patients < 65 years old and >360nmol/L in patients > 65 years old. Serum B12 values <107pmol/L were considered B12 deficient. The prevalence of B12 deficiency was 33% in patients with CD (n=371, median holoTC 48, IQR 33-70pmol/L) compared to 16% of UC patients (n = 141, median holoTC 67, IQR 46-95pmol/L, p < 0.0001). Amongst 89 CD patients undergoing paired testing, serum B12 identified B12 deficiency in 4/89 (5%) compared to 13/89 (15%) using holoTC alone; the latter increased to 28/89 (32%) when intermediate range holoTC results were analysed by MMA. 1/4 (25%) of deficient patients with serum B12 were found to be replete with holoTC/MMA. On multivariate analysis, increasing ileal resection length (OR 3.0, 95% CI: 1.5-6, p = 0.002 and OR: 6.7, 95% CI: 3.0–15.0, p < 0.0001 for ≤ 20 and >20 cm, respectively) and ileal inflammation (endoscopy/imaging, OR: 3.9, 95% CI: 2.2–6.9, p <0.0001) were independent predictors of B12 deficiency. Amongst the 168/381 (44%) of patients who underwent MRI; univariate predictors of B12 deficiency were active terminal ileal inflammation (OR 2.3, 95% CI: 1.2-4.7, p = 0.02), pre-stenotic dilatation (OR 2.9, 95% CI: 1.3-6.8, p = 0.01) and segmental small bowel disease (>1skip lesion) (OR 2.3, 95% CI: 1.2-4.4, p = 0.01). Length of inflamed ileum was greater in patients with B12 deficiency compared with those without (14.1 vs 8.6cm, p = 0.04).

This study, the first to assess the prevalence of vitamin B12 deficiency in patients with CD using holoTC, found B12 deficiency in 33% of patients. Further, holoTC coupled with MMA identified B12 deficiency in patients considered replete with traditional serum measurements. In keeping with other studies, prior surgery and ileal inflammation were predictors of B12 deficiency. Using MRI, we identified terminal ileal active inflammation, skip lesions and pre-stenotic dilatation were associated with B12 deficiency. HoloTC should be considered as the first line screening test for B12 assessment in patients with CD in order to optimize this micronutrient deficiency.

SECTION 2. OPTIMISATION OF THIOPURINES

CHAPTER 2:

Review: Optimisation of immunomodulators when used as combination therapy with anti-tumour necrosis factor agents in the management of inflammatory bowel disease

World Journal of Gastroenterology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i40.11331

World J Gastroenterol 2015 October 28; [redacted]
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Inflammatory Bowel Disease

How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease?

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Author contributions: All authors substantially contributed to the writing of this article and approved the final manuscript.

Conflict-of-interest statement: The authors have no conflict of interest to report.

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Received: June 1, 2015
Peer-review started: June 3, 2015
First decision: June 23, 2015
Revised: July 14, 2015
Accepted: September 14, 2015
Article in press: September 15, 2015
Published online: October 28, 2015

Abstract

In the last 15 years the management of inflammatory bowel disease has evolved greatly, largely through the increased use of immunomodulators and, especially, anti-tumor necrosis factor (anti-TNF) biologic agents. Within this time period, confidence in the use of anti-TNFs has increased, whilst, especially in recent years, the efficacy and safety of thiopurines has been questioned. Yet despite recent concerns regarding the risk: benefit profile of thiopurines, combination therapy with an immunomodulator and an anti-TNF has emerged as the recommended treatment strategy for the majority of patients with moderate-severe disease, especially those who are recently diagnosed. Concurrently, therapeutic drug monitoring has emerged as a means of optimizing the dosage of both immunomodulators and anti-TNFs. However the recommended therapeutic target levels for both drug classes were largely derived from studies of monotherapy with either agent, or studies underpowered to analyze outcomes in combination therapy patients. It has been assumed that these target levels are applicable to patients on combination therapy also, however there are few data to support this. Similarly, the timing and duration of treatment with immunomodulators when used in combination therapy remains unknown. Recent attention, including post hoc analyses of the pivotal registration trials, has focused on the optimization of anti-TNF agents, when used as either monotherapy or combination therapy. This review will instead focus on how best to optimize immunomodulators when used in combination therapy, including an evaluation of recent data addressing unanswered questions regarding the optimal timing, dosage and duration of immunomodulator therapy in combination therapy patients.

Key words: Inflammatory bowel disease; Thiopurines; Drug monitoring; Tumor necrosis factor-alpha; Combination therapy

Core tip: Clinicians managing inflammatory bowel disease frequently have to decide whether to use anti-tumor necrosis factor (anti-TNF) therapy alone or in combination with immunomodulators (IM), which requires an assessment of patient factors and the risk/benefit profile of each treatment strategy. Once a decision is made to use combination therapy, questions on how best to optimize IMs must be addressed. Thiopurines, rather than methotrexate, (MTX) are more efficacious and easier to administer, whereas in certain population groups, MTX may be safer. The effective dose of IM may be lower in combination therapy and combination therapy is probably most important in the first 12 mo of treatment. Withdrawing IMs is best done when the patient is in deep remission, ideally supported by the use of therapeutic drug monitoring of anti-TNFs.

Ward MG, Irving PM, Sparrow MP. How should immunomodulators be optimized when used as combination therapy with anti-tumor necrosis factor agents in the management of inflammatory bowel disease? *World J Gastroenterol* 2015; 21(40): 11331-11342 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i40/11331.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i40.11331

INTRODUCTION

Inflammatory bowel disease (IBD) namely Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions characterized by an exaggerated host immune response to an as yet unidentified antigen, leading to relapsing and remitting inflammation resulting in damage to the gastrointestinal tract. Despite access to an expanding therapeutic armamentarium with the arrival of gut-specific therapies such as vedolizumab and other novel agents targeting key pro-inflammatory cytokines, clinicians still largely rely on the conventional immunomodulators, (IMs) azathioprine, (AZA) mercaptopurine, (MP) and methotrexate, (MTX) and/or anti-tumor necrosis factor (anti-TNF) therapy, (infliximab, (IFX) adalimumab, (ADA) certolizumab pegol and to a lesser extent, golimumab) to treat these diseases. Much has been learnt over the last 15 years of the relative risks and benefits of using these agents, either alone or in combination, however gaps in our knowledge remain as to how IMs are best optimized once a decision has been made to combine them with anti-TNF therapy. This review article begins with a brief outline of the efficacy and safety issues surrounding combination therapy (IM + anti-TNF) and then draws on the available evidence to address some of these unanswered questions (Table 1).

Table 1 Summary and key points

Combination therapy (thiopurines with anti-TNF) is more efficacious than either agent alone in thiopurine-naïve patients with IBD
Combination therapy confers an increased risk of adverse events, of which NMSC, melanoma and lymphoma are the best studied
The benefit of combination therapy is probably due to both an improvement in anti-TNF pharmacokinetics (reduced immunogenicity and improvement in drug levels) and an independent effect of the IM on disease activity
The pharmacokinetic benefits of combination therapy are most important during the first 12 mo of therapy, but may persist beyond this
The optimal dose of IM in this setting may be lower than that used for IM monotherapy, however further studies are needed to confirm this
The risk of relapse after IM withdrawal is highest amongst patients with active disease and positive biomarkers of inflammation or unfavorable anti-TNF pharmacokinetic profiles
Withdrawal of IM should be considered in patients in deep remission after a period of 12 (or perhaps 24 mo) of combination therapy

TNF: Tumor necrosis factor; IBD: Inflammatory bowel disease; IMs: Immunomodulators; NMSC: Non-melanoma skin cancer.

BENEFITS OF COMBINATION THERAPY *VS* ANTI-TNF MONOTHERAPY

The arrival of IFX, and subsequently ADA, both effective therapies for induction and maintenance of remission for luminal and fistulizing CD and UC, revolutionized the management of IBD[1-9]. A common issue faced by clinicians is under what circumstances does combination therapy with an IM offer benefit over anti-TNF monotherapy. Amongst IM naïve patients with moderate-severe CD, the SONIC study (508 treatment naïve CD patients randomized to AZA, IFX or combination therapy) showed that combination therapy was superior to IFX monotherapy with respect to corticosteroid-free clinical remission (56.8% *vs* 44.4%, $P = 0.02$) and mucosal healing (43.9% *vs* 30.1%, $P = 0.06$)[10]. Similar results in moderate-severe UC were seen in the UC-SUCCESS trial, favoring combination therapy (AZA + IFX) over IFX monotherapy for clinical remission, (39.7% *vs* 22.1%, $P = 0.017$) and complete mucosal healing, (29.5% *vs* 11.7%, $P = 0.006$) at week 16[11]. These results should be interpreted with caution as this study was terminated early, and therefore underpowered, and week 16 may be too early for thiopurines to be efficacious; however combination therapy was as effective as, or superior to, IFX monotherapy across a range of secondary endpoints. COMMIT, a 50 wk randomized placebo-controlled trial of CD patients initiated on prednisolone found no benefit of MTX and IFX combination therapy ($n = 63$) over IFX monotherapy ($n = 63$) for the primary endpoint, defined as failure to enter steroid-free clinical remission at week 14, (78% *vs* 76%, P = NS) or failure to maintain remission through week 50, (57% *vs* 56%, P = NS)[12]. When reconciling the opposing findings of combination therapy *vs* anti-TNF monotherapy of SONIC/SUCCESS *vs* COMMIT, several

key differences in study design should be considered. COMMIT used a high dose corticosteroid induction regimen that may have obscured a true benefit of MTX combination therapy over IFX monotherapy. Further, the primary end-point of corticosteroid free remission may have been seen equally between treatment arms due to the enrolment of patients with milder CD activity, a proportion of which may have never failed treatment according to clinical (CDAI) criteria. Of note, in COMMIT, patients randomized to combination therapy had higher median trough drug levels compared to IFX monotherapy (6.35 μg/mL *vs* 3.75 μg/mL, $P = 0.08$), suggesting a beneficial effect of combination therapy on IFX pharmacokinetics.

Sub-group analyses of RCTs of IFX and ADA for both CD and UC, stratified according to baseline IM use, have failed to show a benefit of combination therapy over anti-TNF monotherapy in achieving clinical remission[1,4,6,7,9,13]. However, a large percentage of patients entered these studies already failing IMs, a key difference from the low proportion of previous IM use in SONIC, SUCCESS and COMMIT. Further, in the ADA RCTs there were high rates of previous IFX failure, (CHARM 49%[6], ULTRA-2 41%[9]) therefore these patients may represent a more treatment-refractory cohort. Data from observational studies has been conflicting with some supporting combination therapy over anti-TNF monotherapy[14-19], whereas others do not[20-23]. Differences in study design; patient populations and endpoints all hamper the strength of conclusions that can be drawn from these studies.

A post-hoc analysis of patient level data, (published in abstract form only) taken from 11 anti-TNF RCTs (IFX, ADA, and certolizumab pegol) found that combination therapy was more efficacious than monotherapy for 6 mo clinical remission in those treated with IFX (OR = 1.79; 95%CI: 1.06-3.01) but not ADA (OR = 0.88; 95%CI: 0.58-1.35) or certolizumab (OR = 0.93; 95%CI: 0.65-1.34)[24]. This may be explained as IFX, a chimeric anti-TNF is more immunogenic than the humanized ADA. A "SONIC-type" study comparing ADA monotherapy to ADA+IM combination therapy is needed before we can say with certainty that combination therapy is more efficacious in this setting.

Taken together the literature suggests that in IM naïve patients with moderate to severe IBD, combination therapy is more efficacious and should be considered over monotherapy with an anti-TNF, and that in IM refractory patients, combination therapy may be important for at least the first 12 mo of anti-TNF treatment.

RISKS OF COMBINATION THERAPY *VS* MONOTHERAPY

Infections and malignancy

Any putative increase in efficacy through the use of combination therapy must be balanced against the risk of adverse events, and infectious complications and malignancy in particular[25]. Randomized controlled trials in IBD have shown no significant increase in infections in patients treated with combination therapy compared with anti-TNF monotherapy. A pooled analysis of 1383 patients, randomized to receive either placebo or IFX, of which 40% received concomitant immunomodulation with AZA, MP or MTX from the landmark ACCENT Ⅰ and ACCENT Ⅱ (luminal and fistulizing CD respectively), and ACT Ⅰ and ACT Ⅱ (UC), studies showed similar rates of both infections (44.1% *vs* 44.5%) and serious infections (3.7% *vs* 3.2%) in those treated with immunomodulator co-therapy *vs* those treated with IFX monotherapy[26]. Similarly, in SONIC serious infections were seen in 4.9% *vs* 3.9%, ($P = 0.79$) of those treated with IFX monotherapy and combination therapy, respectively[10]. In COMMIT, respiratory infections occurred in 46% of patients treated with combination therapy compared with 41.3% of those treated with IFX (P = NS), although all patients also received an induction course of corticosteroids which may have contributed to these very high infection rates[12]. Despite these reassuring findings it must be emphasized that follow-up of these trials was relatively short (generally limited to 52 wk), and they were underpowered to detect uncommon opportunistic infections. Retrospective observational studies have reported conflicting infectious complication rates in anti-TNF monotherapy compared with combination therapy. Osterman and colleagues found an increased rate of opportunistic bacterial and fungal infections (HR = 2.64; 95%CI: 1.21-5.73) and herpes zoster (HR = 3.16; 95%CI: 1.25-7.97) amongst 577 patients who "stepped up" to ADA or IFX from IMs (92% thiopurines) over a median follow-up of 1.4-1.7 years, but no increase in the rate of serious infections amongst combination therapy compared with anti-TNF monotherapy[27]. Other studies have shown no increase in infections amongst combination therapy compared with anti-TNF monotherapy[28]. Despite these conflicting data on infection rates, an unequivocal signal from randomized controlled trials and observational studies is that corticosteroids impart a significant additive infective risk for both anti-TNF monotherapy and combination therapy exposed patients[29,30].

MALIGNANCY

It is accepted that thiopurines are associated with an increased risk of non-melanoma skin cancer, (NMSC) (basal cell carcinoma and squamous cell carcinoma) in post-transplant recipient patients[31]. Three large observational studies have demonstrated that thiopurine therapy confers a 4-6 fold increase in NMSC amongst patients with IBD and that this risk remains elevated compared to age-matched thiopurine naïve

patients with IBD even after stopping thiopurines[32-34]. In IBD there are no well-designed studies assessing the risk of NMSC in anti-TNF monotherapy, primarily because of confounding due to prior or concomitant thiopurine exposure. A meta-analysis of anti-TNF monotherapy use amongst patients with rheumatoid arthritis demonstrated an increased risk of NMSC (1.45, 95%CI: 1.15-1.76)[35]. In a nested case-control claim database amongst 3288 matched IBD patients, (3288 NMSC matched to 12945 controls) sub-group analysis of patients with ⩾ 1 year drug use demonstrated the greatest risk amongst combination thiopurines and anti-TNF, (adjusted OR = 3.89, 95%CI: 2.33-6.46) compared to thiopurine monotherapy (adjusted OR = 2.72, 95%CI: 2.27-3.26) and anti-TNF monotherapy (adjusted OR = 1.63, 1.12-2.36)[34]. Amongst patients with less than 12 mo anti-TNF use there was no association with NMSC. A pooled analysis of 1594 CD patients who participated in the landmark RCTs of ADA demonstrated no increased risk of NMSC in ADA monotherapy, compared with an increased risk of NMSC, and other malignancies, in thiopurine combination therapy (adjusted RR = 4, 95%CI: 1.23-13.0)[36]. Taken together, these results suggest that combination therapy increases the risk of NMSC above and beyond the risk of both thiopurine and anti-TNF monotherapy. Despite an apparent increased risk of melanoma amongst patients with IBD[34,37], thiopurine use does not seem to increase the risk further[34]. Anti-TNF therapy, in contrast, appears to double the risk of melanoma[34]. Similar associations between anti-TNF use and melanoma in RA have been observed[35,38,39]. As with NMSC, drawing firm associations between anti-TNF monotherapy exposure and melanoma risk are limited by current or past exposure to IMs.

Determining the influence of IM monotherapy *vs* combination therapy on lymphoma development is difficult due to the relatively uncommon occurrence of this event and the short follow-up period of RCTs. Pooled data from 7054 IBD patients from 11 RCTs, (IFX, ADA, certolizumab and golimumab) followed for 1 year, showed no cases of lymphoma amongst anti-TNF treated patients, compared to 3 placebo arm patients, (although 2 of these had received induction with anti-TNF)[40]. Other pooled analyses have demonstrated an increased risk of lymphoma with combination therapy, however these have not detected cases of lymphoma amongst those treated with anti-TNF monotherapy. This limits the strength of conclusions on the risk of lymphoma development between the two treatment strategies. Accordingly, data from large population-based observational cohort studies must be considered. In CESAME, a prospective observational cohort study of 19 486 IBD patients, the risk of lymphoma was higher amongst patients using thiopurines in combination with anti-TNF compared to thiopurines alone, [standardized incidence ratio, (SIR) = 10.2, 95%CI: 1.24-36.9, $P < 0.04$] *vs* 6.53, 95%CI: 3.48-11.2, $P < 0.0001$, respectively)[41]. Anti-TNF monotherapy did not increase the risk of lymphoma, (SIR = 4.5, 95%CI: 0.6-16.4, $P = 0.1$). Similarly a retrospective cohort study of 36891 Veteran Affairs UC patients, of which 4734 were treated with thiopurines for one year found an increased risk of lymphoma amongst thiopurine users (HR = 4.2, 95%CI: 2.5-6.8, $P < 0.001$)[42]. Subgroup analysis demonstrated a non-significant increased incidence rate ratio, (IRR) amongst thiopurine/IFX combination therapy (IRR = 3.84, 95%CI: 0.8-44.2) compared with thiopurine monotherapy (IRR = 3.6, 95%CI: 2.2-6.0) however only 1 case of lymphoma was diagnosed in the combination group, implying this study was underpowered to detect a true difference. The findings from other studies have been conflicting[27,43-48]. In general, observational studies and meta-analyses have shown that combination therapy increases the risk of lymphoma, however the magnitude of this risk is similar to that seen with IM monotherapy.

UNANSWERED QUESTIONS REGARDING THE OPTIMIZATION OF IMMUNOMODULATORS WHEN USED AS COMBINATION THERAPY

Which immunomodulator should be used - thiopurines or methotrexate?

The evidence as to which IM, thiopurines or MTX, to choose in combination therapy is limited, although there are more data relating to the use of thiopurines. Randomized controlled trials (RCTs) in both CD (SONIC)[10] and UC (SUCCESS)[11] demonstrate superiority of thiopurine-based combination therapy over anti-TNF monotherapy. In contrast, combination therapy with MTX has not been proven to be superior to monotherapy in CD (COMMIT)[12], and there are a lack of high quality data to support the use of MTX in UC when given as monotherapy, with no combination therapy data available[49]. However, given differing trial designs and endpoints, direct comparison of these RCTs must be interpreted with caution.

The benefits of adding an immunomodulator to anti-TNF therapy, even in patients who have previously failed immunomodulators, are presumably due to both a reduction in immunogenicity with a resultant increase in serum anti-TNF levels, and also a direct effect in reducing disease activity. Both thiopurines and MTX have beneficial effects on the pharmacokinetics of anti-TNF agents when used in combination therapy. In a retrospective, single-centre study of 174 CD patients treated with episodic IFX, AZA and MTX were equally effective in preventing immunogenicity (antibodies to IFX, (ATIs) 48% in AZA group *vs* 44% in MTX group, P = NS) and infusion reactions (18% *vs* 14% in AZA and MTX groups respectively, P = NS), and in increasing serum IFX levels (6.15 μg/mL *vs* 5.65 μg/

mL in AZA and MTX groups respectively, P = NS)[50]. The presence of ATI was associated with a shorter duration of response in patients not taking IM (median 11.7 wk) as compared to those taking IM (median 13.8 wk, P = 0.006) although numbers were small. In SONIC, patients on combination therapy with AZA had significantly higher IFX levels than monotherapy patients at week 30 (3.5 μg/mL *vs* 1.6 μg/mL, P < 0.0001)[10]. In the COMMIT study patients on combination therapy with MTX had lower rates of ATI formation than monotherapy patients (4% *vs* 20%, P = 0.01) and a trend to higher serum IFX levels (6.35 μg/mL *vs* 3.75 μg/mL, P = 0.08)[12].

Another advantage of thiopurines is the oral route of administration, compared to MTX, where only parenteral monotherapy in CD has been consistently demonstrated to be effective[51,52]. If used in therapeutic doses in combination therapy, presumably parenteral MTX is the best option. However if used primarily to reduce immunogenicity then rheumatologic data suggests that low dose oral MTX may be adequate. Published only in abstract form, it was demonstrated that the addition of MTX to maintenance ADA increased ADA levels from 5 μg/mL to between 8-9 μg/mL[53]. More recently in the CONCERTO trial 395 RA patients were randomized to open-label ADA 40 mg alternate weekly, and blinded oral MTX at doses or 2.5, 5, 10 and 20 mg weekly. ADA serum concentrations increased with increasing MTX doses up to 10 mg weekly, above which there was no dose response. Anti-adalimumab antibody prevalence was also similar between the 10 and 20 mg MTX groups, suggesting that in RA patients 10 mg MTX orally weekly is the correct dose to optimize ADA pharmacokinetics[54]. Whether these data are applicable to IBD is unknown. Similarly, thiopurines have consistently been shown to increase serum anti-TNF levels when given as combination therapy[10,55], although there are no data delineating an optimal weight-based thiopurine dose needed to achieve maximal serum anti-TNF concentrations.

Another consideration in the choice of concomitant immunomodulator is the small, but real, increased risk of lymphoma associated with thiopurines in IBD. The most recent meta-analysis of both population and referral-based IBD studies demonstrated a SIR of lymphoma of 4.92 (95%CI: 3.10-7.78) amongst thiopurine-exposed patients. The risk was highest amongst males currently receiving thiopurines for at least one year[48]. A similar increased magnitude of risk has been demonstrated in other recent population-based studies and meta-analyses[41,44]. Of particular concern is the association between thiopurine use and hepatosplenic T cell lymphoma (HSTCL), especially in young males under 35 years of age[56]. By contrast there are no studies showing an increased risk of lymphoma with MTX use in IBD, although it must be recognized that this is largely due to a lack of data rather than there being studies definitively showing no association. Studies in rheumatoid arthritis show conflicting data as to whether MTX use is associated with an increased lymphoma risk, either as monotherapy or when combined with anti-TNF agents[57-59]. In considering these data it would seem reasonable to consider MTX as the immunomodulator of choice when lymphoma risk is highest, such as in young males, whereas for other patients the benefits of thiopurines will usually outweigh the small lymphoma risk. Finally MTX is teratogenic and is contraindicated during pregnancy. Due to its long half-life it is recommended to stop MTX 3-6 mo pre-conception in females[60]. Its effects on male fertility and spermatogenesis are controversial; some experts recommend withdrawal in males 3 mo prior to trying to conceive[60].

When should immunomodulators be commenced when used as combination therapy?

The SONIC study demonstrated in a randomized controlled trial that clinical and endoscopic remission occurs most frequently when immunomodulators and IFX are commenced simultaneously in treatment-naïve patients[10]. Pharmacokinetic data from observational single-centre studies has subsequently emerged to support this practice.

In a retrospective study of 217 patients on anti-TNF therapy (108 IFX, 109 ADA) concomitant IMs improved pharmacokinetic outcomes for patients on IFX (83.1% thiopurines, 16.9% MTX), but not ADA (83.3% thiopurines, 16.7% MTX). For IFX, trough levels were significantly higher in the combination therapy group compared to monotherapy patients (7.5 μg/mL *vs* 4.6 μg/mL, P = 0.04), while for ADA no difference was seen (13.1 μg/mL *vs* 11.5 μg/mL respectively, P = 0.5). Similarly, combination therapy patients were less likely to have ATIs than monotherapy patients for IFX (5.7% *vs* 29.8%, P = 0.001), but not ADA (17.2% *vs* 21.6%, P = 0.6). Regarding the timing of introduction of the IM, IFX patients in whom IMs were started at the same time as the anti-TNF were less likely to develop ATIs than patients in whom IMs were started later (2.4% *vs* 18.2%, P = 0.04); again no difference was seen in ADA patients. Interestingly, there was no association between IM dose and IFX trough levels, and in fact counter-intuitively patients with suboptimal IM doses had higher trough levels (9.81 *vs* 5.36, P = 0.02). This study suggests that immunogenicity occurs early in the treatment course of anti-TNFs and that perhaps a lower dose of IM may be sufficient to prevent anti-drug antibody formation and optimize trough levels[61]. It is important to note that this pharmacokinetic study did not assess clinical outcomes, hence it is unclear whether the favorable effect of combination therapy on improving drug levels and reducing ATIs conferred a clinical benefit. Consistent with these results, in a prospective observational study of 125 patients treated

with IFX (98 CD, 27 UC), 46% of patients developed ATIs. Of these, 90% of patients who developed permanent ATIs did so within 12 mo of starting IFX, whilst transient, and clinically non-significant, antibodies developed at any time during therapy (P < 0.001). Patients on combination therapy had a longer ATI-free survival compared to monotherapy patients (P = 0.003, log rank test)[17]. Low IFX trough levels and high ATI titers were significantly more prevalent amongst patients with clinical loss of response, P < 0.001. These data therefore also demonstrate that IMs are most effective at reducing immunogenicity in the first 12 mo of anti-TNF therapy, suggesting that the two classes of therapy should be commenced simultaneously.

What dose of immunomodulator should be used when used as combination therapy - are lower doses equally effective and safer?

To date most studies of combination therapy have used full weight-based thiopurine doses (AZA-2.0-2.5 mg/kg per day, MP-1.0-1.5 mg/kg per day), with or without further dose-optimization aiming for therapeutic metabolite levels [6-thioguanine nucleotide, (6-TGN) 235-450 pmol/8 × 10^8 RBC]. However, more recently, definite signals of thiopurine toxicity have been confirmed in large population-based studies, in particular the risk of infections, NMSC and lymphoma[32,41]. Of these adverse events, infection risk is definitely dose-dependent, however most population-based studies of NMSC and lymphoma risk have not included thiopurine doses in their analyses[32,48]. This raises the question of whether lower thiopurine doses can be used in combination therapy with equal efficacy and pharmacokinetic benefits on serum anti-TNF levels, and presumably, less toxicity. Recent retrospective and observational studies have explored the effect of thiopurine dose on outcomes when used in combination therapy, analyzing by mg/kg daily doses or surrogate measures of 6-TGN levels and changes in mean corpuscular volume (MCV) in thiopurine-treated patients.

In the Dutch retrospective study assessing pharmacokinetic outcomes of combination therapy (predominantly with thiopurines) there was no correlation between IM dose and anti-TNF levels, suggesting that lower IM doses in combination therapy may be equally effective[61]. More recently, in a single centre cross-sectional study of 72 patients (45 CD, 27 UC) on combination therapy with scheduled maintenance IFX and thiopurines, thiopurine metabolite levels were correlated with IFX levels and ATIs. There was a moderate correlation between 6-TGN concentrations and IFX levels (rho - 0.53, P < 0.0001). The 6-TGN cut off that best predicted higher IFX levels was 125 pmol/8 × 10^8 RBCs (AUROC - 0.86, P < 0.001). Patients with 6-TGN levels below this cut off had IFX levels similar to patients on monotherapy (4.3 μg/mL *vs* 4.8 μg/mL, P = 0.8). Similarly, patients with 6-TGN levels below this threshold were more likely to have ATIs (OR = 1.3, 95%CI: 2.3-72.5, P < 0.01). These results provide the first signal that lower thiopurine doses, as measured by metabolite levels, may be equally effective as therapeutic doses in optimizing serum anti-TNF levels, however they must be interpreted with caution. The primary endpoint was IFX levels, with mucosal healing as a secondary endpoint, and IFX levels of > 8.3 μg/mL were associated with mucosal healing. When dichotomized above and below this cutoff, a mean 6-TGN level of 223 pmol/8 × 10^8 RBCs was required to achieve an IFX level of 8.3 μg/mL, compared to mean 6-TGN levels of 128 pmol/8 × 10^8 RBCs for IFX levels < 8.3 μg/mL (P < 0.001). Similarly, undetectable *vs* detectable ATIs were associated with mean 6-TGN levels of 117 pmol/8 × 10^8 RBCs and 193 pmol/8 × 10^8 RBCs respectively (P = 0.024). Therefore, while a 6-TGN level of 125 pmol/8 × 10^8 RBCs best predicted increased IFX levels, very similar 6-TGN levels were associated with a lack of mucosal healing and the development of ATIs - this disparity may in part be explained by the high IFX cut off of 8.3 μg/mL that was used, for which sensitivity and specificity were only moderate (71% and 73% respectively)[62]. Similar findings were observed in a single centre cross-sectional study of 269 IBD patients treated with IFX who underwent TDM with a drug-tolerant mobility shift assay[63]. Patients co-treated with AZA/MP, [n = 99 (37%)] and MTX [n = 32 (12%)] were more likely to have therapeutic IFX levels than those on monotherapy, (P = 0.05 and P = 0.04 for thiopurines and MTX, respectively). Regression analysis did not demonstrate a relationship between AZA dose and drug levels (P = 0.88) nor was an association seen between weight based dose (mg/kg) and drug levels when analysed by quartiles (P = 0.87).

The change in MCV with thiopurine therapy has been correlated with 6-TGN levels, with a delta MCV of at least 7 fL being associated with therapeutic 6-TGN levels and improved clinical outcomes[64,65]. A post hoc analysis of the SONIC study [which included only patients with normal thiopurine methyltransferase, (TPMT) activity] investigated the relationship between the change in MCV (dichotomized to above and below 7 fL) and outcomes in patients receiving combination therapy with AZA and IFX. An increase in MCV of at least 7 fL was associated with mucosal healing at week 26 (75% *vs* 47.1% if delta MCV < 7 fL, P = 0.02) and IFX levels > 3.0 μg/mL (68.4% *vs* 38.8% if delta MCV < 7 fL, P = 0.003). On multivariate analysis, delta MCV > 7 fL was associated with mucosal healing (OR = 3.86, 96%CI: 1.05-14.19, P = 0.04). Interestingly, patients with a delta MCV > 7 fL had less infectious adverse events (26.5% *vs* 49.2% if delta MCV < 7 fL, P = 0.008). No correlation between changes in MCV and mg/kg thiopurine doses was performed and thiopurine

Amendment: Column 2 line 3 OR should read 13 not 1.3

metabolites were not measured[66]. These results represent progress in optimizing thiopurines when used in combination therapy, although the optimal mg/kg dose, or surrogate measure of efficacy, remain to be determined.

Similarly, for MTX there are few data to guide clinicians as to the optimal dose, and route, to use in combination therapy with anti-TNF agents in IBD. In rheumatoid arthritis, 10 mg MTX orally weekly was the optimal dose to increase serum adalimumab levels in a MTX dose-escalation study[54]. In the COMMIT study subcutaneous MTX was commenced at 10 mg weekly and increased to 25 mg weekly by week 5, with the mean MTX dose at week 50 being 22.3 mg. At this dose, combination therapy patients compared to monotherapy patients had less ATIs (4% *vs* 20%, $P = 0.01$), numerically higher IFX trough levels (6.35 μg/mL *vs* 3.75 μg/mL, $P = 0.08$) and were more likely to have detectable IFX trough levels (52% *vs* 44%, $P = 0.84$). Even at this high dose, there was no difference in adverse event rates between the two groups[12]. More recently, in a single referral-centre retrospective study of combination MTX and anti-TNF therapy, outcomes were compared between patients on low dose (< 12.5 mg weekly) and high-dose (15-25 mg weekly) MTX. 73 IBD patients with active disease were included (CD-54, UC-16, indeterminate colitis - 3), of which 71% received high-dose and 29% low-dose MTX. The anti-TNF was ADA in 49% of patients, IFX in 40% of patients and certolizumab in 11% of patients, and MTX was given orally in 75% of patients. 46 of 73 (62%) patients went into remission and were followed and included in the primary analysis of duration of remission maintenance. High-dose MTX combination therapy patients were less likely to relapse (log-rank test, $P < 0.01$), and although rates of adverse events (33% *vs* 12%, $P = 0.13$) and discontinuations (14% *vs* 6%, $P = 0.34$) were higher in the high-dose MTX group, these differences did not reach significance. There were no differences when analyzed by the anti-TNF used in combination therapy (log-rank test, $P = 0.58$), diagnosis (log-rank test, $P = 0.78$), or mode of MTX administration (log-rank test, $P = 0.56$). Therapeutic drug monitoring was not performed[67].

Although a lower dose of concurrent IM would be hoped to be safer, in particular resulting in fewer infections and malignancies, there are few data to support this assumption. Studies amongst non-IBD populations have found a relationship between rates of malignancy and total thiopurine dose, thiopurine metabolite levels and TPMT activity[68-70]. Caution must be exercised before extrapolating these findings to the setting of combination therapy in IBD. Thiopurines are associated with increased infections, and viral infections in particular, (as outlined above) although a post-hoc analysis did not find a difference in infection risk between patients on high dose *vs* low dose thiopurines[27]. Similarly, the risk of NMSC and lymphoma associated with thiopurines has never been demonstrated to be dose-dependent in IBD, however most studies addressing these questions have not included IM dose[32,44,48]. From these data, which are mainly retrospective or post hoc analyses, it is not possible to conclude whether a lower dose of concurrent IM is equally efficacious and safer in combination therapy. For thiopurines, "therapeutic" 6-TGN levels were required to achieve IFX levels associated with mucosal healing, while a rise in MCV of > 7 fL may be a useful surrogate target if replicated in other studies. For MTX, unlike rheumatologic studies where lower doses appear adequate to maximize anti-TNF levels, in IBD higher doses (15-25 mg weekly) were required to maintain remission. Therefore until well-designed prospective studies prove otherwise, using full doses of IMs as combination therapy appears to be the best option for clinicians.

CAN IMMUNOMODULATORS BE STOPPED AT ANY TIME WHEN USED IN COMBINATION THERAPY?

In combination therapy patients with a high risk of adverse events to continuing therapy and a low risk of disease relapse on treatment withdrawal, cessation of therapy can be considered. Either the anti-TNF or the IM can be stopped, although relapse rates after IM withdrawal are generally lower than relapse rates after anti-TNF discontinuation, making IM withdrawal the more logical strategy[71]. Another rationale for stopping the IM comes from recent data showing that the risk of malignancy with thiopurines, and lymphoma in particular, is associated with the duration of therapy and reduces, or even normalizes, after IMs are ceased. In the CESAME cohort the hazard ratio for lymphoma was 5.28 (95%CI: 2.01-13.9, $P = 0.0007$) for those continuing thiopurines, but became insignificant (HR = 1.02, 95%CI: 2.01-13.9, $P = 0.98$) after they were ceased[41]. More recently in a retrospective cohort study of 36,891 veterans with UC the hazard ratio for developing lymphoma in patients on thiopurines was 4.2 (95%CI: 2.5-6.8, $P < 0.0001$), but reduced to 0.5 (95%CI: 0.2-1.3, $P = 0.17$) after thiopurines were discontinued[42]. In the most-recent meta-analysis combining 18 population-based and referral-centre studies lymphoma risk became significant after 1 year of thiopurine exposure. Amongst population studies standardized incidence ratios (SIR) were increased amongst current (SIR = 5.71, 95%CI: 3.72-10.1), but not former users (SIR = 1.42, 95%CI: 0.86-2.34)[48]. Similar trends of a reduction in malignancy risk after cessation of therapy have been demonstrated in some thiopurine-associated NMSC cohorts[32,72].

The first well-designed, albeit open-label, study of IM withdrawal (the IMID Study) came from the Leuven group in which 80 CD patients in remission on combination therapy for at least 6 mo were randomized to continue or stop IM therapy, with both

groups continuing scheduled maintenance IFX for 2 years. There was no difference in the primary endpoint of patients requiring a decrease in IFX dosing interval (60% in patients continuing IMs *vs* 55% in patients stopping IMs, P = 0.65) or stopping IFX (27.5% *vs* 22.5% respectively, P = NS). Mucosal healing rates were also similar between groups. However patients continuing on IMs had significantly higher trough IFX levels (2.87 μg/mL *vs* 1.65 μg/mL, $P < 0.0001$) and correspondingly lower levels of CRP (1.6 mg/L *vs* 2.8 mg/L, $P < 0.005$), suggesting the possibility of differing outcomes between groups over a longer period of follow up[55]. In a single-centre observational study of 48 CD patients on combination therapy for at least 6 mo in whom AZA was stopped, survival without IFX failure was 85% at 12 mo and 41% at 24 mo. Predictors of IFX failure were a duration of combination therapy less than 27 mo (HR = 7.46, 95%CI: 1.64-33.85, P = 0.01) and presence of inflammation at the time of IM withdrawal (CRP > 5 mg/L, HR = 4.79, 95%CI: 1.52-15.10, P = 0.008, and platelet count > 298 (HR = 4.75, 95%CI: 1.28-17.57, P = 0.02)[73]. More recently, in another single-centre, retrospective study the Leuven group assessed the effect of IM withdrawal on IFX trough levels and immunogenicity. Of 158 patients on combination therapy for at least 6 mo (median 13 mo), IM were withdrawn in 117 patients who were followed for a median of 29 mo. Of patients stopping IMs 38% required an increase in IFX dosing interval and 18% stopped IFX. However IFX trough levels were unchanged before and after IM withdrawal (3.2 μg/mL *vs* 3.7 μg/mL respectively, P = 0.70). Low IFX trough levels and high CRP at the time of IM withdrawal, and previous IFX dose-escalation prior to IM withdrawal were predictors of subsequent IFX monotherapy failure. Interestingly, no patients with an IFX trough level > 5 μg/mL at the time of IM withdrawal relapsed during the follow up period[74]. From these three studies it can be concluded that the lowest risk of relapse is in patients who are in deep remission (clinical remission and normalized biomarkers including mucosal healing), with good anti-TNF drug levels, after a prolonged period of combination therapy (ideally at least 12 mo) before IMs are withdrawn. Patients with active disease who withdraw IM are more likely to flare and subsequently require optimization of treatment.

Hopefully the upcoming international BIOCYCLE study, which aims to compare outcomes of treatment cycles in patients on combination therapy to outcomes when either the anti-TNF or IM is withdrawn will provide further clarification of the safety of de-escalation strategies in individual patients.

Of relevance to the issue of de-escalation of therapy, two small recent studies have shown that in patients losing response to anti-TNF monotherapy the re- addition of an IM can overcome immunogenicity and recapture response in some patients. In a small series of 5 patients losing response to IFX due to immunogenicity the addition of an IM (thiopurines in 3 patients, MTX in 2 patients) was successful in overcoming ATIs, increasing serum IFX levels and restoring clinical response in all patients[75]. Similar results were demonstrated when thiopurines were added to five patients failing ADA monotherapy, all of whom had previously failed thiopurine monotherapy. Clinical improvement was noted in all patients and repeat endoscopy was performed in four patients, all of whom showed improvement[76].

CONCLUSION

Over the last 15 years there have been great advances in the understanding of the relative roles IMs and anti-TNFs play in the modern management of IBD. It has become recognized that amongst thiopurine naïve patients, combination therapy is more efficacious than monotherapy with either thiopurines or anti-TNF alone, albeit at an increased risk of adverse events, most important of which are infection and malignancy. However questions remain as to how best to position IM use in those who require treatment with an anti-TNF, particularly in IM failures. Many of these are being addressed as we learn more about the pharmacokinetic relationship between anti-TNF and IM use and clinical outcomes. Combination therapy is associated with higher anti-TNF drug levels and less anti-drug antibody production, especially during the first 12 mo. Higher drug levels, in turn, measured post-induction[77-80] and during maintenance therapy[81-84], are associated with favorable clinical outcomes. Whereas it is tempting to equate the beneficial effects of combination therapy solely to an improvement in anti-TNF pharmacokinetics, it must be recognized that this conclusion is at present intuitive rather than evidence based. Prospective studies are needed that assess differences in efficacy, safety and costs between combination therapy *vs* anti-TNF monotherapy with anti-TNF dose-adjustments to achieve similar drug levels[85]. Further research is also needed to determine the effect of varying thiopurine and MTX doses on anti-TNF pharmacokinetics, incorporating both weight-based and metabolite-based (thioguanine nucleotides and MTX polyglutamates[86], for thiopurines and MTX respectively) dose-optimization strategies.

REFERENCES

1 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P; ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/S0140-6736(02)08512-4]

2 **Present DH**, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-1405 [PMID: 10228190 DOI: 10.1056/NEJM199905063401804]

3 **Targan SR**, Hanauer SB, van Deventer SJ, Mayer L, Present DH,

Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997; **337**: 1029-1035 [PMID: 9321530 DOI: 10.1056/NEJM199710093371502]

4 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885 [PMID: 14985485 DOI: 10.1056/NEJMoa030815]

5 **Hanauer SB**, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, Panaccione R, Wolf D, Pollack P. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323-333; quiz 591 [PMID: 16472588 DOI: 10.1053/j.gastro.2005.11.030]

6 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65 [PMID: 17241859 DOI: 10.1053/j.gastro.2006.11.041]

7 **Sandborn WJ**, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, Panaccione R, Wolf D, Kent JD, Bittle B, Li J, Pollack PF. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007; **56**: 1232-1239 [PMID: 17299059 DOI: 10.1136/gut.2006.106781]

8 **Reinisch W**, Sandborn WJ, Hommes DW, D'Haens G, Hanauer S, Schreiber S, Panaccione R, Fedorak RN, Tighe MB, Huang B, Kampman W, Lazar A, Thakkar R. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011; **60**: 780-787 [PMID: 21209123 DOI: 10.1136/gut.2010.221127]

9 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-265. e1-e3 [PMID: 22062358 DOI: 10.1053/j.gastro.2011.10.032]

10 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]

11 **Panaccione R**, Ghosh S, Middleton S, Márquez JR, Scott BB, Flint L, van Hoogstraten HJ, Chen AC, Zheng H, Danese S, Rutgeerts P. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 2014; **146**: 392-400.e3 [PMID: 24512909 DOI: 10.1053/j.gastro.2013.10.052]

12 **Feagan BG**, McDonald JW, Panaccione R, Enns RA, Bernstein CN, Ponich TP, Bourdages R, Macintosh DG, Dallaire C, Cohen A, Fedorak RN, Paré P, Bitton A, Saibil F, Anderson F, Donner A, Wong CJ, Zou G, Vandervoort MK, Hopkins M, Greenberg GR. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. *Gastroenterology* 2014; **146**: 681-688.e1 [PMID: 24269926 DOI: 10.1053/j.gastro.2013.11.024]

13 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

14 **Sokol H**, Seksik P, Carrat F, Nion-Larmurier I, Vienne A, Beaugerie L, Cosnes J. Usefulness of co-treatment with immunomodulators in patients with inflammatory bowel disease treated with scheduled infliximab maintenance therapy. *Gut* 2010; **59**: 1363-1368 [PMID: 20587545 DOI: 10.1136/gut.2010.212712]

15 **Peters CP**, Eshuis EJ, Toxopeüs FM, Hellemons ME, Jansen JM, D'Haens GR, Fockens P, Stokkers PC, Tuynman HA, van Bodegraven AA, Ponsioen CY; North Holland GUT club. Adalimumab for Crohn's disease: long-term sustained benefit in a population-based cohort of 438 patients. *J Crohns Colitis* 2014; **8**: 866-875 [PMID: 24491515 DOI: 10.1016/j.crohns.2014.01.012]

16 **Eshuis EJ**, Peters CP, van Bodegraven AA, Bartelsman JF, Bemelman W, Fockens P, D'Haens GR, Stokkers PC, Ponsioen CY. Ten years of infliximab for Crohn's disease: outcome in 469 patients from 2 tertiary referral centers. *Inflamm Bowel Dis* 2013; **19**: 1622-1630 [PMID: 23552767 DOI: 10.1097/MIB.0b013e318281f4c4]

17 **Ungar B**, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, Kopylov U, Eliakim R, Ben-Horin S; ABIRISK consortium. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014; **63**: 1258-1264 [PMID: 24041539 DOI: 10.1136/gutjnl-2013-305259]

18 **Reenaers C**, Louis E, Belaiche J, Seidel L, Keshav S, Travis S. Does co-treatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? *Aliment Pharmacol Ther* 2012; **36**: 1040-1048 [PMID: 23061650 DOI: 10.1111/apt.12076]

19 **Kiss LS**, Szamosi T, Molnar T, Miheller P, Lakatos L, Vincze A, Palatka K, Barta Z, Gasztonyi B, Salamon A, Horvath G, Tóth GT, Farkas K, Banai J, Tulassay Z, Nagy F, Szenes M, Veres G, Lovasz BD, Vegh Z, Golovics PA, Szathmari M, Papp M, Lakatos PL. Early clinical remission and normalisation of CRP are the strongest predictors of efficacy, mucosal healing and dose escalation during the first year of adalimumab therapy in Crohn's disease. *Aliment Pharmacol Ther* 2011; **34**: 911-922 [PMID: 21883326 DOI: 10.1111/j.1365-2036.2011.04827.x]

20 **Moss AC**, Kim KJ, Fernandez-Becker N, Cury D, Cheifetz AS. Impact of concomitant immunomodulator use on long-term outcomes in patients receiving scheduled maintenance infliximab. *Dig Dis Sci* 2010; **55**: 1413-1420 [PMID: 19533357 DOI: 10.1007/s10620-009-0856-7]

21 **Oussalah A**, Evesque L, Laharie D, Roblin X, Boschetti G, Nancey S, Filippi J, Flourié B, Hebuterne X, Bigard MA, Peyrin-Biroulet L. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. *Am J Gastroenterol* 2010; **105**: 2617-2625 [PMID: 20736936 DOI: 10.1038/ajg.2010.345]

22 **Regueiro M**, Siemanowski B, Kip KE, Plevy S. Infliximab dose intensification in Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 1093-1099 [PMID: 17480021 DOI: 10.1002/ibd.20177]

23 **Schnitzler F**, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, Hoffman I, Van Steen K, Vermeire S, Rutgeerts P. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. *Gut* 2009; **58**: 492-500 [PMID: 18832518 DOI: 10.1136/gut.2008.155812]

24 **Jones J**, Kaplan GG, Peyrin-Biroulet L, Baidoo L, Devlin S, Melmed GY, Tanyingoh D, Raffals LH, Irving PM, Kozuch PL, Sparrow M, Velayos FS, Bressler B, Cheifetz AS, Colombel J-F, Siegel CA. Impact of Concomitant Immunomodulator Treatment on Efficacy and Safety of Anti-TNF Therapy in Crohn's Disease: A Meta-Analysis of Placebo Controlled Trials With Individual Patient-Level Data. *Gastroenterology* 2013; **144**: S179-179

25 **Siegel CA**. Review article: explaining risks of inflammatory bowel disease therapy to patients. *Aliment Pharmacol Ther* 2011; **33**: 23-32 [PMID: 21083583 DOI: 10.1111/j.1365-2036.2010.04489.x]

26 **Lichtenstein GR**, Diamond RH, Wagner CL, Fasanmade AA, Olson AD, Marano CW, Johanns J, Lang Y, Sandborn WJ. Clinical trial: benefits and risks of immunomodulators and maintenance infliximab for IBD-subgroup analyses across four randomized trials. *Aliment Pharmacol Ther* 2009; **30**: 210-226 [PMID: 19392858 DOI: 10.1111/j.1365-2036.2009.04027.x]

27 **Osterman MT**, Haynes K, Delzell E, Zhang J, Bewtra M, Brensinger CM, Chen L, Xie F, Curtis JR, Lewis JD. Effectiveness and Safety of Immunomodulators With Anti-Tumor Necrosis Factor Therapy in Crohn's Disease. *Clin Gastroenterol Hepatol* 2015; **13**: 1293-1301.e5; quiz e70, e72 [PMID: 25724699 DOI: 10.1016/j.cgh.2015.02.017]

28 **Deepak P**, Stobaugh DJ, Ehrenpreis ED. Infectious complications of TNF-α inhibitor monotherapy versus combination therapy with immunomodulators in inflammatory bowel disease: analysis of the Food and Drug Administration Adverse Event Reporting System. *J Gastrointestin Liver Dis* 2013; **22**: 269-276 [PMID: 24078983]

29 **Schneeweiss S**, Korzenik J, Solomon DH, Canning C, Lee J, Bressler B. Infliximab and other immunomodulating drugs in patients with inflammatory bowel disease and the risk of serious bacterial infections. *Aliment Pharmacol Ther* 2009; **30**: 253-264 [PMID: 19438424 DOI: 10.1111/j.1365-2036.2009.04037.x]

30 **Toruner M**, Loftus EV, Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, Colombel JF, Egan LJ. Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology* 2008; **134**: 929-936 [PMID: 18294633 DOI: 10.1053/j.gastro.2008.01.012]

31 **Gutierrez-Dalmau A**, Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients: a systematic review. *Drugs* 2007; **67**: 1167-1198 [PMID: 17521218 DOI: 10.2165/00003495-200767080-00006]

32 **Peyrin-Biroulet L**, Khosrotehrani K, Carrat F, Bouvier AM, Chevaux JB, Simon T, Carbonnel F, Colombel JF, Dupas JL, Godeberge P, Hugot JP, Lémann M, Nahon S, Sabaté JM, Tucat G, Beaugerie L; Cesame Study Group. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. *Gastroenterology* 2011; **141**: 1621-1628.e1-e5 [PMID: 21708105 DOI: 10.1053/j.gastro.2011.06.050]

33 **Singh H**, Nugent Z, Demers AA, Bernstein CN. Increased risk of nonmelanoma skin cancers among individuals with inflammatory bowel disease. *Gastroenterology* 2011; **141**: 1612-1620 [PMID: 21806945 DOI: 10.1053/j.gastro.2011.07.039]

34 **Long MD**, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. *Gastroenterology* 2012; **143**: 390-399.e1 [PMID: 22584081 DOI: 10.1053/j.gastro.2012.05.004]

35 **Mariette X**, Matucci-Cerinic M, Pavelka K, Taylor P, van Vollenhoven R, Heatley R, Walsh C, Lawson R, Reynolds A, Emery P. Malignancies associated with tumour necrosis factor inhibitors in registries and prospective observational studies: a systematic review and meta-analysis. *Ann Rheum Dis* 2011; **70**: 1895-1904 [PMID: 21885875 DOI: 10.1136/ard.2010.149419]

36 **Osterman MT**, Sandborn WJ, Colombel JF, Robinson AM, Lau W, Huang B, Pollack PF, Thakkar RB, Lewis JD. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. *Gastroenterology* 2014; **146**: 941-949 [PMID: 24361468 DOI: 10.1053/j.gastro.2013.12.025]

37 **Singh S**, Nagpal SJ, Murad MH, Yadav S, Kane SV, Pardi DS, Talwalkar JA, Loftus EV. Inflammatory bowel disease is associated with an increased risk of melanoma: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 210-218 [PMID: 23644389 DOI: 10.1016/j.cgh.2013.04.033]

38 **Askling J**, Fahrbach K, Nordstrom B, Ross S, Schmid CH, Symmons D. Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. *Pharmacoepidemiol Drug Saf* 2011; **20**: 119-130 [PMID: 21254282 DOI: 10.1002/pds.2046]

39 **Wolfe F**, Michaud K. Biologic treatment of rheumatoid arthritis and the risk of malignancy: analyses from a large US observational study. *Arthritis Rheum* 2007; **56**: 2886-2895 [PMID: 17729297 DOI: 10.1002/art.22864]

40 **Williams CJ**, Peyrin-Biroulet L, Ford AC. Systematic review with meta-analysis: malignancies with anti-tumour necrosis factor-α therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2014; **39**: 447-458 [PMID: 24444171 DOI: 10.1111/apt.12624]

41 **Beaugerie L**, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F; CESAME Study Group. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; **374**: 1617-1625 [PMID: 19837455 DOI: 10.1016/S0140-6736(09)61302-7]

42 **Khan N**, Abbas AM, Lichtenstein GR, Loftus EV, Bazzano LA. Risk of lymphoma in patients with ulcerative colitis treated with thiopurines: a nationwide retrospective cohort study. *Gastroenterology* 2013; **145**: 1007-1015.e3 [PMID: 23891975 DOI: 10.1053/j.gastro.2013.07.035]

43 **Dulai PS**, Thompson KD, Blunt HB, Dubinsky MC, Siegel CA. Risks of serious infection or lymphoma with anti-tumor necrosis factor therapy for pediatric inflammatory bowel disease: a systematic review. *Clin Gastroenterol Hepatol* 2014; **12**: 1443-1451; quiz e88-9 [PMID: 24462626 DOI: 10.1016/j.cgh.2014.01.021]

44 **Siegel CA**, Marden SM, Persing SM, Larson RJ, Sands BE. Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2009; **7**: 874-881 [PMID: 19558997 DOI: 10.1016/j.cgh.2009.01.004]

45 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Langholff W, Londhe A, Sandborn WJ. Drug therapies and the risk of malignancy in Crohn's disease: results from the TREAT ™ Registry. *Am J Gastroenterol* 2014; **109**: 212-223 [PMID: 24394749 DOI: 10.1038/ajg.2013.441]

46 **Herrinton LJ**, Liu L, Weng X, Lewis JD, Hutfless S, Allison JE. Role of thiopurine and anti-TNF therapy in lymphoma in inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 2146-2153 [PMID: 22031357 DOI: 10.1038/ajg.2011.283]

47 **Nyboe Andersen N**, Pasternak B, Basit S, Andersson M, Svanström H, Caspersen S, Munkholm P, Hviid A, Jess T. Association between tumor necrosis factor-α antagonists and risk of cancer in patients with inflammatory bowel disease. *JAMA* 2014; **311**: 2406-2413 [PMID: 24938563 DOI: 10.1001/jama.2014.5613]

48 **Kotlyar DS**, Lewis JD, Beaugerie L, Tierney A, Brensinger CM, Gisbert JP, Loftus EV, Peyrin-Biroulet L, Blonski WC, Van Domselaar M, Chaparro M, Sandilya S, Bewtra M, Beigel F, Biancone L, Lichtenstein GR. Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: a meta-analysis. *Clin Gastroenterol Hepatol* 2015; **13**: 847-858.e4; quiz e48-e50 [PMID: 24879926 DOI: 10.1016/j.cgh.2014.05.015]

49 **Carbonnel F**. OP023. Methotrexate for corticosteroid-dependent ulcerative colitis: results of a placebo randomized controlled trial. *J Crohns Colitis* 2015; **9** Suppl 1: S14-S15

50 **Vermeire S**, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* 2007; **56**: 1226-1231 [PMID: 17229796 DOI: 10.1136/gut.2006.099978]

51 **Feagan BG**, Rochon J, Fedorak RN, Irvine EJ, Wild G, Sutherland L, Steinhart AH, Greenberg GR, Gillies R, Hopkins M. Methotrexate for the treatment of Crohn's disease. The North American Crohn's Study Group Investigators. *N Engl J Med* 1995; **332**: 292-297 [PMID: 7816064 DOI: 10.1056/NEJM199502023320503]

52 **Feagan BG**, Fedorak RN, Irvine EJ, Wild G, Sutherland L, Steinhart AH, Greenberg GR, Koval J, Wong CJ, Hopkins M, Hanauer SB, McDonald JW. A comparison of methotrexate with placebo for the maintenance of remission in Crohn's disease. North American Crohn's Study Group Investigators. *N Engl J Med* 2000; **342**: 1627-1632 [PMID: 10833208 DOI: 10.1056/NEJM200006013422202]

53 **Seitz K**, Zhou H. Pharmacokinetic drug-drug interaction potentials for therapeutic monoclonal antibodies: reality check. *J Clin Pharmacol* 2007; **47**: 1104-1118 [PMID: 17766698 DOI: 10.1177/0091270007306958]

54 **Burmester GR**, Kivitz AJ, Kupper H, Arulmani U, Florentinus S, Goss SL, Rathmann SS, Fleischmann RM. Efficacy and safety of ascending methotrexate dose in combination with adalimumab: the randomised CONCERTO trial. *Ann Rheum Dis* 2015; **74**: 1037-1044 [PMID: 24550168 DOI: 10.1136/annrheumdis-2013-204769]

55 **Van Assche G**, Magdelaine-Beuzelin C, D'Haens G, Baert F, Noman M, Vermeire S, Ternant D, Watier H, Paintaud G, Rutgeerts

P. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology* 2008; **134**: 1861-1868 [PMID: 18440315 DOI: 10.1053/j.gastro.2008.03.004]

56 **Kotlyar DS**, Osterman MT, Diamond RH, Porter D, Blonski WC, Wasik M, Sampat S, Mendizabal M, Lin MV, Lichtenstein GR. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011; **9**: 36-41.e1 [PMID: 20888436 DOI: 10.1016/j.cgh.2010.09.016]

57 **Buchbinder R**, Barber M, Heuzenroeder L, Wluka AE, Giles G, Hall S, Harkness A, Lewis D, Littlejohn G, Miller MH, Ryan PF, Jolley D. Incidence of melanoma and other malignancies among rheumatoid arthritis patients treated with methotrexate. *Arthritis Rheum* 2008; **59**: 794-799 [PMID: 18512713 DOI: 10.1002/art.23716]

58 **Wolfe F**, Michaud K. The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. *Arthritis Rheum* 2007; **56**: 1433-1439 [PMID: 17469100 DOI: 10.1002/art.22579]

59 **Salliot C**, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. *Ann Rheum Dis* 2009; **68**: 1100-1104 [PMID: 19060002 DOI: 10.1136/ard.2008.093690]

60 **Vermeire S**, Carbonnel F, Coulie PG, Geenen V, Hazes JM, Masson PL, De Keyser F, Louis E. Management of inflammatory bowel disease in pregnancy. *J Crohns Colitis* 2012; **6**: 811-823 [PMID: 22595185 DOI: 10.1016/j.crohns.2012.04.009]

61 **van Schaik T**, Maljaars JP, Roopram RK, Verwey MH, Ipenburg N, Hardwick JC, Veenendaal RA, van der Meulen-de Jong AE. Influence of combination therapy with immune modulators on anti-TNF trough levels and antibodies in patients with IBD. *Inflamm Bowel Dis* 2014; **20**: 2292-2298 [PMID: 25230167 DOI: 10.1097/MIB.0000000000000208]

62 **Yarur AJ**, Kubiliun MJ, Czul F, Sussman DA, Quintero MA, Jain A, Drake KA, Hauenstein SI, Lockton S, Deshpande AR, Barkin JS, Singh S, Abreu MT. Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol* 2015; **13**: 1118-1124.e3 [PMID: 25562796 DOI: 10.1016/j.cgh.2014.12.026]

63 **Cahill J**, Zadvornova Y, Naik AS, Agrawal D, Best K, Stein D. Tu1282 Azothioprine or 6-Mercaptopurnine Dose Does Not Effect Serum Infliximab Level or Rate of Antibody to Infliximab Formation. *Gastroenterology* 2015; **148**: S-847-847 [DOI: 10.1016/S0016-5085(15)32870-5]

64 **Thomas CW**, Lowry PW, Franklin CL, Weaver AL, Myhre GM, Mays DC, Tremaine WJ, Lipsky JJ, Sandborn WJ. Erythrocyte mean corpuscular volume as a surrogate marker for 6-thioguanine nucleotide concentration monitoring in patients with inflammatory bowel disease treated with azathioprine or 6-mercaptopurine. *Inflamm Bowel Dis* 2003; **9**: 237-245 [PMID: 12902847 DOI: 10.1097/00054725-200307000-00004]

65 **Decaux G**, Prospert F, Horsmans Y, Desager JP. Relationship between red cell mean corpuscular volume and 6-thioguanine nucleotides in patients treated with azathioprine. *J Lab Clin Med* 2000; **135**: 256-262 [PMID: 10711864 DOI: 10.1067/mlc.2000.105215]

66 **Bouguen G**, Sninsky C, Tang KL, Colombel JF, D'Haens G, Kornbluth A, Mantzaris GJ, Rachmilewitz D, Reinisch W, Rutgeerts P, Molenda M, Jannekevan der Woude C, Sandborn WJ. Change in erythrocyte mean corpuscular volume during combination therapy with azathioprine and infliximab is associated with mucosal healing: a post hoc analysis from SONIC. *Inflamm Bowel Dis* 2015; **21**: 606-614 [PMID: 25581826 DOI: 10.1097/MIB.0000000000000302]

67 **Colman RJ**, Rubin DT. Optimal doses of methotrexate combined with anti-TNF therapy to maintain clinical remission in inflammatory bowel disease. *J Crohns Colitis* 2015; **9**: 312-317 [PMID: 25616487 DOI: 10.1093/ecco-jcc/jjv027]

68 **Lennard L**, Thomas S, Harrington CI, Maddocks JL. Skin cancer in renal transplant recipients is associated with increased concentrations of 6-thioguanine nucleotide in red blood cells. *Br J Dermatol* 1985; **113**: 723-729 [PMID: 3913458 DOI: 10.1111/j.1365-2133.1985.tb02408.x]

69 **Silman AJ**, Petrie J, Hazleman B, Evans SJ. Lymphoproliferative cancer and other malignancy in patients with rheumatoid arthritis treated with azathioprine: a 20 year follow up study. *Ann Rheum Dis* 1988; **47**: 988-992 [PMID: 3207388 DOI: 10.1136/ard.47.12.988]

70 **Bo J**, Schrøder H, Kristinsson J, Madsen B, Szumlanski C, Weinshilboum R, Andersen JB, Schmiegelow K. Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells: relation to thiopurine metabolism. *Cancer* 1999; **86**: 1080-1086 [PMID: 10491537]

71 **Pariente B**, Laharie D. Review article: why, when and how to de-escalate therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2014; **40**: 338-353 [PMID: 24957164 DOI: 10.1111/apt.12838]

72 **Abbas AM**, Almukhtar RM, Loftus EV, Lichtenstein GR, Khan N. Risk of melanoma and non-melanoma skin cancer in ulcerative colitis patients treated with thiopurines: a nationwide retrospective cohort. *Am J Gastroenterol* 2014; **109**: 1781-1793 [PMID: 25244964 DOI: 10.1038/ajg.2014.298]

73 **Oussalah A**, Chevaux JB, Fay R, Sandborn WJ, Bigard MA, Peyrin-Biroulet L. Predictors of infliximab failure after azathioprine withdrawal in Crohn's disease treated with combination therapy. *Am J Gastroenterol* 2010; **105**: 1142-1149 [PMID: 20389296 DOI: 10.1038/ajg.2010.158]

74 **Drobne D**, Bossuyt P, Breynaert C, Cattaert T, Vande Casteele N, Compernolle G, Jürgens M, Ferrante M, Ballet V, Wollants WJ, Cleynen I, Van Steen K, Gils A, Rutgeerts P, Vermeire S, Van Assche G. Withdrawal of immunomodulators after co-treatment does not reduce trough level of infliximab in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2015; **13**: 514-521.e4 [PMID: 25066841 DOI: 10.1016/j.cgh.2014.07.027]

75 **Ben-Horin S**, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, Awadie H, Weiss B, Chowers Y. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013; **11**: 444-447 [PMID: 23103905 DOI: 10.1016/j.cgh.2012.10.020]

76 **Ong DE**, Kamm MA, Hartono JL, Lust M. Addition of thiopurines can recapture response in patients with Crohn's disease who have lost response to anti-tumor necrosis factor monotherapy. *J Gastroenterol Hepatol* 2013; **28**: 1595-1599 [PMID: 23662928 DOI: 10.1111/jgh.12263]

77 **Bortlik M**, Duricova D, Malickova K, Machkova N, Bouzkova E, Hrdlicka L, Komarek A, Lukas M. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013; **7**: 736-743 [PMID: 23200919 DOI: 10.1016/j.crohns.2012.10.019]

78 **Cornillie F**, Hanauer SB, Diamond RH, Wang J, Tang KL, Xu Z, Rutgeerts P, Vermeire S. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014; **63**: 1721-1727 [PMID: 24474383 DOI: 10.1136/gutjnl-2012-304094]

79 **Adedokun OJ**, Sandborn WJ, Feagan BG, Rutgeerts P, Xu Z, Marano CW, Johanns J, Zhou H, Davis HM, Cornillie F, Reinisch W. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. *Gastroenterology* 2014; **147**: 1296-1307.e5 [PMID: 25173754 DOI: 10.1053/j.gastro.2014.08.035]

80 **Arias MT**, Vande Casteele N, Vermeire S, de Buck van Overstraeten A, Billiet T, Baert F, Wolthuis A, Van Assche G, Noman M, Hoffman I, D'Hoore A, Gils A, Rutgeerts P, Ferrante M. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. *Clin Gastroenterol Hepatol* 2015; **13**: 531-538 [PMID: 25117777 DOI: 10.1016/j.cgh.2014.07.055]

81 **Paul S**, Del Tedesco E, Marotte H, Rinaudo-Gaujous M, Moreau A, Phelip JM, Genin C, Peyrin-Biroulet L, Roblin X. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013; **19**: 2568-2576 [PMID: 24013361 DOI: 10.1097/MIB.0b013e3182a77b41]

82 **Roblin X**, Marotte H, Rinaudo M, Del Tedesco E, Moreau A, Phelip JM, Genin C, Peyrin-Biroulet L, Paul S. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014; **12**: 80-84.e2 [PMID: 23891927 DOI: 10.1016/j.cgh.2013.07.010]

83 **Maser EA**, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1248-1254 [PMID: 16931170 DOI: 10.1016/j.cgh.2006.06.025]

84 **Mazor Y**, Almog R, Kopylov U, Ben Hur D, Blatt A, Dahan A, Waterman M, Ben-Horin S, Chowers Y. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. *Aliment Pharmacol Ther* 2014; **40**: 620-628 [PMID: 25039584 DOI: 10.1111/apt.12869]

85 **Hanauer SB**. Heading back to the trough (levels of biologics in IBD). *Clin Gastroenterol Hepatol* 2015; **13**: 548-551 [PMID: 25311382 DOI: 10.1016/j.cgh.2014.10.007]

86 **Halilova KI**, Brown EE, Morgan SL, Bridges SL, Hwang MH, Arnett DK, Danila MI. Markers of treatment response to methotrexate in rheumatoid arthritis: where do we stand? *Int J Rheumatol* 2012; **2012**: 978396 [PMID: 22844292 DOI: 10.1155/2012/978396]

P- Reviewer: Iizuka M, Sorrentino D, Yuksel I
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

CHAPTER 3:

When concomitantly used with adalimumab: Metabolite-adjusted thiopurine dosing in combination with adalimumab is superior to adalimumab monotherapy during induction and maintenance in Crohn's disease

Metabolite-adjusted thiopurine dosing in combination with adalimumab is superior to adalimumab monotherapy during induction and maintenance in Crohn's disease

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Non standard Abbreviations:

CIM : concomitant immunomodulation

ADA : adalimumab

TGN : thioguanine nucleotides

IFX : infliximab

MTX : Methotrexate

AZA : azathioprine

MP : mercaptopurine

TPMT : thiopurine-S-methyltransferase

MeMP : methylated metabolites

CRP – C reactive protein

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Keywords: Crohn's disease, thiopurine, combination therapy

Conference presentations:

Digestive Disease week 2014 – Oral presentation

Australian Gastroenterology Week 2013 – Oral presentation

ABSTRACT

Background and Aims: The benefit of concomitant immunomodulation (CIM) with adalimumab (ADA) in Crohn's disease is poorly understood. We aimed to compare ADA monotherapy with combination therapy with thiopurines, stratified by thioguanine nucleotides (TGNs).

Methods: Retrospective observational study of ADA induction and maintenance. Thiopurines were classified according to TGNs (>235 pmol/8x10^8RBC therapeutic).

Results: At induction, response was more frequent in combination than ADA monotherapy (83 vs 61%, p = 0.02) and with therapeutic compared to sub-therapeutic TGNs (87 vs 70% p = 0.011). Amongst 280 maintenance semesters 91 patients; remission was higher with combination than monotherapy (81 vs 60%, p < 0.0001) and therapeutic vs sub-therapeutic TGNs (85 vs 58%, p = 0.004). Therapeutic TGN (OR 4.32, 95% CI: 1.41–13.29, p = 0.01) and albumin (OR 1.09, 95% CI: 1.01–1.18, p = 0.03) were predictors of response to induction. Therapeutic TGN (OR 3.71, 95% CI: 1.87–7.34, p < 0.0001) and ileal disease (OR 0.21, 95% CI: 0.08–0.57, p = 0.002) were predictors of remission semesters. CIM at induction was associated with longer time to failure (69 vs 36 months, p = 0.009). Therapeutic TGN at induction (p = 0.03) and male gender (p = 0.026) were associated with time to failure.

Conclusion: Combination therapy was superior to ADA monotherapy for induction and during maintenance. This benefit was increased further when thiopurines resulted in therapeutic TGNs. Early use of adequately dosed thiopurines (≥3 months prior to starting ADA) was associated with improved clinical outcomes.

INTRODUCTION

Adalimumab, (ADA, Humira, Abbott Laboratories, Abbott Park, IL) a fully humanised monoclonal IgG antibody directed against tumor necrosis factor alpha, is effective at inducing and maintaining remission in patients with moderate-to-severe Crohn's disease.[1-5] However, a proportion of patients fail to respond to ADA. Of those that do respond, approximately 30% lose response by 12 months, with a further 10% losing response annually thereafter.[5-7] Accordingly, there is a need to understand whether there are factors that are associated with response and loss of response to improve outcomes.

Immunogenicity is a well recognised mechanism implicated in ADA failure[8]. Antibodies to ADA have been shown to negatively influence the pharmacokinetics of ADA, leading to increased drug clearance and lower ADA levels.[9] The use of concomitant immunomodulation, (CIM) with anti-TNF agents decreases anti-drug antibody formation.[10-12] In the case of infliximab, (IFX) combination therapy is superior to monotherapy, both for patients with Crohn's disease and those with ulcerative colitis UC.[11-13] However, there is less evidence for a similar effect with ADA, in part because of a lack of randomized controlled trials designed to address this question. Accordingly, supportive evidence regarding the need for CIM when using ADA is based on sub-analysis of randomized controlled trials and retrospective studies.[8,14] The results of these studies are conflicting, suggesting that further data would be of use. Further, there are no studies in ADA-treated patients assessing whether the intensity of CIM, (in the case of thiopurines measured using 6-thioguainine nucleotide metabolites (TGN)) is of importance, an area recently addressed in two IFX-treated cohorts.[15,16]

The aim of this study was, therefore, to investigate the influence of CIM on clinical outcomes in a well characterized and prospectively assessed cohort of Crohn's disease patients treated with ADA. In addition, we aimed to assess whether therapeutic TGN concentrations were associated with improved outcomes compared with sub-therapeutic TGNs in patients on thiopurine combination therapy.

METHODS

Study Design

We performed a retrospective single-centre cohort study of consecutive patients with moderate-to-severe Crohn's disease who commenced ADA at Guy's and St. Thomas' Inflammatory Bowel Disease Centre between January 2006 and June 2013.

Study population

The diagnosis of Crohn's disease was based on standard endoscopic, histological and radiological criteria.[17] Only patients who commenced ADA at our centre were included. Data were collected prospectively from January 2009 through our Virtual Biologic Clinic which has been described previously.[18] Within this setting, patients are reviewed prior to commencing ADA and subsequently every 3-6 months, unless indicated earlier. All other data were retrieved from the electronic patient record.

All patients initiated ADA at standard induction dosing, (160mg/80mg weeks 0 and 2) followed by maintenance (40mg every other week). In those with an incomplete response after induction or secondary loss of response, ADA was intensified to 40mg each week. Dose reduction back to 40mg every other week was considered after attainment of remission, based on a combination of clinical, biochemical, endoscopic and radiological parameters.

Methotrexate (MTX) or thiopurines (azathioprine (AZA), mercaptopurine (MP) and thioguanine were commenced at the treating physician's discretion. MTX was dosed at 15-25mg weekly orally with folic acid supplementation[19] and thioguanine at 20-40mg. AZA and MP were dosed according to body weight (2-2.5mg/kg AZA, 1-1.5mg/kg MP) after measurement of thiopurine-S-methyltransferase (TPMT) activity[20-22] with dose reduction by 50% in TPMT heterozygotes.[23] Therapeutic drug monitoring using TGN and methylated metabolites was performed[24]; a TGN of 230-450 pmol/8x10^8RBC was considered therapeutic.[25] Patients with sub-therapeutic TGNs or evidence of hepatotoxicity or intolerance with a

metabolite profile consistent with hypermethylation, were initiated on allopurinol 100mg, with thiopurine dose reduction to 25-33% of the original dose.[26]

Assessment of response - Induction

Response to induction was assessed at 12 weeks and was classified as either primary non-response, partial response or complete response by physician global assessment after consideration of clinical activity, (Harvey-Bradshaw Index[27]) and biomarkers (C-reactive protein, (CRP), faecal calprotectin) in conjunction with imaging and/or endoscopy, where available. Patients maintained on a stable dose of immunomodulator ≥ 3 months prior to ADA induction and who continued for a ≥ 6 months after induction were defined as CIM at induction. All other patients were classified as not being on CIM at induction. Patients taking thiopurines were further classified according to TGN levels; > 235 was considered therapeutic.

Assessment of response – Maintenance

Beginning after the first 12 months of treatment, patients were assessed for long-term clinical response, according to 6-monthly semesters. Semesters with ≥ 3 months of CIM therapy were considered CIM semesters. Patients on thiopurines were again stratified according to TGNs measured from each semester, where available.

Semesters were classified according to one of three definitions:

Flare semester: active clinical disease resulting in treatment intensification (ADA dose escalation, new corticosteroids or addition of CIM), hospital admission due to active Crohn's disease, new perianal disease or Crohn's disease-related surgery not leading to ADA withdrawal.

Remission semester: semester without a flare either on every other week or weekly dosing.

Failure semester: Failure, defined as withdrawal of ADA due to primary non-response, secondary loss of response despite dose-intensification, or due to development of adverse effects or complications.

Factors associated with clinical response.

Covariates that were assessed for response to induction, ADA failure, dose intensification and semester outcomes included: gender, disease duration, age at diagnosis, disease location and behaviour as per Montreal classification[28], smoking status, weight, previous anti-TNF exposure, previous surgery, CIM 3 ≥ months prior to starting therapy, CIM status during semester, and CRP and Harvey-Bradshaw Index at commencing ADA. Interactions between weight and need for ADA dose intensification were also explored.

Statistical analysis.

Categorical variables are presented as number and percentage, and quantitative data as mean with standard deviation or median with interquartile range, as appropriate. Between group comparisons were performed using Pearson's Chi-squared, independent sample t-test, or Mann–Whitney U test. Multivariate analysis was performed using Cox regression for time to failure and binary logistic regression for factors associated with induction outcome, dose escalation and semesters of remission. Covariates identified *a priori* with $p < 0.1$ on univariate analysis were entered into a multivariate backward stepwise model. Variables with $p < 0.05$ were retained in the final model and reported as adjusted hazard ratios (HRs) in the Cox regression and odds ratios (ORs) in logistic regression with 95% confidence intervals (CIs). Time to ADA failure was calculated using Kaplan-Meir survival analysis and comparisons between groups were made using the log-rank test. Two-sided P-values < 0.05 were considered significant. Statistical analyses were carried out using SPSS v23.0 (SPSS Inc., Chicago, IL).

Ethical Consideration

According to the guidelines of the U.K. Health Research Authority as the data collected were done so as part of routine clinical care and were evaluated retrospectively, ethical approval was not required.[29]

RESULTS

Patient characteristics

156 patients commenced ADA between January 2006 and June 2013; 123 met inclusion criteria for the induction analysis (Fig 1). Patient characteristics are shown in Table 1. CIM was prescribed for ≥ 3 months prior to starting ADA in 77/123 (63%); thiopurines were used in 67/77, MTX in 6, thioguanine in 3 and mycophenolate mofetil in 1. 57 and 59% of patients had previously been exposed to anti-TNF in the CIM, and no CIM cohorts, respectively. No significant differences in baseline CRP, ($p = 0.49$), albumin ($p = 0.19$) or Harvey-Bradshaw Index ($p = 0.052$) were observed between CIM and no-CIM groups. Follow-up was similar in both groups (20 vs 22 months, $p = 0.4$)

280 semesters amongst 91 patients were available for the maintenance analysis; 201 (72%) were classified as CIM semesters (143 with immunomodulators ≥ 3 months prior to starting ADA vs. 58 who were not) compared with 79 (28%) ADA monotherapy semesters (20 in patients treated with immunomodulators ≥ 3 months prior to starting ADA vs. 59 who were not) ($p < 0.001$). Thiopurines were used in 84% of semesters, of these TGNs were available in 92%. 135 (88%) were therapeutic and 19 (12%) sub-therapeutic.

Primary response

Complete response was seen in 92/123 (75%) at week 12; response was higher amongst those treated with CIM compared to those not treated with CIM (83 vs 61%, $p = 0.02$). In addition, the rate of primary non-response was significantly lower among patients treated with CIM (12 vs 30%, $p = 0.02$) (Fig 2).

Most, (97%) patients treated with thiopurines had TGNs prior to starting ADA; 16% were sub-therapeutic. Response to induction was seen in 48 (87%), 7 (70%) and 28 (61%) of those with therapeutic TGNs, sub-therapeutic TGNs and no CIM, respectively ($p = 0.011$) (Fig 3).

In univariate analysis CIM use at induction and serum albumin were significantly associated with response at week 12 (Table 2). On multivariate analysis, therapeutic TGN levels (OR 4.32, 95% CI: 1.41-13.29, p = 0.01) and albumin level (OR 1.09, 95% CI: 1.01-1.18, p = 0.03) were independent predictors of response to induction. (Table 2).

Semester analysis:

Of 280 semesters, every other week dosing was observed in 200 (72%) and weekly in 80 (29%). A similar proportion of CIM and non-CIM semesters were observed in each dosing regimen (every other week 74 vs weekly 68%, p = 0.31). More CIM semesters were classified as remission compared to non-CIM semesters (81 vs 60%, p < 0.0001, Fig 3). Considering CIM semesters, patients with therapeutic TGNs were more likely to be in remission compared to those with sub-therapeutic TGNs (86 vs 58%, p = 0.004) (Fig 4.)

In univariate analysis, ileal location (p = 0.001), extra-intestinal manifestations of disease (p = 0.03, and semesters with therapeutic TGNs (p < 0.0001) were associated with remission (Table 3). These covariates remained significant after multivariate analysis (ileal disease location: OR 0.21, 95%CI: 0.08-0.57, p = 0.002, therapeutic TGN: OR 3.71 95% CI: 1.87-7.34, p < 0.0001).

Factors associated with ADA failure

35/123 (29%) ceased ADA during the study; 5/35 withdrew due to sustained clinical remission. A further 2/35 prescribed ADA to down-stage inflammation pre-operatively were not continued post-operatively. Hence, 28 patients were subsequently analysed with regards to ADA failure. Mean time to failure was 58 months (95% CI: 50.5–66.3). CIM ≥ 3 months prior to ADA was associated with longer time to failure compared to those not treated with CIM (68.5 vs 35.7 months, p = 0.009 $_{\text{log rank}}$) (Fig 5.)

On univariate analysis, male gender (p = 0.033) and therapeutic TGN (p = 0.03) were associated with time to failure (Table 4). Therapeutic TGN ≥ 3 months prior to ADA (HR 0.37, 95%CI: 0.15–0.89, p = 0.026) and male gender (HR 0.39, 95% CI: 0.17–0.91, p = 0.028) were independently associated with time to failure on Cox regression analysis. Dose escalation did not predict subsequent ADA failure (p = 0.20). CIM ≥ 3 months prior to ADA was independently associated with time to failure (HR 0.37, 95% CI 0.17–0.81, p = 0.012).

Dose escalation and factors associated with dose escalation

ADA was escalated to weekly dosing in 34/123 (28%) patients. Mean time to dose escalation was 12.5 months (SD 8.7). All base line characteristics were considered for univariate analysis (Supplement 1). On multivariate analysis CIM ≥ 3 months prior to starting adalimumab was not associated with time to dose escalation (HR 0.55, 95%CI: 0.28-1.09, p = 0.088). Baseline CRP (HR 1.01, 95% CI 1.001–1.024, p = 0.035) and 5-ASA treatment at ADA initiation (HR 3.97, 95%CI 1.68–9.40, p = 0.002) were significant independent predictors associated with time to dose escalation on multivariate analysis.

Adverse events

Serious adverse events occurred in 5 patients during the study. 2 malignancies occurred; metastatic breast cancer after 19 months of combination treatment with thioguanine and ADA and transitional cell carcinoma of the bladder after 27 months of ADA monotherapy. A 25-year-old male treated with thioguanine and ADA developed primary EBV infection and recovered after discontinuing both drugs. Two patients developed intra-abdominal sepsis, 4 and 10 months into treatment; one was on ADA monotherapy, the other on combination therapy with azathioprine.

DISCUSSION

We have demonstrated that in patients with Crohn's disease starting ADA, combination therapy with an immunomodulator was associated with higher rates of clinical response after induction compared to ADA monotherapy, and observed lower rates of subsequent ADA failure. During maintenance, combination therapy was associated with a decrease in the proportion of flare semesters. We assessed the relationship of thiopurines stratified according to TGN levels, not previously reported in the literature, and found that sub-therapeutic TGNs at induction and during maintenance therapy were associated with worse clinical outcomes and an increased risk of ADA failure compared to patients with therapeutic TGNs.

The situation with regard to combination therapy in patients taking infliximab has been studied extensively. In a retrospective analysis of 584 semesters amongst 121 patients with IBD, Sokol et al found a significantly decreased incidence of flares (32 vs 19%), perianal complications (12 vs 4%), and mean CRP (11 vs 9%) in those treated with combination therapy compared with infliximab monotherapy.[30] Many of the patients in this cohort started infliximab upon failure of immunomodulator therapy and continued CIM after initiating infliximab, suggesting that there is a benefit of combination therapy in all patients starting IFX, not just those naïve to immunomodulators. This has also been supported by a recent meta-analyses of patient level data in the biologic registration trials.[31] In addition, combination therapy has been shown to improve rates of deep remission, (defined as clinical remission together with normalization of CRP and mucosal healing) compared to infliximab monotherapy in patients who were previously naïve to both drugs (65 vs 25%, $p = 0.037$).[16]

Although the benefits of combination therapy with infliximab appear robust, evidence to support the same benefit with ADA is relatively sparse. The same meta-analysis of randomized controlled trials demonstrating a benefit of combination therapy in induction of clinical remission at 6 months with IFX, found no such association for combination therapy with ADA (OR 0.88, 95%CI: 0.58–1.35).[31] Presented in abstract form, a recent prospective study randomizing treatment naïve patients with moderate-to-severe Crohn's disease to either ADA monotherapy, or combination therapy with a thiopurine found no difference in clinical remission at week 26 between the two treatment arms (72 vs

68%) although an improvement in endoscopic activity at week 26 and higher ADA trough levels were observed in those treated with combination therapy.[32]

Conversely, a recent meta-analysis amongst patients with CD found that ADA monotherapy was slightly inferior to combination therapy for induction of remission (OR 0.78, 95%CI: 0.64–0.96, p= 0.02) although no such benefit was seen for maintenance of clinical remission (OR 1.08, 95% CI: 0.79–1.48, p = 0.48) nor was combination therapy superior to monotherapy in terms of need for dose escalation (OR 1.13, 95%CI: 0.69–1.85, p = 0.62).[33]

Our study builds on previously published open data. A retrospective study from two large centres described 207 patients with Crohn's disease and found that CIM maintained for 3 months or more within 6 months of initiating ADA was associated with a lower risk of ADA failure and fewer flare semesters during maintenance.[14] CIM was not, however, associated with improved rates of response to induction therapy, nor was ongoing CIM associated with fewer semesters of flare nor with a lower risk of ADA failure. Semesters in which ADA was dosed weekly, rather than every other week, were classified as flares, even if the patient remained well during the semester, which may have influenced these results. It is recognised that secondary loss of response occurs in a significant proportion of patients during ADA maintenance and that dose escalation can recapture response in many.[6] It is possible to argue that patients who regain response on dose escalation and remain well on weekly dosing are, therefore, not treatment failures but, rather, represent a subgroup of patients who require higher dosing to achieve therapeutic drug levels to maintain remission.[34] In the current study, therefore, a semester requiring dose escalation was classified as a flare; subsequent semesters were classified according to clinical status and were not automatically recorded as flare semesters based on the need for weekly dosing. Interestingly, dose escalation was not associated with time to failure, supporting our study design.

For the first time we report the association between adequate dosing of thiopurines (TGN > 235 pmol/8x10^8RBC) and clinical response. We found significantly higher response rates in patients with therapeutic compared with sub-therapeutic TGNs at both induction (88 vs 70%) and during maintenance

(85 vs 58%). In this regard, data are beginning to emerge demonstrating that the intensity of concomitant immunomodulation influences the pharmacokinetics of anti-TNF therapy and subsequent clinical outcomes. A Dutch group found that MTX reduced immunogenicity to IFX in a dose-dependent manner, with the odds of developing anti-drug antibodies being 0.36 (95% CI: 0.18–0.74) in the 5-10mg/week, 0.22 (95% CI: 0.10–0.46) in the 12.5-20mg/week and 0.14 (95% CI 0.07–0.28) in patients on >22.5mg/week) relative to patients not treated with MTX.[35] In addition, in a post-hoc analysis of the SONIC study, patients on combination therapy with azathioprine with an increase of 7 femtoliters in the mean corpuscular volume (delta MCV), used as a surrogate marker for therapeutic TGN levels, were more likely to achieve mucosal healing (75 vs 47% for delta MCV >7, p = 0.017) and maintain therapeutic trough infliximab levels > 3 μg/mL at week 30 (68 vs 39% for delta MCV >7, p = 0.003).[36] Similarly, in a cross-sectional analysis of 72 patients with inflammatory bowel disease, IFX drug levels were higher amongst those on combination therapy with a thiopurine compared with IFX monotherapy (13 vs 4.8 μg/mL,) and a TGN cut-off of 125 pmol/8x10^8RBC best predicted a significantly higher IFX trough level.[15] Taken together with the findings that higher anti-TNF drug levels are associated with improved rates of remission[37,38] these findings suggest that the dose of thiopurine may be of significant importance.

The utility of measuring TGN in patients taking thiopurines as combination therapy is perhaps even greater when one considers rates of non-adherence and the impact of hypermethylation. Adherence to thiopurines is a well-recognised problem in inflammatory bowel disease.[39] Similarly, under dosing with thiopurines has been reported in 29% when weight based dosing is employed.[24] Thiopurine hypermethylation, whereby shunting occurs away from the therapeutic TGNs towards a methylated metabolite profile, is seen in 15-20% and is associated with an inability to achieve therapeutic TGN.[40] Without thiopurine metabolite testing a large proportion of patients will fail to achieve a therapeutic TGN; the structured approach to optimisation of thiopurines in our cohort may explain why a greater benefit of CIM was observed compared with other cohorts.

The development of antibodies against ADA has been implicated as a mechanism leading to secondary

loss of response and treatment failure.[41,42] Combination therapy can improve the pharmacokinetics of infliximab by increasing drug levels[43] and by decreasing anti-infliximab antibody production (RR: 0.50, 95% CI: 0.42 – 0.59, p < 0.00001).[43] There is convincing evidence that CIM can prevent immunogenicity in Crohn's disease[8]. In a retrospective analysis of 536 samples collected from 148 patients analysed using a drug tolerant homogenous mobility shift assay, antibodies to ADA were detected in 20% after a median of 34 weeks[8]. CIM was associated with decreased antibody formation (HR: 0.23, 95% CI: 0.06–0.86) and antibodies were associated with future elevated CRP (p = 0.0013) and discontinuation of ADA due to loss of response (OR 3.04, 95%CI: 1.039–9.093). Such immunogenicity occurs early in the course of ADA therapy. A prospective observational cohort study of 272 patients treated with ADA for rheumatoid arthritis found antibodies to ADA in 28% over a 3 year follow-up; in 67% antibodies occurred within the first 28 weeks of therapy.[44] Similarly, antibodies to IFX have also been found to occur early. In a prospective observational study of 125 patients with IBD, anti-drug antibodies occurred in 46% at a median time of 1.5 months (IQR 0.5–5.5); 90% developed within 12 months and anti-drug antibody free survival was longer in patients taking combination therapy compared with IFX monotherapy (p = 0.003).[45] These findings suggest that early concomitant immunomodulation, perhaps even prior to starting anti-TNF therapy is important, as has previously been shown in murine models.[46] Thiopurines have a slow onset of action, with a mean time to response of 3.1 months.[21] Therefore, it is possible that some of the beneficial effects of combination therapy may be greater in those patients who are established on therapy prior to starting ADA.

Given the findings from our study (and some others) that early combination therapy is beneficial, and that immunogenicity occurs largely in the first 12 months of ADA therapy, a key question is whether combination therapy should be continued during maintenance. Such a decision must weigh up the benefits and risks of continued combination therapy against withdrawal to ADA monotherapy. In this regard we demonstrated higher rates of remission semesters in those treated with CIM vs ADA monotherapy (81 vs 60%) and in those with therapeutic compared with non-therapeutic TGNs (85 vs 58%). Further, CIM use during a semester was an independent predictor of remission (OR 2.92, 95% CI: 1.62–5.25, p < 0.0001). Our results are in agreement with those from the Oxford/Liege cohort,

where combination therapy beyond 6 months was associated with fewer semesters with flares (14 vs 36%, p = 0.02, OR = 0.31).[14] Recent studies have called into question the benefit of continued concomitant immunomodulation during maintenance therapy, suggesting that a lower dose of thiopurine may be equally efficacious as full weight-based dosing.[47] We were unable to explore this association in the current study as only a small proportion of patients (3/65) were found to have TGNs <125. The benefits of combination therapy must, of course, be balanced with the risks particularly in light of recent safety signals regarding the use of thiopurines.[48,49]

We acknowledge several limitations with the study. First patients were not randomized to combination therapy or ADA monotherapy, hence despite the groups being similarly matched in terms of phenotype, previous anti-TNF exposure and disease severity they are not directly comparable. As we did not measure ADA drug levels or antibodies to ADA we cannot prove that the benefit seen with CIM was due to improvements in ADA pharmacokinetics and reductions in immunogenicity. Third, assessment of response to induction was made using a combination of Harvey-Bradshaw Index, CRP and faecal calprotectin. Fourth, a relatively high number of patients were treated with corticosteroids during induction (53%) which may contribute to the relatively high response rate seen overall (75%), although there was no difference in corticosteroid use in patients treated with combination or monotherapy. Finally, a relatively small proportion of patients had sub-therapeutic TGNs during induction (15%) and maintenance (12%), hence the conclusion that response rates are superior with therapeutic compared with sub-therapeutic TGNs should be interpreted with caution until it has been confirmed in other cohorts.

Conclusion

Combination therapy was found to be superior to ADA monotherapy in this cohort of patients with moderate-to-severe Crohn's disease with improved response at induction, more semesters in remission and a longer time to ADA failure. Further, adequately dosed thiopurines when used as concomitant immunomodulation was associated with improved clinical outcomes. We propose that, after carefully

balancing the risk and benefit and noting the association of increased risks of lymphoma, non-melanoma skin cancer and possibly other malignancies,[48] immunomodulators should be initiated early when considering ADA therapy, dosed to a TGN > 235, and continued during maintenance therapy. Further randomized controlled studies are needed that incorporate thiopurine metabolite testing during both induction and maintenance.

Competing interests: VCK has received lecture fees from Janssen, Ferring, Shire and Takeda and financial support from Ferring, Abbvie for service development. MGW has served as a speaker for Janssen, AbbVie, Ferring and Takeda and has received research funding from AbbVie. PAB, KVP, RG and JDS have no disclosures. PMI has received lecture fees from Abbvie, Warner Chilcott, Ferring, Falk Pharma, Takeda, MSD, Johnson and Johnson, Shire and financial support for research from MSD and Takeda and advisory fees from Abbvie, Warner Chilcott, Takeda, MSD, Vifor Pharma, Pharmacosmos, Topivert, Genentech, Hospira and Samsung Bioepis.

Funding: The design, data analysis and manuscript writing was performed independently by the authors without external funding.

Contributors: VCK and MGW contributed equally to study design, data acquisition, analysis, and wrote and revised the manuscript. PAB, KVP and RG contributed to data acquisition and manuscript revision. PMI and JDS contributed to study design, manuscript revision and intellectual content. All authors approved the final version of the manuscript.

REFERENCES

1. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology 2006;130:323–33– quiz 591.

2. Rutgeerts P, Van Assche G, Sandborn WJ, et al. Adalimumab Induces and Maintains Mucosal Healing in Patients With Crohn's Disease: Data From the EXTEND Trial. Gastroenterology 2012;142:1102–1111.e2.

3. Colombel J-F, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology 2007;132:52–65.

4. Sandborn W, Rutgeerts P, Enns R, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab. Ann Intern Med 2007;146:829.

5. Panaccione R, Colombel J-F, Sandborn WJ, et al. Adalimumab maintains remission of Crohn's disease after up to 4 years of treatment: data from CHARM and ADHERE. Aliment Pharmacol Ther 2013;38:1236–1247.

6. Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of Response and Need for Adalimumab Dose Intensification in Crohn's Disease: A Systematic Review. Am J Gastroenterol 2011;106:674–684.

7. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33:987–995.

8. Baert F, Kondragunta V, Lockton S, et al. Antibodies to adalimumab are associated with future inflammation in Crohn's patients receiving maintenance adalimumab therapy: a post hoc analysis of the Karmiris trial. Gut 2016;65:1126–1131.

9. Ternant D, Karmiris K, Vermeire S, et al. Pharmacokinetics of adalimumab in Crohn's disease. Eur J Clin Pharmacol 2015;71:1155–1157.

10. Baert F, Glorieus E, Reenaers C, et al. Adalimumab dose escalation and dose de-escalation success rate and predictors in a large national cohort of Crohn's patients. Journal of Crohn's and Colitis 2013;7:154–160.

11. Colombel J, Sandborn W, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. New England Journal of Medicine 2010;362:1383.

12. Christophorou D, Funakoshi N, Duny Y, et al. Systematic review with meta-analysis: infliximab and immunosuppressant therapy vs. infliximab alone for active ulcerative colitis. Aliment Pharmacol Ther 2015;41:603–612.

13. Panaccione R, Ghosh S, Middleton S, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. Gastroenterology 2014;146:392–400.e3.

14. Reenaers C, Louis E, Belaiche J, et al. Does co-treatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? Aliment Pharmacol Ther 2012;36:1040–1048.

15. Yarur AJ, Kubiliun MJ, Czul F, et al. Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. Clin. Gastroenterol. Hepatol. 2015;13:1118–1124.e3.

16. Colombel J-F, Reinisch W, Mantzaris GJ, et al. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC post hoc analysis. Aliment Pharmacol Ther 2015;41:734–746.

17. Dignass A, Van Assche G, Lindsay JO, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. In: Vol 4.

2010:28–62.

18. Duncan J, Caulfield S, Clark A. A multidisciplinary virtual biologics clinic: is it worthwhile? Gut 2010;59:A152.

19. Feagan BG, Rochon J, Fedorak RN, et al. Methotrexate for the treatment of Crohn's disease. N Engl J Med 1995;332:292–297.

20. Summers RW, Switz DM, Sessions Jr JT, Becktel JM, Best WR, Kern Jr F, Singleton JW. National Cooperative Crohn's Disease Study: results of drug treatment. Gastroenterology. 1979 Oct 1;77(4 Pt 2):847-69.

21. Present DH, Korelitz BI, Wisch N, et al. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. New England Journal of Medicine 1980;302:981–987.

22. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology 2000;118:705–713.

23. Kaskas BA, Louis E, Hindorf U, et al. Safe treatment of thiopurine S-methyltransferase deficient Crohn's disease patients with azathioprine. Gut 2003;52:140–142.

24. Haines ML, Ajlouni Y, Irving PM, et al. Clinical usefulness of therapeutic drug monitoring of thiopurines in patients with inadequately controlled inflammatory bowel disease. Inflamm Bowel Dis 2011;17:1301–1307.

25. Smith M, Blaker P, Patel C, et al. The impact of introducing thioguanine nucleotide monitoring into an inflammatory bowel disease clinic. Int. J. Clin. Pract. 2013;67:161–169.

26. Smith MA, Blaker P, Marinaki AM, et al. Optimising outcome on thiopurines in inflammatory bowel disease by co-prescription of allopurinol. Journal of Crohn's and Colitis 2012;6:905–

912.

27. Harvey RF, Bradshaw JM. A simple index of Crohn's-Disease Activity. The Lancet 1980;315:514.

28. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005;19:5–36.

29. Health Research Authority. Defining research. 2013:1–4. Available at: http://www.hra.nhs.uk/documents/2013/09/defining-research.pdf. Accessed - Nov 2015. 2013; 1–4.

30. Sokol H, Seksik P, Carrat F, et al. Usefulness of co-treatment with immunomodulators in patients with inflammatory bowel disease treated with scheduled infliximab maintenance therapy. Gut 2010;59:1363–1368.

31. Jones JL, Kaplan GG, Peyrin-Biroulet L, et al. Effects of Concomitant Immunomodulator Therapy on Efficacy and Safety of Anti–Tumor Necrosis Factor Therapy for Crohn's Disease: A Meta-analysis of Placebo-controlled Trials. Clinical Gastroenterology and Hepatology 2015:1–10.

32. Matsumoto T, Motoya S, Watanabe K. OP011. Comparison of adalimumab monotherapy and a combination with azathioprine for patients with Crohn's disease: a prospective, multicentre, open-labelled clinical trial (DIAMOND study). Journal of Crohn's and Colitis 2016;10:S8–S8.

33. Kopylov U, Al-Taweel T, Yaghoobi M, et al. Adalimumab monotherapy versus combination therapy with immunomodulators in patients with Crohn's disease: A systematic review and meta-analysis. Journal of Crohn's and Colitis 2014;8:1632–1641.

34. Sandborn WJ, Colombel J-F, D'haens G, et al. Association of baseline C-reactive protein and prior anti-tumor necrosis factor therapy with need for weekly dosing during maintenance

therapy with adalimumab in patients with moderate to severe Crohn's disease. Curr Med Res Opin 2013;29:483–493.

35. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. Ann Rheum Dis 2012;71:1914–1915.

36. Bouguen G, Sninsky C, Tang KL, et al. Change in Erythrocyte Mean Corpuscular Volume During Combination Therapy with Azathioprine and Infliximab Is Associated with Mucosal Healing. Inflamm Bowel Dis 2015;21:606–614.

37. Paul S, Moreau AC, Del Tedesco E, et al. Pharmacokinetics of Adalimumab in Inflammatory Bowel Diseases. Inflamm Bowel Dis 2014;20:1288–1295.

38. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. Journal of Crohn's and Colitis 2016;10:619–625.

39. Goodhand JR, Kamperidis N, Sirwan B, et al. Factors associated with thiopurine non-adherence in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2013;38:1097–1108.

40. Blaker PA, Arenas-Hernandez M, SMITH MA, et al. Mechanism of allopurinol induced TPMT inhibition. Biochem. Pharmacol. 2013;86:539–547.

41. Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology 2009;137:1628–1640.

42. West RL, Zelinkova Z, Wolbink GJ, et al. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Aliment Pharmacol Ther 2008;28:1122–1126.

43. Lee LYW, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. Eur J Gastroenterol Hepatol 2012;24:1078–1085.

44. Bartelds GM, Krieckaert CLM, Nurmohamed MT, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA 2011;305:1460–1468.

45. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut 2014;63:1258–1264.

46. Garman RD, Munroe K, Richards SM. Methotrexate reduces antibody responses to recombinant human alpha-galactosidase A therapy in a mouse model of Fabry disease. Clin Exp Immunol 2004;137:496–502.

47. Del Tedesco E, Paul S, Marotte H, et al. 693 Azathioprine Dose Reduction in Inflammatory Bowel Disease Patients on Combination Therapy: A Prospective Study. Gastroenterology 2016;150:S143–S144.

48. Louis E, Irving P, Beaugerie L. Use of azathioprine in IBD: modern aspects of an old drug. Gut 2014;63:1695–1699.

49. Dulai PS, Siegel CA, Colombel J-F, et al. Systematic review: monotherapy with antitumour necrosis factor α agents versus combination therapy with an immunosuppressive for IBD. 2014.

FIGURES

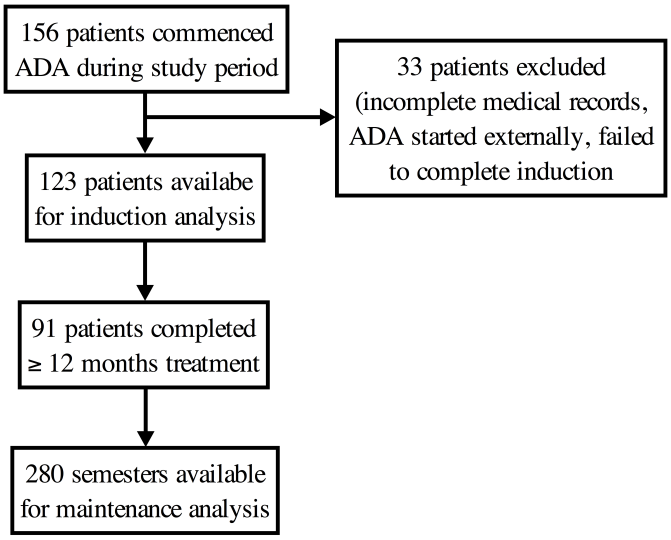

Figure 1.

Figure 1. Flow diagram of patient recruitment. ADA = adalimumab

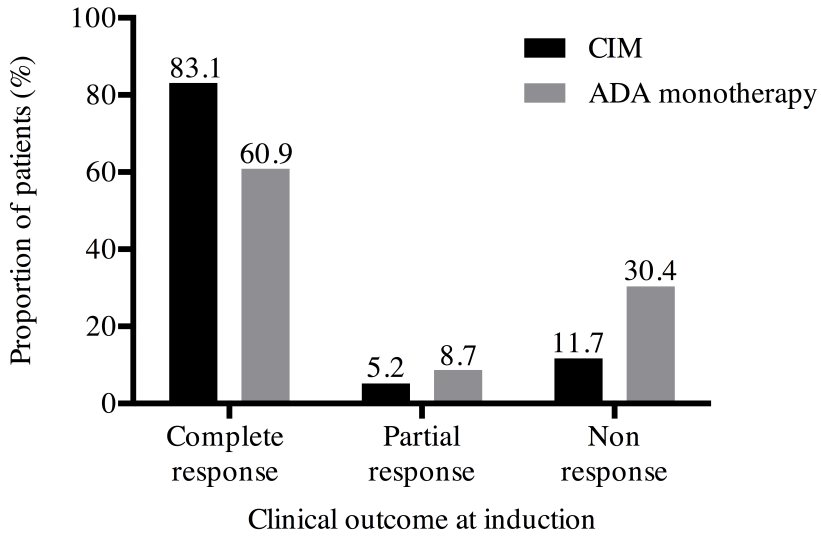

Figure 2.

Figure 2. Clinical response after induction comparing concomitant immunomodulation to adalimumab monotherapy. Complete response to induction was observed more frequently in patients treated with ADA and CIM compared to ADA monotherapy (83.1 vs 60.9%, p = 0.02) CIM = concomitant immunomodulation, ADA = adalimumab

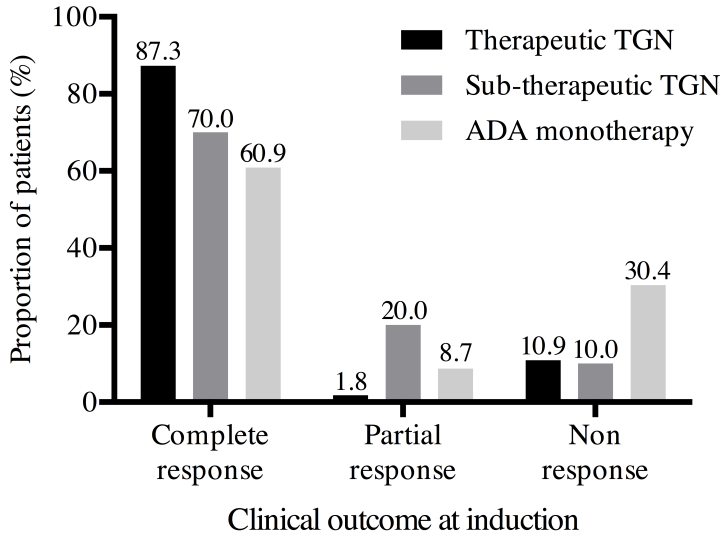

Figure 3.

Figure 3. Clinical response after induction stratified by TGN and ADA monotherapy. Complete response was observed more frequently in patients with therapeutic TGN vs sub-therapeutic TGN vs ADA monotherapy (87.3 vs 70.0 vs 60.9%, p = 0.011). TGN = thioguanine nucleotide, ADA = adalimumab monotherapy

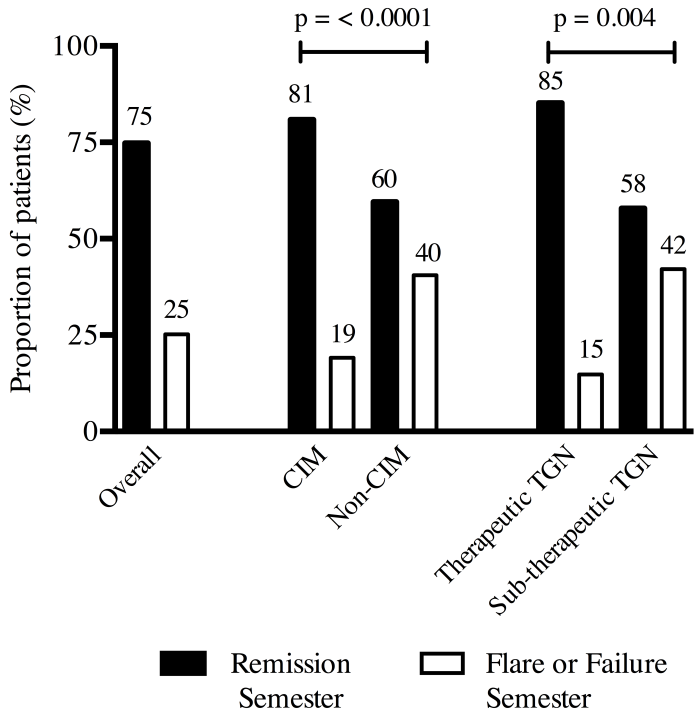

Figure 4.

Figure 4. Association between semester outcomes overall, and according to CIM and TGN status.

CIM = concomitant immunomodulation, TGN = thioguanine nucleotide level

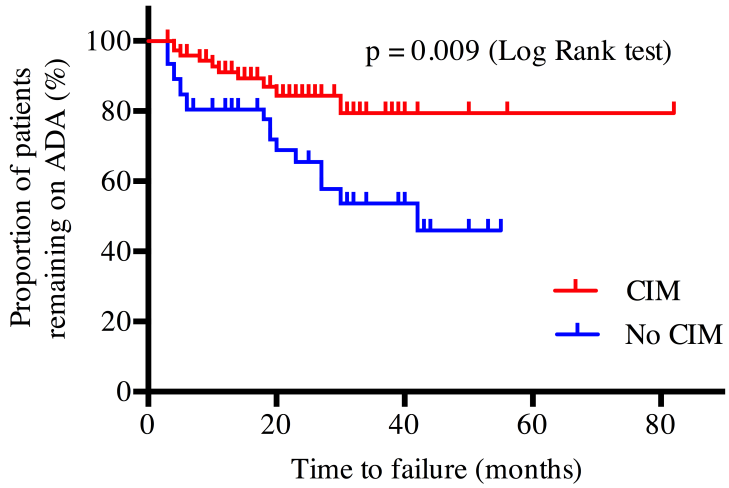

Figure 5.

Figure 5. Time to adalimumab failure. Kaplan-Meier analysis illustrating time to ADA failure (months) in patients treated (n = 77) and not treated (n = 46) with CIM for ≥ 3 months prior to commencing ADA (and continued for first 6 months). CIM = concomitant immunomodulation, ADA = adalimumab

Table 1. Baseline characteristics at adalimumab (ADA) initiation (n = 123)			
Characteristic	**CIM (n = 77)**	**No CIM (n = 46)**	**p value**
Male, number (%)	40 (51.9)	25 (54.3)	0.79
Age at diagnosis, median (IQR)	21 (17-28)	22 (18-29)	0.32
Disease duration years, median (IQR)	11 (4.5-16)	9 (3.5-17.2)	0.46
Location L1:L2:L3 (%)	15.6; 19.5; 64.9	10.9; 28.3; 60.9	0.46
Upper GI involvement (%)	16.9	19.6	0.71
Behavior B1:B2:B3 (%)	36.4; 44.2; 19.4	37.0; 43.5; 19.5	0.99
Perianal disease (%)	31.2	41.3	0.25
EIM (%)	19.7	21.7	0.79
Weight kg, median (IQR)	66.0 (54.4-78.9)	69.5 (59.5-83.5)	0.35
Current smoker (%)	10.6	22.0	0.28
Family history (no:1st deg:other) (%)	90.3; 6.5; 3.2	84.6; 12.8; 2.6	0.54
Prior surgery, number (%)	41(53.2)	18 (39.1)	0.13
Perianal surgery, number (%)	13 (16.9)	7 (15.2)	0.81
Steroids at ADA induction (%)	19.5	41.3	0.01
5-ASA (%)	6.5	17.4	0.06
Prior anti-TNF exposure (%)	55.8	58.7	0.76
IFX/ADA/both (%)	50.0; 2.6; 3.9	45.7; 2.2; 10.9	0.52
CRP mg/L mean (SD)	20.7 (28.5)	25.1 (30.3)	0.49
Albumin (g/L) mean (SD)	42.3 (6.6)	42.0 (4.1)	0.20
HBI, mean (SD)	7.1 (4.4)	9.0 (4.5)	0.05

CIM: concomitant immunomodulation, ADA: adalimumab, EIM: extra-intestinal manifestation, CRP: c-reactive protein, HBI: Harvey-Bradshaw Index, SD: standard deviation, IQR: interquartile range

Table 2: Univariate and multivariate predictors of response at week 12				
Covariant	**Univariate**		**Multivariate**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Gender	0.51 (0.20-1.28)	0.15		
Age at diagnosis	1.02 (0.97-1.08)	0.46		
Disease duration at start of ADA	1.00 (0.95-1.05)	0.99		
Montreal location (reference L3)				
L1	0.65 (0.18-2.31)	0.51		
L2	0.73 (0.25-2.16)	0.57		
Montreal location L4	1.04 (0.32-3.43)	0.94		
Montreal behaviour (reference B3)				
B1	1.22 (0.35-4.23)	0.76		
B2	1.16 (0.35-3.85)	0.81		
EIM	1.91 (0.52-7.00)	0.33		
Weight (kg)	1.02 (0.98-1.05)	0.31		
Current smoker	0.83 (0.45-1.54)	0.55		
Family history of IBD	1.39 (0.36-5.33)	0.63		
Prior bowel resection	1.01 (0.41-2.49)	0.98		
Exposure to anti-TNF	1.02 (0.41-2.54)	0.97		
Steroids at induction	0.66 (0.25-1.73)	0.39		
5-ASA at induction	0.74 (0.19-2.94)	0.67		
CIM (reference no CIM)				
Sub-therapeutic TGN	3.94 (0.45-34.12)	0.21	3.36 (0.38-29.79)	0.28
Therapeutic TGN	3.57 (1.24-10.26)	0.18	4.32 (1.41-13.29)	0.01
CRP at induction	0.99 (0.98-1.00)	0.09	Removed	0.35

Albumin at induction	1.08 (1.01-1.17)	0.03	1.09 (1.01-1.18)	0.03
HBI at induction	0.95 (0.86-1.05)	0.95		

ADA = adalimumab, EIM = extra-intestinal manifestation, IBD = inflammatory bowel disease, CIM = concomitant immunomodulation, TGN = thioguanine nucleotide, CRP = c-reactive protein, HBI = Harvey-Bradshaw Index

Table 3: Univariate and multivariate predictors of remission semesters				
Covariant	**Univariate**		**Multivariate**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Gender	1.69 (0.97-2.95)	0.06	1.77 (0.91-3.44)	0.09
Age at diagnosis	0.99 (0.97-1.02)	0.72		
Disease duration at start of ADA	1.03 (0.99-1.07)	0.09	Removed	0.26
Montreal location (reference L3)				
L1	0.25 (0.11-0.56)	0.001	0.21 (0.08-0.57)	0.002
L2	0.57 (0.29-1.09)	0.09	0.50 (0.24-1.04)	0.064
Montreal location L4	0.83 (0.43-1.59)	0.57		
Montreal behavior (reference B3)				
B1	0.54 (0.23-1.31)	0.17	Removed	0.49
B2	0.46 (0.20-1.07)	0.07		0.25
EIM	2.08 (1.07-4.07)	0.03	Removed	0.11
Weight (kg)	1.00 (0.98-1.02)	0.87		
Current smoker	0.93 (0.63-1.36)	0.69		
Family history of IBD	0.97 (0.53-1.93)	0.97		
Prior bowel resection	0.91 (0.53-1.56)	0.73		
Prior perianal surgery	0.47 (0.24-0.91)	0.25	Removed	0.99
Exposure to anti-TNF	0.79 (0.44-1.42)	0.44		
Steroids at induction	0.76 (0.43-1.33)	0.33		
5-ASA at induction	0.50 (0.24-1.03)	0.06	Removed	0.15
CIM induction (reference no CIM)				

Sub therapeutic TGN *Therapeutic TGN*	0.71 (0.24-2.05) 1.58 (0.83-3.02)	0.52 0.17		
Semester CIM (reference no CIM) *Sub therapeutic TGN* *Therapeutic TGN*	 0.94 (0.34-2.58) 3.91 (2.04-7.53)	 0.90 <0.0001	 1.11 (0.37-3.26) 3.71 (1.87-7.34)	 0.86 <0.0001
CRP at induction	0.56 (0.97-1.01)	0.56		
Albumin at induction	1.01 (0.86-1.06)	0.81		
ADA = adalimumab, EIM = extra-intestinal manifestation, IBD = inflammatory bowel disease, CIM = concomitant immunomodulation, TGN = thioguanine nucleotide, CRP = c-reactive protein, HBI = Harvey-Bradshaw Index				

Table 4: Univariate and multivariate predictors of time to ADA failure				
Covariant	**Univariate**		**Multivariate**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Gender (reference female)	0.42 (0.19-0.93)	0.03	0.39 (0.17-0.91)	0.028
Age at diagnosis	0.99 (0.96-1.04)	0.94		
Disease duration at start of ADA	1.01 (0.97-1.06)	0.60		
Montreal location (reference L3) *L1* *L2*	 1.74 (0.63-4.80) 1.34 (0.55-3.26)	 0.28 0.51		
Montreal location L4 *Montreal behavior (reference B3)* *B1* *B2*	0.82 (0.45-2.75) 0.49 (0.17-1.39) 0.84 (0.34-2.09)	0.82 0.18 0.71		
Perianal disease	1.15 (0.54-2.47)	0.72		
EIM	0.40 (0.12-1.32)	0.13		
Weight (kg)	0.98 (0.95-1.01)	0.18		
Current smoker	1.07 (0.64-1.78)	0.80		
Family history of IBD	0.32 (0.52-2.02)	0.23		
Prior bowel resection	0.73 (0.34-1.55)	0.41		
Prior perianal surgery	1.10 (0.42-2.89)	0.85		
Exposure to anti-TNF	1.19 (0.54-2.60)	0.67		
Steroids at induction	1.61 (0.76-3.42)	0.21		
5-ASA at induction	1.81 (0.73-4.48)	0.20		

CIM induction (reference no CIM)				
Sub-therapeutic TGN	0.31 (0.04-2.38)	0.52	0.42 (0.04-2.38)	0.263
Therapeutic TGN	0.38 (0.16-0.91)	0.03	0.37 (0.15-0.89)	0.026
CRP at induction	1.01 (0.99-1.02)	0.37		
Albumin at induction	0.98 (0.92-1.03)	0.40		
HBI at induction	1.00 (0.91-1.10)	0.99		
ADA dose escalation	0.46 (0.19-1.11)	0.08	Removed	0.203

ADA = adalimumab, EIM = extra-intestinal manifestation, IBD = inflammatory bowel disease, CIM = concomitant immunomodulation, TGN = thioguanine nucleotide, CRP = c-reactive protein, HBI = Harvey-Bradshaw Index

Supplement 1: Univariate and multivariate predictors of ADA dose escalation

Covariant	**Univariate**		**Multivariate**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Gender (reference female)	1.18 (0.60-2.33)	0.63		
Age at diagnosis	1.01 (0.98-1.05)	0.57		
Disease duration at start of ADA	0.98 (0.94-1.02)	0.38		
Montreal location (reference L3)				
L1	1.41 (0.52-3.81)	0.50		
L2	1.64 (0.74-3.62)	0.22		
Montreal location L4	0.84 (0.35-2.04)	0.70		
Montreal behavior (reference B3)				
B1	1.20 (0.46-3.14)	0.71		
B2	0.97 (0.37-2.53)	0.94		
Perianal disease	1.04 (0.50-2.15)	0.92		
EIM	0.95 (0.43-2.12)	0.91		
Weight (kg)	0.99 (0.97-1.02)	0.89		
Weight at dose escalation (Kg)	1.01 (0.98-1.03)	0.64		
Current smoker	0.95 (0.58-1.57)	0.85		
Family history of IBD	1.31 (0.59-2.87)	0.51		
Prior bowel resection	0.84 (0.43-1.66)	0.62		
Prior perianal surgery	1.07 (0.44-2.63)	0.88		
Exposure to anti-TNF	1.37 (0.68-2.78)	0.38		
Steroids at induction	1.72 (0.86-3.45)	0.12		
5-ASA at induction	2.54 (1.15-5.65)	0.02	3.98 (1.68-9.40)	0.002
CIM induction (reference no CIM)				
Sub-therapeutic TGN	0.23 (0.03-1.71)	0.15	Removed	0.30
Therapeutic TGN	0.51 (0.23-1.09)	0.08		
CRP at induction	1.01 (1.00-1.02)	0.03	1.01(1.001-1.02)	0.035
Albumin at induction	0.98 (0.93-1.03)	0.33		
HBI at induction	1.05 (0.98-1.12)	0.20		
ADA dose escalation	0.46 (0.19-1.11)	0.08		

CHAPTER 4:

When other thiopurines have failed: Thioguanine in inflammatory bowel disease: Long-term efficacy and safety

UNITED EUROPEAN GASTROENTEROLOGY ueg journal

Original Article

United European Gastroenterology Journal
0(0) 1–8

DOI: 10.1177/2050640616663438
ueg.sagepub.com
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Thioguanine in inflammatory bowel disease: Long-term efficacy and safety

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Abstract

Background: Thioguanine (TG) is efficacious in inflammatory bowel disease (IBD), but its toxicity, particularly nodular regenerative hyperplasia (NRH) of the liver, has limited its use. We assessed the long-term clinical outcomes and safety of TG in patients whom were intolerant or refractory to conventional immunomodulators.

Methods: This is a retrospective, single-centre study of IBD patients treated with TG from 2001–2013. Response was defined as clinical remission (Harvey–Bradshaw Index < 5 for Crohn's disease (CD), Simple Clinical Colitis Activity Index < 4 for ulcerative colitis (UC)) without corticosteroids or, if receiving anti-tumour-necrosis-factor (anti-TNF) therapy, absence of dose escalation. We recorded TG failure, withdrawal and adverse events. Patients were monitored with biochemistry, liver biopsy and/or magnetic resonance imaging (MRI).

Results: 54 patients (47 CD and 7 UC) whom received TG (mean dose: 27 mg/d (range: 20–40 mg/d)) as monotherapy ($n = 36$) or concomitantly with anti-TNF ($n = 18$) for a median inter-quartile range of 16 (5–37) months (126 patient-years of follow-up). 32 (59%) patients responded to TG at 6 months and 23 (43%) at 12 months. Pancreatitis did not recur amongst the 19 patients with prior thiopurine-induced pancreatitis. 16 (30%) patients ceased TG due to intolerance or toxicity (four serious); NRH was not observed. 6-thioguanine nucleotide concentrations did not correlate with efficacy nor with toxicity.

Conclusions: TG was efficacious and well tolerated in one out of two patients who had previously failed conventional immunomodulators. NRH did not occur.

Keywords

Crohn's disease, thioguanine, thiopurine, toxicity, ulcerative colitis

Received: 25 February 2016; accepted: 17 July 2016

Introduction

Immunomodulation remains the first-line therapy in inflammatory bowel disease (IBD). The conventional thiopurines, azathioprine (AZA) and 6-mercaptopurine (MP), are efficacious in maintaining steroid-free remission in IBD.[1] A substantial proportion of patients with Crohn's disease (CD) require treatment with a thiopurine; however, approximately 20–30% of these patients discontinue due to intolerance.[2] A further 30–40% withdraw treatment due to non-response, in part because an effective therapeutic dose measured by 6-thioguanine nucleotides (TGNs) cannot be achieved.[3] Pharmacogenetic differences in thiopurine metabolism contribute to intolerance and non-response.[4]

After ingestion, AZA is converted to MP, which then undergoes metabolism via the purine salvage pathway, to pharmacologically-active TGN. Concurrently, competitive metabolism by reduction to thiouric acid (via xanthine oxidase) and methylation to

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methyl-mercaptopurine (MMP) via thiopurine-*S*-methyltransferase (TPMT) determines the ultimate level of TGN. Despite targeted weight-adjusted dosing regimens, wide individual variation in the production of TGN and MMP has been observed, reflecting in part individual differences in the relative activities of the enzymes involved.[5] For many, intolerance or non-response to AZA/MP can be circumvented for a favourable clinical outcome.[4] However, in a proportion, intolerance is unavoidable and an alternative agent is needed. In particular, this applies to thiopurine-induced pancreatitis, where recurrence on re-challenge is likely.[6] Furthermore, certain patients, despite switching to MP or low-dose thiopurine and allopurinol, remain intolerant, due to nausea or flu-like symptoms.[4]

Thioguanine, (TG) a purine analogue of the nucleobase guanine, is a less frequently utilised non-conventional thiopurine. It is converted directly to TGN by hypoxanthine phosphoribosyltransferase (HPRT), circumventing numerous intermediate metabolites involved in the conventional thiopurine pathway.[7] Despite being a substrate for TPMT with similar enzyme kinetics to MP, the putative advantages of TG include avoiding potential toxicity and adverse effects, which likely occur from intermediate metabolites generated with treatment by conventional thiopurines.[7]

TG has been demonstrated in small, uncontrolled studies to be effective at inducing and maintaining clinical remission in CD and ulcerative colitis (UC).[8,9] Initial interest in TG was tempered by high rates of hepatotoxicity, particularly nodular regenerative hyperplasia (NRH), which was reported in 18–76% of recipients.[10,11] It has been suggested that TG-induced NRH may be dose-dependent,[12] with few cases arising on low-dose TG.[13] Splitting the dose of TG may also reduce the rate of NRH.[14]

Overall, the literature on TG in IBD remains sparse, and, despite the drug being a logical alternative in cases of thiopurine toxicity or refractoriness, the use of TG is limited by uncertainties over the risk of toxicity. TG has been in regular use at our institution for 15 years. Here we report our long-term efficacy and safety data regarding TG in IBD.

Methods

Patient population

We performed a single-centre retrospective study of all IBD patients treated with TG between 2001–2013 at Guy's and St. Thomas Hospitals, in London, UK. We previously reported the short-term outcomes of the first 30 patients.[15] Patients were identified by pharmacy dispensing records. Their diagnosis of IBD was based on standard criteria[1,16] and confirmed after review of the patients' medical records. Patients were included if they took a single dose of TG. Those with incomplete medical records were excluded.

Indications for TG were classified as active disease (induction of remission), as steroid-sparing in steroid dependency (>6 months corticosteroids or relapse on corticosteroid withdrawal), in order to maintain remission with episodic infliximab (IFX) or concomitant immunomodulation with scheduled maintenance IFX or adalimumab (ADA). Active disease was defined as a Harvey–Bradshaw index (HBI)[17] ≥ 5 (CD) or a Simple Clinical Colitis Activity Index (SCCAI) ≥ 4 (UC)[18] and was documented in the medical record at each clinical visit, as is our standard practice. Prior immunomodulator treatment and intolerance was documented. Where TG therapy was interrupted, only the first period of TG was reported.

Clinical response and failure

Clinical response was assessed at 6 and 12 months after commencing TG. Response was defined clinically (HBI < 5, SCCAI < 4). For steroid sparing, the response was only met if steroids were withdrawn within 6 months and maintained 12 months after starting TG. We performed a sub-group analysis of the patients treated with TG and a biologic, and their response was defined as not failing biologic therapy. In those patients treated episodically with a biologic, a response was defined as not requiring further doses. Failure was defined as TG withdrawal due to adverse effects, new corticosteroids, unplanned IBD-related surgery or biologic dose intensification or switching. Adherence was determined by pharmacy-initiated tablet counting and at clinical appointments. To represent real-world clinical practice, patients could have met criteria for failing TG, yet continued the drug, hence data was also collected pertaining to the timing and reason for subsequent TG cessation.

Monitoring for toxicity

After recognising the association between NRH and TG,[10] we advised patients (between 2001–2004) to undergo percutaneous liver biopsy after treatment for ≥ 12 months, performed under radiologic guidance using an 18G Trucut needle. Biopsies were stained with haematoxylin and eosin, reticulin silver, connective tissue trichrome stain and chromotrope aniline blue, and the slides were reviewed by an experienced histopathologist. After 2004, screening for NRH was recommended every 24 months with liver magnetic resonance imaging (MRI).

Upon starting TG, a full blood count and liver function tests (LFTs) were performed each fortnight for the first six weeks, and then every three months, unless

otherwise indicated. Toxicity was defined as LFTs 2 × upper limit of normal (ULN), thrombocytopenia ($<150 \times 10^9$) or leucopenia (white cell count (WCC) $< 3.5 \times 10^9$). Red blood cell (RBC) TGNs were collected every six months and analysed by ultra high-performance liquid chromatography (HPLC), based on a method described elsewhere.[19]

Statistics

Continuous variables were reported as the mean (SD) or the median inter-quartile range (IQR), and for categorical variables as the number (percent). We used Pearson's correlation for relationships between parameters. Comparisons between clinical outcomes and TGNs were performed using the Mann–Whitney U-test. The cumulative probability of failing or withdrawing TG was calculated using the Kaplan-Meier method. The significance threshold was 0.05. Statistics were performed using Prism 6.0 (San Diego, USA).

Ethical considerations

According to the guidelines of the UK Health Research Authority, as the data were collected as part of routine clinical care and were evaluated retrospectively, the study was considered a review of clinical practice and ethical approval was not required.[20] This study was conducted in accordance with the Declaration of Helsinki.[21]

Results

Study population

There were 58 patients treated with TG, but four were excluded due to incomplete medical records; thus, 54 patients were included. Their characteristics are summarised in Table 1. Median age at TG initiation was 35 years (IQR 27–49) and disease duration was 9.5 years (IQR 5–18). 47 out of 54 (87%) patients had CD and 7 out of 54 (13%) had UC. 19/54 (35.2%) had prior AZA-induced pancreatitis. All were intolerant of, or refractory to, thiopurines or methotrexate (MTX). The indications for TG were active disease (24/54 (44.4%)), steroid sparing (12/54 (22.2)), concomitant immunomodulation (9/54 (16.7%)) and to maintain remission with episodic anti-TNF therapy (9/54 (16.7%)).

Table 1. Patient demographics.

Patient characteristics	Frequency
Gender (male), *n* (%)	19 (35.2)
Age at starting thioguanine, years (IQR)	35 (27–49)
Disease duration, years (IQR)	9.5 (5–18)
Diagnosis CD: UC; *n* (%)	47 (87) : 7 (13)
CD Montreal Location L1:L2:L3, *n* (%)	5 (9.3) : 10 (18.5) : 32 (59.3)
CD Montreal upper modifier, *n* (%)	5 (9.3)
CD Montreal Behaviour B1:B2:B3, *n* (%)	17 (31.5) : 23 (42.6) : 7(13)
CD Montreal perianal modifier, *n* (%)	15 (27.8)
UC location E1:E2:E3, *n* (%)	0 (0) : 2 (3.7) : 5 (9.3)
Previous thiopurine induced pancreatitis, *n* (%)	19 (35.2)
Previous azathioprine intolerant/refractory *n* (%)	23 (42.6) / 12 (22.2)
Previous mercaptopurine intolerant/refractory *n* (%)	12 (22.2) / 2 (3.7)
Previous methotrexate intolerant/refractory *n* (%)	8 (14.8) / 28 (51.9)

CD: Crohn's disease; IQR: inter-quartile range; UC: ulcerative colitis.

Thioguanine dose

TG was started at 20 mg in 36 out of 54 patients and 40 mg in 18 out of 54 patients, and was continued as a single daily dose. The mean daily dose of TG was 27 mg (SD 8.4, range 20–40), equating to 0.44 mg/kg body weight (SD 0.19, range 0.2–0.9). The median cumulative dose of TG was 13 g (IQR 4.1–26). Considering the entire cohort, the median duration of treatment was 16 months (IQR 5–37). Of 34 out of 54 (63%) patients continuing TG for ≥12 months, the median duration was 26 months (range: 12–132).

Median TGNs during TG were 740 pmol per 8×10^8 RBC (IQR 445–1078). There was a trend towards a correlation between the dose and TGN ($r = 0.28$ and $p = 0.06$), but not between the normalised dose/body weight and TGN ($r = 0.026$, $p = 0.87$).

Response

Clinical response was observed in 32/54 patients (59.3%) and 23/54 patients (42.6%) at six and 12 months, respectively. Response by indication is shown in Table 2. Amongst CD patients with active disease, pre-treatment HBI did not predict response at six months (median HBI non-responders 11, versus responders 9.5, $p = 0.17$) or at 12 months (HBI non-responders 11 versus responders 8.5, $p = 0.1$). In responders with CD and active disease, median HBI decreased from 9 to 3 at six months ($p = 0.004$), and to 2 at 12 months ($p = 0.008$). There was no difference

Table 2. Response rate by indication.

Indication	Response at 6 months	Response at 12 months
All patients, *n* (%)	32/54 (59.3)	23/54 (42.6)
Active disease,[a] *n* (%)	15/24 (62.5)	9/24 (37.5)
Steroid sparing, *n* (%)	2/12 (16.7)	3/12 (25)
Concomitant immunomodulation,[a] *n* (%)	7/9 (77.8)	4/9 (44.4)
Avoid further episodic anti-TNF,[a] *n* (%)	8/9 (88.9)	7/9 (77.8)

[a]One patient with stoma for each indication.
TNF: Tumor necrosis factor.

Table 3. Causes of thioguanine failure.

Cause of failure	*n* (%)
Did not fail	14 (25.9)
New corticosteroids	7 (13.0)
Escalation to or intensification of biologic	10 (18.5)
Unplanned IBD-related surgery	7 (13.0)
Withdrawn due to adverse effect	14 (25.9)
Withdrawn due to biochemical abnormality	2 (3.7)

IBD: inflammatory bowel disease.

in TGN over the first 12 months between responders and non-responders (833 versus 938 pmol per 8×10^8 RBC, $p = 0.62$). Response rates at six and 12 months were lower amongst those treated with TG for active disease or as steroid sparing, compared with those where the indication was maintaining response with a biologic (47.2% versus 83.3% and 33.3% versus 61.1%, respectively).

Failure and withdrawal

25 out of 54 patients (46.3%) failed TG by 12 months, and 40 out of 54 patients (74.1%) had failed by the end of the study period. Amongst those failing TG, the median time to failure was 7.5 months (IQR 2–16). Causes of failure are shown (Table 3). Mean TGNs were not different between patients who were failing and not failing TG (743 versus 607 pmol per 8×10^8 RBC; $p = 0.74$). A Kaplan–Meier survival curve depicting the proportion of patients failing TG over the study period is shown. Within the entire cohort, the median time-to-failure was 15 months (Figure 1(a)).

There were 18 out of 54 patients (33.3%) who continued TG to the end of the study period (median 32 months, range 12–132. The causes of TG withdrawal amongst 36/54 patients (66.7%) are shown in Table 4. Amongst those who were intolerant to TG, four out of nine patients (44.4%) developed rash and five out of nine (55.6%), nausea or vomiting. Both patients with biochemical abnormalities leading to drug withdrawal developed persisting elevations in ALP and GGT, which normalised after stopping TG. TG was withdrawn in three patients due to infection (two were co-treated with ADA and one had TG monotherapy). Causes for discontinuation amongst the remaining seven out of 54 patients (13%) included: Withdrawal due to remission ($n = 1$), pregnancy or family planning ($n = 3$), enteropathic arthritis ($n = 1$), TG shortage ($n = 1$) and portal hypertensive syndrome ($n = 1$). Amongst the patients who ceased TG, the median time to withdrawal was 8.5 months (IQR 2–20). A Kaplan–Meier survival curve depicting the cumulative rate of remaining free of TG withdrawal is shown. Amongst all 54 patients, the median time to drug withdrawal was 19 months (Figure 1(b)).

Biochemical abnormalities

In two patients treated for two and five months, biochemistry was unavailable (performed externally). 18 out of 52 patients (34.6%) returned abnormal blood tests at least once during treatment. These were: deranged LFTs ($n = 9$), pancytopenia ($n = 2$), lymphopenia ($n = 7$) and neutropenia ($n = 1$). Abnormal biochemistry led to TG withdrawal in two patients and a dose reduction in one out of 18 patients. In the remaining 15 patients abnormal biochemistry was transient and resolved without dose modification.

Screening for hepatic complications

34 out of 54 patients treated with TG for ≥ 12 months were recommended screening for hepatotoxicity: 24/34 (70.6%) underwent MRI liver at a median of 20 months (IQR 12.3–29 months) after starting TG. 19/24 (79.2%) were normal and 4/24 (16.7%) demonstrated fatty liver disease, of which two were seen on ultrasound prior to TG. A single patient with portal hypertensive syndrome demonstrated splenomegaly, but no other abnormal findings. There were 11 out of 34 patients (32.4%) who underwent liver biopsy: four were normal, two had steatohepatitis and mild fibrosis, three had mild steatosis, one had solitary granuloma and one had a single focus of inflammation. There were no confirmed cases of NRH.

Safety

Four serious events occurred amongst 126 patient-years of treatment. Two patients developed

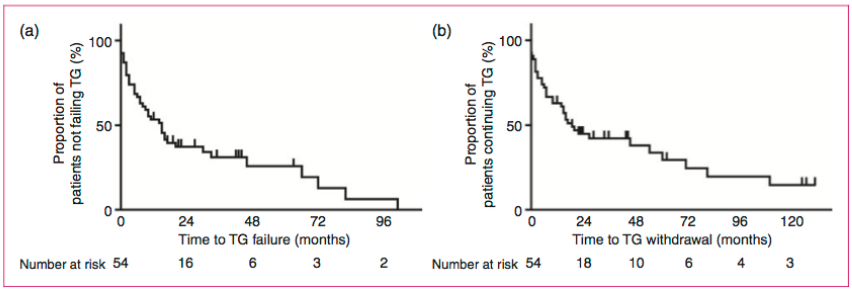

Figure 1. (a) Kaplan–Meier survival plot of 54 patients, showing the time to TG failure in months. The median time to TG failure was 15 months. Vertical lines represent the censored cases. (b) Kaplan–Meier survival plot of 54 patients, showing the time to TG withdrawal in months. Median time to TG withdrawal was 19 months. Vertical lines represent the censored cases.
TG: thioguanine

Table 4. Causes of thioguanine withdrawal.

Cause of withdrawal	*n* (%)
Continued thioguanine	18 (33.3)
Intolerant	9 (16.7)
Biochemical abnormality	2 (3.7)
Infection	3 (5.6)
Malignancy	2 (3.7)
Unplanned surgery	12 (22.2)
Non-responsive	1 (1.9)
Other	7 (13)

malignancy: a 54-year-old male with gastric cancer, who died 15 months after starting TG (he had been intolerant of thiopurines and was biologic naïve), and a 61-year-old female, who developed metastatic breast cancer 10 months after co-treatment with TG and ADA. Both agents were withdrawn and she remains in remission after chemotherapy. A 57-year-old female previously on AZA and episodic IFX, with no history of liver disease, developed spider naevi, jaundice, ascites and cholestatic LFTs 13 months after starting TG. The cumulative TG dose was 16.8 g, the mean daily dose 40 mg and median TGN 1071 pmol per 8×10^8 RBC. MRI revealed splenomegaly with normal portal/hepatic venous flow and liver parenchyma. She declined a liver biopsy and recovered after ceasing TG. A patient treated with ADA and TG developed neutropenic sepsis and pneumonia, and recovered after interruption of immunosuppression.

Discussion

AZA and MP remain first-line immunomodulators in IBD and are important as concomitant immunomodulation to limit loss of response to anti-TNF agents.[2,22,23] However, a proportion of patients fail to tolerate conventional thiopurines[2,22] and typically are considered for MTX and in some, immunomodulation is no longer possible. In this setting, TG circumvents most adverse effects encountered with conventional thiopurines, but has been largely ignored. This likely relates to concerns regarding the risk of NRH[10,11] and, in some, resigns the patient to anti-TNF monotherapy, despite an anticipated higher rate of loss of response.[23]

In this study, we describe the largest series of IBD patients, with the longest period of follow-up after treatment with TG, whom were refractory or intolerant to conventional immunosuppression. Overall, clinical response to TG was seen in 59.3% and 42.6% of patients at six and 12 months, with a median exposure of 16 months. This compares favourably to response rates seen with conventional thiopurines, despite all having previously failed at least one immunomodulator. It is significant that within this difficult-to-treat cohort, 50% of patients continued TG for 19 months. Adverse events with conventional immunosuppression did not recur in the majority. At inclusion, 77.8% were intolerant of AZA, yet only 29.6% discontinued TG because of an adverse event or biochemical abnormality, demonstrating improved tolerability of TG. Myelotoxicity and hepatotoxicity were generally transient and resolved spontaneously, with TG withdrawn in only two patients.

Serious events occurred in four patients over the 126 patient-years of follow-up. One developed a portal hypertensive syndrome, resolving on TG withdrawal. Two developed solid-organ malignancy (both previously exposed to AZA and one also with anti-TNF): one gastric and one breast cancer. TG has historically been used as treatment for breast cancer,[24] and the

causes of solid-organ malignancy are multi-factorial; hence, the aetiology of malignancy with thiopurines remains in debate. Aside from the recognised risk of lymphoma[25] and non-melanoma skin cancer,[26] thiopurines are probably associated with a modest increased risk of solid organ malignancy.[27] Of note, in our cohort, we observed no cases of lymphoma nor non-melanoma skin cancer, although given the small patient numbers, our study was underpowered to detect such a relationship.

Approximately 3% of patients treated with thiopurines developed pancreatitis.[6] Hypersensitivity to a component of AZA/MP is likely, supported by the association of thiopurine-induced pancreatitis with the HLA-DQA1*02:01–HLA-DRB1*07:01 haplotype.[28] The mechanism remains unclear; however, importantly pancreatitis did not recur with TG, as was demonstrated in our series where 35% of our patients had prior pancreatitis secondary to AZA. This implicates a metabolite of AZA/MP earlier in the purine salvage pathway, or the parent molecules themselves, as the cause of pancreatitis. Thiopurine-induced pancreatitis is therefore a key setting in which to consider TG.

The incidence of NRH with TG in IBD varies between 0–62%.[9–11,13,29] Early studies report significant rates of NRH, confirmed by liver biopsy. This was not replicated in subsequent series using low-dose TG, and earlier concerns regarding TG and NRH may be overestimated. No study has performed baseline MRI or liver biopsy prior to TG. In the study reporting the highest frequency of NRH,[10] most patients had significant exposure to conventional thiopurines prior to TG, and TGNs on TG were significantly higher than in subsequent series, where NRH was not encountered.[7,13,29] This implies a relationship between NRH and higher TG doses (>40 mg/day) and hence, higher TGN levels. Studies with TGNs < 1200 pmol per 8×10^8 RBC report no NRH.[13,29] In our cohort, which is the longest follow-up reported to date, 33.3% of patients had a median exposure of 32 months, and 20 patients >35 months, without NRH.

Furthermore, NRH is associated with conventional thiopurines,[30] IBD itself,[31] and with other chronic inflammatory conditions.[32] A study utilising intra-operative liver biopsies from thiopurine-naïve IBD patients detected NRH in 6% of CD and 33% of UC patients.[31] Autopsy studies indicate rates of up to 2.6%.[33] Therefore, particularly with lower doses of TG, NRH appears unlikely to be more frequent than the inherent background IBD risk.

The optimal surveillance strategy for NRH is debated. At our institution prior to 2004, patients treated with TG for >12 months were recommended liver biopsy. Subsequently, screening with three monthly LFTs and platelet monitoring, in conjunction with MRI every 24 months, is offered. Screening by biochemistry alone is insufficient; three of nine patients biopsied in the Dubinsky series associating NRH with TG had normal biochemistry.[10] The sensitivity and specificity of liver MRI for NRH is 77% and 72% respectively[11] and is our preferred screening tool, with biopsy being recommended if MRI or biochemistry raise the possibility of NRH.

No correlation between TGNs and response, or between TGNs and side-effects, was seen. We aim for target TGNs of 600–1000 pmol per 8×10^8 RBC, on the grounds that NRH has not been reported with <1200 pmol/8×10^8 RBC. TGNs on TG are higher than those found with AZA/MP, most likely due to rapid uptake by circulating mature RBCs, rather than TGN formation by erythroid precursors in bone marrow. In contrast to the relationship between high TGNs and myelotoxicity seen with conventional thiopurines, high TGNs with TG do not typically cause bone marrow suppression, likely due to differences in thiopurine metabolism in leucocytes between therapies.[34] This is reflected in our study, where significant myelotoxicity was seen in only one patient. Therefore, TGNs on TG cannot be interpreted in the same way as AZA/MP, and future studies involving TG should involve measurement of TGNs in the target cells, namely leukocytes.

We acknowledge several limitations in this study. Clinical efficacy was assessed retrospectively using HBI/SCCAI, and, despite our practice to document this prospectively at every consultation, the response data must be interpreted with caution. Clinical indices (including Crohn's disease activity index) correlate poorly with mucosal inflammation.[35] The indication for using TG was as concomitant immunomodulation, to maintain response to scheduled or episodic biologic use in a significant proportion of patients (33%). The response rates within this cohort were 83% and 61% at six and 12 months, respectively, which was higher than those seen when TG was prescribed as monotherapy for active disease or steroid sparing (47.2% and 33.3% at six and 12 months, respectively). Whilst this definition of 'response' is questionable, it does represent real-world clinical practice. As many patients did not have C-reactive protein measured routinely, we could not assess the biochemical response to TG. NRH was not formally excluded in all; hence, the true rate of NRH may be underestimated. One patient developed a portal hypertensive syndrome, but did not undergo liver biopsy; this could have represented NRH. Where TG was interrupted, we did not report efficacy nor the safety data of subsequent TG therapy, although the numbers were small; hence, the true duration of drug tolerance may have been longer than that reported.

Conclusions

In summary, we report our long-term experience of TG in a cohort of IBD patients' refractory to, or intolerant of, conventional immunomodulators. The efficacy and side-effects were comparable to conventional immunomodulators and the safety data for TG is reassuring. We acknowledge that the latter, rather than the clinical effectiveness of TG, is the key message that clinicians may find most useful when translating our findings to everyday clinical practice. Thiopurine-induced pancreatitis did not recur with TG and the majority of patients who previously discontinued conventional thiopurines tolerated TG.

In contrast to earlier studies with TG, we did not observe NRH in this cohort after long-term follow-up (the largest reported in the literature). NRH may relate partly to dose and elevated TGNs; hence, TGN measurement and vigilance in monitoring for NRH are key issues when using TG. We recommend doses ≤40 mg/day, with dose adjustments based on TGN levels.

Head to head studies of TG versus conventional thiopurines would help establish the safety and efficacy of TG in IBD, but are unlikely to be undertaken. Nonetheless, TG remains an acceptable alternative in patients with IBD who have not tolerated or have failed conventional immunomodulators.

Acknowledgements

MW contributed to study design, data acquisition, analysis, and wrote and revised the manuscript. KP, VK, RG, BW, PB and TE contributed to data acquisition, manuscript writing and revision. PI, AM and JS contributed to study design, manuscript revision and intellectual content. All authors approved the final version of the manuscript.

Declaration of conflicting interests

There is no conflict of interest for any author to declare.

Funding

This research received no specific grant from any funding agency in the public, commercial or non-for-profit sectors.

References

1. Dignass A, Van Assche G, Lindsay J, et al. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; 4: 28–62.
2. Chande N, Tsoulis DJ and Macdonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2013; 4: CD000545.
3. Sparrow MP, Hande SA, Friedman S, et al. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine. *Clin Gastroenterol Hepatol* 2007; 5: 209–214.
4. Smith MA, Marinaki AM, Arenas M, et al. Novel pharmacogenetic markers for treatment outcome in azathioprine-treated inflammatory bowel disease. *Aliment Pharmacol Ther* 2009; 30: 375–384.
5. Lowry PW, Franklin CL, Weaver AL, et al. Measurement of thiopurine methyltransferase activity and azathioprine metabolites in patients with inflammatory bowel disease. *Gut* 2001; 49: 665–670.
6. Kennedy NA, Rhatigan E, Arnott IDR, et al. A trial of mercaptopurine is a safe strategy in patients with inflammatory bowel disease intolerant to azathioprine: An observational study, systematic review and meta-analysis. *Aliment Pharmacol Ther* 2013; 38: 1255–1266.
7. Derijks LJJ, Gilissen LPL, Engels LGJB, et al. Pharmacokinetics of 6-thioguanine in patients with inflammatory bowel disease. *Ther Drug Monit* 2006; 28: 45–50.
8. Dubinsky MC, Hassard PV, Seidman EG, et al. An open-label pilot study using thioguanine as a therapeutic alternative in Crohn's disease patients resistant to 6-mercaptopurine therapy. *Inflamm Bowel Dis* 2001; 7: 181–189.
9. Van Asseldonk DP, Jharap B, Kuik DJ, et al. Prolonged thioguanine therapy is well tolerated and safe in the treatment of ulcerative colitis. *Dig Liver Dis* 2011; 43: 110–115.
10. Dubinsky MC, Vasiliauskas EA, Singh H, et al. 6-Thioguanine can cause serious liver injury in inflammatory bowel disease patients. *Gastroenterology* 2003; 125: 298–303.
11. Seiderer J, Zech CJ, Reinisch W, et al. A multicenter assessment of liver toxicity by MRI and biopsy in IBD patients on 6-thioguanine. *J Hepatol* 2005; 43: 303–309.
12. Oancea I, Png CW, Das I, et al. A novel mouse model of veno-occlusive disease provides strategies to prevent thioguanine-induced hepatic toxicity. *Gut* 2013; 62: 594–605.
13. De Boer NKH, Zondervan PE, Gilissen LPL, et al. Absence of nodular regenerative hyperplasia after low-dose 6-thioguanine maintenance therapy in inflammatory bowel disease patients. *Dig Liver Dis* 2008; 40: 108–113.
14. Pavlidis P, Ansari A, Duley J, et al. Splitting a therapeutic dose of thioguanine may avoid liver toxicity and be an efficacious treatment for severe inflammatory bowel disease: A 2-center observational cohort study. *Inflamm Bowel Dis* 2014; 20: 2239–2246.
15. Ansari A, Elliott T, Fong F, et al. Further experience with the use of 6-thioguanine in patients with Crohn's disease. *Inflamm Bowel Dis* 2008; 14: 1399–1405.
16. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 2: Current management. *J Crohns Colitis* 2012; 6: 991–1030.
17. Harvey RF and Bradshaw MJ. Measuring Crohn's disease activity. *Lancet* 1980; 1: 1134–1135.
18. Walmsley RS, Ayres RC, Pounder RE, et al. A simple clinical colitis activity index. *Gut* 1998; 43: 29–32.
19. Dervieux T and Boulieu R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 1998; 44: 551–555.

20. National Health Service of UK. http://www.hra.nhs.uk/documents/2016/06/defining-research.pdf (2013, accessed November 2014), pp.1–4.
21. Morris K. Revising the Declaration of Helsinki. *Lancet* 328: 1889–1890.
22. Timmer A, McDonald JWD, Tsoulis DJ, et al. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2012; 9: CD000478.
23. Hazlewood GS, Rezaie A, Borman M, et al. Comparative effectiveness of immunosuppressants and biologics for inducing and maintaining remission in Crohn's disease: A network meta-analysis. *Gastroenterology* 2015; 148: 344–354.
24. Ingle JN, Twito DI, Suman VJ, et al. Evaluation of intravenous 6-thioguanine as first-line chemotherapy in women with metastatic breast cancer. *Am J Clin Oncol* 1997; 20: 69–72.
25. Beaugerie L, Brousse N, Bouvier AM, et al. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: A prospective observational cohort study. *Lancet* 2009; 374: 1617–1625.
26. Peyrin-Biroulet L, Khosrotehrani K, Carrat F, et al. Increased risk for non-melanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. *Gastroenterology* 2011; 141: 1621–1628.
27. Pasternak B, Svanström H, Schmiegelow K, et al. Use of azathioprine and the risk of cancer in inflammatory bowel disease. *Am J Epidemiol* 2013; 177: 1296–1305.
28. Heap GA, Weedon MN, Bewshea CM, et al. HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants. *Nat Genet* 2014; 46: 1131–1134.
29. Almer SHC, Hjortswang H and Hindorf U. 6-Thioguanine therapy in Crohn's disease: Observational data in Swedish patients. *Dig Liver Dis* 2009; 41: 194–200.
30. Vernier-Massouille G, Cosnes J, Lemann M, et al. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. *Gut* 2007; 56: 1404–1409.
31. De Boer NKH, Tuynman H, Bloemena E, et al. Histopathology of liver biopsies from a thiopurine-naïve inflammatory bowel disease cohort: Prevalence of nodular regenerative hyperplasia. *Scand J Gastroenterol* 2008; 43: 604–608.
32. Musumba CO. Review article: The association between nodular regenerative hyperplasia, inflammatory bowel disease and thiopurine therapy. *Aliment Pharmacol Ther* 2013; 38: 1025–1037.
33. Wanless IR. Micronodular transformation (nodular regenerative hyperplasia) of the liver: A report of 64 cases among 2500 autopsies and a new classification of benign hepatocellular nodules. *Hepatology* 1990; 11: 787–797.
34. Lancaster DL, Patel N, Lennard L, et al. Leucocyte versus erythrocyte thioguanine nucleotide concentrations in children taking thiopurines for acute lymphoblastic leukaemia. *Cancer Chemother Pharmacol* 2002; 50: 33–36.
35. Rutgeerts P, Diamond RH, Bala M, et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. *Gastrointest Endosc* 2006; 63: 433–442.

SECTION 3. OPTIMISATION OF BIOLOGIC ANGENTS THAT INHIBIT TUMOUR NECROSIS FACTOR

CHAPTER 5:

Review: Role of therapeutic drug monitoring for biologics in inflammatory bowel disease

LEADING ARTICLE

Role of Therapeutic Drug Monitoring for Biologics in IBD

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The introduction of monoclonal antibody therapy directed against tumor necrosis factor-α revolutionized the modern management of patients with IBD. In spite of this, a proportion of patients do not initially respond to this therapy or, of those that do, some lose response with time. The mechanisms for loss of response include immunogenicity leading to increased drug clearance. In this regard, a growing body of evidence has demonstrated a relationship between circulating drug levels, anti-drug antibodies, and clinical outcomes. This article addresses these issues in the context of therapeutic drug monitoring and aims to give the treating clinician a platform upon which to make clinical decisions to guide management of loss of response. *Inflamm Bowel Dis Monit* 2013;**14**(2):44–53.

The introduction of tumor necrosis factor (TNF) antagonists some 15 years ago revolutionized the modern management of patients with IBD [1,2]. Traditional "step-up" management strategies involved the treatment of acute flares with corticosteroids and then maintenance of clinical remission with aminosalicylates (in the context of ulcerative colitis [UC]) or immunomodulators (in both Crohn's disease and UC). Corticosteroids, although extremely effective in the short-term [3–5], are associated with poor sustained remission rates, low rates of mucosal healing, steroid dependency, and both short- and long-term adverse effects [6]. Meanwhile, thiopurines and methotrexate are slow to work, are often poorly tolerated, and have serious side-effects in some patients. In addition, they are only effective in a proportion of patients and their ability to achieve mucosal healing is only partially understood.

Pivotal trials at the turn of this century demonstrated that infliximab (Remicade®; Centocor, Malvern, PA, USA), a chimeric monoclonal immunoglobulin G1 (IgG1) antibody, and subsequently adalimumab (Humira®; Abbott Laboratories, Abbott Park, IL, USA), a humanized monoclonal IgG antibody, were effective agents for the induction and maintenance of remission in luminal and fistulizing Crohn's disease [7–11]. Certolizumab pegol (Cimzia®; UCB Pharma, Brussels), a Fab' anti-TNF antibody that was pegylated to increase its half-life, has broadly similar response rates to infliximab and adalimumab in Crohn's disease [12–14]. Infliximab and adalimumab have subsequently been shown to induce and maintain remission in moderate-to-severe active UC [15,16].

In Crohn's disease, the integration of biologic therapy into modern treatment strategies has resulted in higher rates of steroid-free remission [17,18] and mucosal healing [18–22], fewer hospitalizations [23,24], and a less frequent need for intestinal resection [23,25]. This has led to improved quality of life [23,26,27] and a reduction in healthcare expenditure compared with conventional therapy [28,29]. Similar benefits have been seen in UC [30], and the use of infliximab as a rescue therapy to avoid colectomy in acute severe UC has also been demonstrated [31,32].

Despite the clear benefits of anti-TNF therapy, it is not a panacea. A small proportion of patients does not improve with induction therapy, and these patients are classified as primary non-responders. In placebo-controlled trials in luminal Crohn's disease, rates of primary non-response (PNR) at week 4 to infliximab and adalimumab were 40% and 41%, respectively [1,7]. In large placebo-controlled maintenance trials, the maximal response rate was seen at week 10 for infliximab and week 12 for adalimumab, with rates of PNR of 29.2% [9] and 21% [11], respectively. Real-life data support the concept,

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Table 1. Causes of loss of response in patients receiving anti-TNF therapy.

Non-inflammatory causes	Non-IBD-related inflammatory causes	IBD-related inflammatory causes
Irritable bowel syndrome	Infection	Non-adherence
Fibrostenotic disease	Ischemia	Loss of response due to immunogenicity (anti-drug antibodies)
Malignancy	Other: vasculitis/amyloidosis	
Bacterial overgrowth		Loss of response due to non-immune increase in drug clearance
Bile salt diarrhea		Shift of disease from TNF-α pathway to involve other mediators

TNF: tumor necrosis factor.

however, that selecting appropriate patients for treatment results in extremely low primary rates of PNR [33,34].

In Crohn's disease, amongst those who exhibit a response to infliximab induction therapy and continue with scheduled maintenance dosing, between 15% and 54% subsequently lose response by 12 months, depending on the definition employed [7,35–38]. A meta-analysis found that the mean percentage loss of response (LOR) was 37%, which equated to a 13% risk per patient-year [39]. Similar rates of secondary LOR have been seen with adalimumab in Crohn's disease [11,40,41].

Unlike other chronic inflammatory conditions such as rheumatoid arthritis and psoriatic arthritis (for which a larger pool of biologics is available) in IBD, treatment options are generally limited to infliximab and adalimumab. Accordingly, management and prevention of LOR to anti-TNF is of critical importance in IBD.

The causes of LOR are protean (**Table 1**). Once non-inflammatory causes have been excluded, several strategies exist. Empiric dose intensification can restore clinical response in 60–90% of patients in the short-term [42]. At 12 months, sustained response to dose-escalation is seen in 35–47% [40,43]. Within-class switching of anti-TNF agents is an alternative option. A systematic review of 1810 patients with Crohn's disease commencing adalimumab after losing response to infliximab found short-term clinical response rates between 41% and 83% and 12-month clinical remission rates of 19–68% [44].

However, such "blind" manipulation of anti-TNF therapy has recently been challenged by evidence supporting the measurement of drug and anti-drug antibody (ADAb) levels in order to guide the appropriate next step; an increasing number of studies has demonstrated a relationship between low serum trough (i.e. pre-dose) levels of infliximab and adalimumab, in the presence or absence of ADAb, and loss of response. As a result, interest in the role of therapeutic drug monitoring (TDM) for anti-TNF therapy has grown rapidly. This article aims to review the commonly available assays measuring drug and ADAb; the relationship between drug levels, ADAb, and clinical outcomes; and how this evidence may be used to incorporate anti-TNF TDM into everyday practice.

Methodology behind commonly used assays

Interpreting the evidence regarding the relationship between drug levels, ADAb, and clinical response requires an understanding of the differences between the available assays. The three most commonly employed assays are enzyme-linked immunosorbent assay (ELISA), radio-immunoassay (RIA), and, more recently, a high-pressure liquid chromatography (HPLC)-based homogeneous mobility shift assay (HMSA). Each of the assays shares similarities but they also have key differences. Moreover, the differing methodology means that direct comparison of results between assays is not straightforward. Indeed, there is also evidence that assays using the same technology may produce discrepant results [45–47].

ELISA

This is the most technically straightforward, cheapest, and most widely available assay, with numerous "in-house" versions and commercial kits being available. A capture moiety, either infliximab or adalimumab for determination of ADAb, or TNF-α for determination of drug levels, is immobilized on a plate. Serum samples from treated patients are applied to the plate and either ADAb or drug binds to the capture moiety, forming a capture moiety–target molecule complex. To measure drug levels, anti-human (or anti-rabbit/goat) IgG bound to color-producing horseradish peroxidase (HRP) is added and the intensity of the color reaction is recorded. For detection of ADAb, a sandwich (double-antigen) principle is employed, whereby infliximab/adalimumab bound to HRP is added to the target–capture moiety complex, and the color change is measured as before. An important caveat to this method is that it can only measure ADAb in the absence of detectable levels of drug, as free drug competitively occupies binding sites on ADAb preventing their capture by immobilized drug on the plate. Negative ADAb findings in the setting of circulating drug are, therefore, termed "inconclusive" and such results have been reported in 25–79% of patients on anti-TNF therapy [18,48,49].

Techniques have evolved to address this problem. Substitution of infliximab-HRP with anti-human λ chain-conjugated antibody in the detector phase resulted in the identification of 6.3% of patients with positive ADAb in the presence of infliximab who had been deemed inconclusive on conventional double-antigen ELISA [50]. Alternatively, acid-dissociation of infliximab–ADAb prior to capture resulted in detection of antibodies to infliximab in 27.6% of samples compared with 3.4% with the conventional assay [51]. However, the clinical significance of ADAb in patients with therapeutic drug levels remains unknown.

Similarly, only free drug rather than that bound to ADAb is measured. However, this is probably of little relevance since it is likely that the amount of free drug in the serum is the clinically important measure rather than the total amount of drug including that portion that is bound to circulating antibody.

In addition, it is important to note that false-positive ADAb results due to binding of other low-affinity antibodies, including heterophilic antibodies, rheumatoid factors, and activated complement, have been reported [52].

RIA

RIA employs a radiolabeled capture moiety to detect drug and ADAb. For drug levels, serum is first incubated with ^{125}I-TNF-α. Rabbit anti-human Fcγ antibody is then added and centrifugation separates out infliximab–^{125}I-TNF-antiFc complexes from free ^{125}I-TNF [53]. Drug concentration is then quantified by measurement of the radioactivity of the precipitants. To measure antibodies to infliximab, chromatography columns lined with anti-human λ light chains are used [54]. This utilizes the fact that infliximab is an IgG construct with murine κ light chains, hence discriminating between free circulating infliximab and that bound to any class of λ-containing human Ig. Another technique substituting anti-human λ chains with ^{125}I-labelled, pepsin-treated infliximab-Fab2 and protein A in rheumatoid arthritis detected similar rates of anti-infliximab antibodies in patients treated with infliximab [55].

HMSA

This recently developed, commercially available method (Prometheus Laboratories Inc., San Diego, CA, USA) was designed to overcome some of the limitations seen with ELISA and RIA assays. The first step involves acid-dissociation, freeing ADAb from drug, thereby allowing the detection of ADAb in the presence of free drug. A capture moiety is then added and incubated to form capture moiety–target complexes (liquid-phase). HPLC is used to identify and quantify the capture moiety–target complex. The advantages of this method include a high sensitivity and specificity, and the ability to detect and to differentiate all isotypes of Ig and subtypes of IgG. Most importantly, however, is its ability to quantify ADAb in the presence of circulating drug [46,56]. The disadvantages include limited availability and high cost.

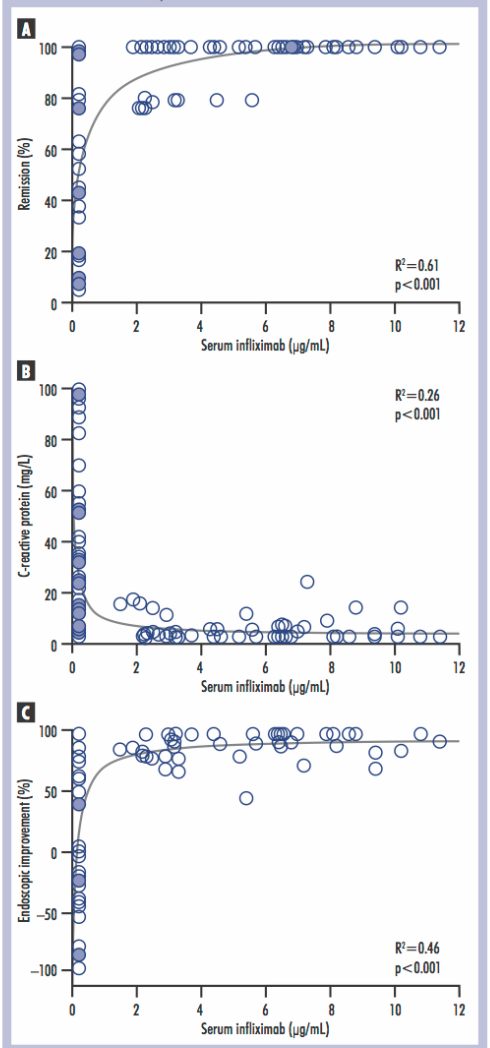
Relationship between drug levels, ADAb status, and clinical outcomes

A growing body of evidence has implicated a relationship between drug levels, ADAb status, and clinical response. Interpretation of the data is limited, however, by many factors including trial design (retrospective vs. *post hoc* analysis), differences in drug-administration schedules (episodic vs. maintenance), variation in the type of assay used, variations in therapeutic cut-offs used, sampling times (trough vs. mid-cycle), and definitions of outcomes.

Infliximab levels and Crohn's disease

Early evidence reporting an association between drug level and clinical response was published by Baert et al. [57]. In their investigation, 125 patients with Crohn's disease were treated with either a single infusion of 5 mg/kg infliximab (for luminal disease) or three doses of 5 mg/kg infliximab at weeks 0, 2, 6 (for fistulizing disease) and followed until clinical relapse when re-treatment was offered. Patients with an infliximab concentration of ≥12.0 μg/mL at week 4 had a greater median duration of response of 81.5 days (95% confidence interval [CI] 68–90 days) compared with 68.5 days (95% CI 52–77 days) in those with an infliximab concentration <12.0 μg/mL at week 4 ($p<0.01$) [57]. Further support for the relationship between drug levels and response was provided by the SONIC (Study of Biologic and Immunomodulator Naïve Patients in Crohn's Disease) trial in which 508 immunosuppressant-naïve patients with moderate-to-severe Crohn's disease were randomized to receive infliximab or azathioprine monotherapy, or both drugs in combination [18]. The study was not designed to investigate the relationship between trough levels and clinical outcome. Nevertheless, a trend to improved rates of corticosteroid-free clinical remission was observed in patients with infliximab trough levels >1 μg/mL at week 30 compared with those with levels of 0–1 μg/mL. Maser et al. investigated this relationship further in a single-center observational study amongst 105 consecutive patients with Crohn's disease who underwent induction with infliximab and then either episodic (n=23) or scheduled therapy (n=82) [58]. The proportion of patients achieving clinical remission (defined as a Harvey–Bradshaw Index [HBI] ≤2) was higher amongst those with detectable trough infliximab compared with undetectable trough infliximab (82% vs. 6%; $p<0.001$). Detectable trough infliximab was also associated with lower median serum C-reactive protein (CRP) level (2.0 mg/L vs. 11.8 mg/L; $p<0.001$), higher rates of normalization of CRP (76% vs. 32%; $p<0.001$), and significant endoscopic improvement (88% vs. 33%; $p<0.001$) (**Figure 1**) [58].

Figure 1. Relation of clinical outcomes to serum infliximab in 105 patients. A: Duration of interval of clinical remission defined as the percentage of time (weeks) between infusions with HBI score of ≤2. **B:** Serum CRP concentration. **C:** Endoscopic improvement defined as percentage change in endoscopic score from the baseline to the follow-up evaluation.



CRP: C-reactive protein; HBI: Harvey–Bradshaw Index.
Closed circles indicate patients who discontinued infliximab before 52 weeks; open circles indicate patients who continued infliximab beyond 52 weeks.
Redrawn with permission from [58].

Each of these studies measured drug levels using ELISAs. The lower end of the therapeutic range is probably somewhere in the region of 2–3 μg/mL although this is yet to be fully defined, with different studies having used different cut-offs for the lower limit of the therapeutic range. Furthermore, it may not be possible to extrapolate ELISA-based cut-offs to other methodologies. Using RIA, a single-center, retrospective analysis of 106 patients with Crohn's disease and UC aimed to quantify therapeutic cut-off levels [59]. Patients with Crohn's disease who maintained a response had significantly higher median trough levels compared with those with loss of response (median infliximab 2.8 μg/mL vs. 0 μg/mL; p<0.0001). An infliximab trough level of <0.5 μg/mL correlated with a sensitivity of 86% and specificity of 85% in predicting loss of response.

Detectable infliximab trough levels after induction have also been shown to predict sustained response. In a retrospective analysis of 84 patients with Crohn's disease who responded to infliximab induction, 56% had a sustained response at a median follow-up of 25 months [60]. A trough level >3 μg/mL at the beginning of the maintenance phase (week 14 or 22) was associated with a decreased risk of treatment failure (hazard ratio 0.34, 95% CI 0.16–0.75).

Adalimumab levels and Crohn's disease

A similar association between trough levels and short-term clinical response can be seen with adalimumab. In CLASSIC I (Clinical Assessment of Adalimumab Safety and Efficacy. Studied as Induction Therapy in Crohn's Disease), 299 patients with moderate-to-severe Crohn's disease naïve to anti-TNF therapy were randomized to three different induction regimens of adalimumab (40 mg/20 mg, 80 mg/40 mg, or 160 mg/80 mg) given at weeks 0 and 2 [9]. Remission rates, defined as a Crohn's Disease Activity Index <150 at week 4, were seen in 18%, 24%, and 36%, respectively, which correlated to mean trough levels of 2.79±1.48 μg/mL, 5.65±3.06 μg/mL, and 12.61±5.25 μg/mL, respectively.

A recent *post hoc* analysis of CLASSIC I and CLASSIC II (a follow-on randomized trial of 276 patients from CLASSIC I) analyzed the relationship between adalimumab concentration and clinical outcome at weeks 4, 24, and 56, and sought to identify a therapeutic threshold that could discriminate between clinical response/remission status [61]. Again, a consistent pharmacokinetic relationship was observed between varying adalimumab doses and median adalimumab concentration. Whilst median adalimumab concentrations were significantly higher in patients achieving clinical remission at week 4 of CLASSIC I, and weeks 4 and 24 of CLASSIC II (p<0.05), there was no difference observed at week 56. Considerable inter- and intra-patient variability and overlap of adalimumab concentrations between remission and active disease meant that a therapeutic cut-off to predict outcome could not be identified. An interesting observation was that there was no significant difference in remission rates between

those patients with undetectable adalimumab compared with those with detectable adalimumab at both week 24 and 56.

The relationship between rates of discontinuation of adalimumab and adalimumab trough levels was prospectively evaluated in a small follow-up series of 22 patients with Crohn's disease who initially responded to adalimumab [62]. Significantly higher adalimumab trough levels were found in patients who remained in remission compared with those who developed mild, moderate, or severe disease activity (p<0.01). Patients discontinuing adalimumab owing to a loss of response had significantly lower adalimumab trough levels compared with patients who remained on adalimumab (2.1 μg/mL vs. 6.7 μg/mL; p<0.01). Conversely, a large observational study of 168 patients treated with adalimumab after secondary failure with infliximab did not demonstrate a relationship between short-term efficacy and adalimumab trough level [40]. However, long-term clinical benefit was significantly decreased in patients with a trough concentration at any time-point of <0.33 μg/mL. Overall, 65% of patients required adalimumab dose-escalation; of these, 72% responded. Adalimumab trough concentrations increased after dose escalation from a median of 4.8 μg/mL (interquartile range [IQR] 2.3–8.9 μg/mL) to 9.4 μg/mL (IQR 1.2–16.4 μg/mL; p=0.001) and this increase correlated well with clinical response to escalation (median increase 5.9 μg/mL [IQR 1.9–8.3 μg/mL] for responders vs. 0.0 μg/mL [IQR 0.0–1.7 μg/mL] for non-responders; p<0.0001).

Anti-TNF levels and UC

A relationship between detectable infliximab levels and clinical outcome has also been demonstrated in UC. Seow et al. reported their single-center experience of 115 consecutive UC patients who underwent induction and maintenance therapy with infliximab [63]. Rates of remission were 32% at week 10 and 37% at week 54. Overall, 40% of patients came to colectomy at a median of 5.4 months. Detectable trough serum infliximab was associated with higher rates of remission (69% vs. 15%; p<0.001) and endoscopic improvement (76% vs. 28%; p<0.001). Undetectable infliximab predicted an increased risk of colectomy (55% vs. 7%, odds ratio [OR] 9.3, 95% CI 2.9–29.9; p<0.001). The same group re-analyzed this cohort with the more sensitive HMSA and found detectable serum infliximab in 54.4% of samples, of which 8.8% had detectable ADAb [64]. A trough level >2 μg/mL, with or without ADAb, was associated with higher rates of steroid-free remission (69% vs. 16%; p<0.001) and lower rates of colectomy (13% vs. 64%; p<0.001). In addition, subanalysis of the landmark trials of adalimumab and infliximab in UC also demonstrated a relationship between increasing quartiles of drug level and remission [65,66].

Anti-TNF levels and mucosal healing

Recent uncontrolled studies have demonstrated a relationship between infliximab and adalimumab trough levels and mucosal healing. Paul et al. prospectively identified 52 patients with Crohn's disease and UC who had undergone dose intensification after relapse on maintenance infliximab [67]. Mucosal healing was measured at 8 weeks, defined as CRP <5 mg/L and fecal calprotectin <250 μg/g in Crohn's disease, and a Mayo endoscopic subscore <2 in UC. Overall, 50% of patients achieved mucosal healing. An increase in the level of infliximab of >0.5 μg/mL was associated with mucosal healing (sensitivity 0.88, specificity 0.77, positive predictive value 0.79, negative predictive value 0.87; p<0.0001). Similarly, Imaeda et al. investigated the relationship between infliximab trough levels and endoscopic activity amongst 45 patients with Crohn's disease [68]. Using a modified Rutgeerts scoring system, 26% of patients had mucosal healing and this was associated with higher infliximab levels compared with patients without mucosal healing (16.2 μg/mL vs. 4.1 μg/mL; p<0.0001). A cut-off of 4.0 μg/mL was identified to predict mucosal healing (area under the curve 0.63, 95% CI 0.56–0.70). A single study has reported higher median adalimumab trough levels amongst IBD patients with mucosal healing compared with those with absence of healing (6.5 μg/mL vs. 4.2 μg/mL; p<0.005) and identified a trough level of <4.9 μg/mL as being associated with absence of healing (likelihood ratio 4.3; sensitivity 66%, specificity 85%) [69].

It is worth noting that in studies demonstrating an association between drug levels and clinical outcomes there are some patients who maintain clinical response [59,61,63] and exhibit mucosal healing with subtherapeutic levels [58], and that conversely, some lose response with therapeutic levels. Further, a small single-center study has shown no correlation between adalimumab levels and clinical outcomes [70]. Nevertheless, the majority of evidence from the studies discussed herein, along with other published results [65,71,72], strongly suggests that a relationship exists between detectable trough level of drug and clinical outcome. This relationship does not imply causation (i.e. that low drug levels drive outcomes *per se*) as other confounding variables, such as the role of ADAb and patient characteristics, may influence drug pharmacokinetics. Indeed, it may be possible that low drug levels are caused by inflammation rather than being responsible for active disease. For example, fecal loss of infliximab has been demonstrated to be significantly higher in non-responding patients with more severe disease than in responders [73]. Similarly, amongst 728 patients with UC treated with infliximab, lower albumin concentrations (a surrogate marker of more severe disease) correlated with lower infliximab concentrations, perhaps suggesting that other proteins such as anti-TNF agents are, akin to albumin, lost in patients with active disease [74].

Recent data from the TAXIT (Trough Level-Adapted Infliximab Treatment) study, however, challenge this theory [75]. In the pre-optimization phase, correction of low drug levels with increased doses of infliximab in patients with

Table 2. Clinical management based on therapeutic drug monitoring.

	ADAb-negative	ADAb-positive
Subtherapeutic drug level	• Dose intensify: shorten dosing schedule and/or increase dose	• Switch agent within class • Add/modify concomitant immunomodulation • Consider dose-escalation NB: Intervention is more likely to be successful with low levels of ADAb
Therapeutic drug level	• Evaluate for active disease • Consider switching agent out of class	• Possible false-positive result, re-test NB: significance in assays measuring total antibody unclear

ADAb: anti-drug antibody.

Crohn's disease resulted in a reduction in their CRP levels and HBI. Whilst this does not prove that active disease results from low drug levels it does suggest that active disease can be improved by increasing drug levels.

ADAb and clinical outcome

Immunogenicity is a recognized result of treatment with biologic therapies. Interpreting the literature linking the incidence of ADAb with clinical outcome poses significant challenges not least because of the limitations of the assays used to measure these antibodies. Nevertheless, a growing body of evidence suggests that the development of ADAb impacts on at least some elements of clinical outcome.

Episodic infliximab administration has been shown to be associated with higher rates of ADAb detection, with high ADAb titers being associated with a shorter time to relapse and a higher risk of infusion reactions [57]. However, in a *post hoc* analysis of ACCENT I (A Crohn's Disease Clinical Study Evaluating Infliximab in a New Long-term Treatment Regimen I), no association was found between ADAb status and clinical outcomes at week 54 (ADAb-positive vs. ADAb-negative for response and remission, 64% vs. 62%, p=0.35; and 41% vs. 39%, p=0.76; respectively) [48]. Likewise, in a prospective, observational cohort study of 168 Crohn's disease patients treated with adalimumab after infliximab failure, ADAb were detected by ELISA over a median of 20.4 months of follow-up in 12 patients (9.2%) [40]. Detection of ADAb was associated with significantly lower median trough adalimumab concentrations (p<0.0001) and was associated with treatment discontinuation.

Whilst the relevance of ADAb is unclear with regards to response, ADAb are more commonly seen in patients who develop infusion reactions to infliximab. A meta-analysis reporting on seven studies that investigated this relationship found that ADAb significantly increased the risk of an infusion reaction (relative risk [RR] 2.07, 95% CI 1.62–2.67; p<0.00001) [76], but were not associated with clinical response. However, a separate meta-analysis of 1378 patients with IBD found that the pooled risk ratio of loss of clinical response to infliximab in the presence of ADAb was 3.2 (95% CI 1.9–5.5; p<0.0001) [77].

Using TDM in clinical practice

TDM is commonly employed with medications with a narrow therapeutic window, such as digoxin, warfarin, and gentamicin. Whilst the evidence described above suggests a fairly robust relationship between anti-TNF drug levels and clinical response, translating this into using drug levels (and perhaps ADAb levels) to guide clinical practice requires further consideration. Taking this into account, in IBD, TDM is most likely to be useful in guiding management decisions in patients with secondary loss of response. In this situation, measurement of trough anti-TNF and ADAb levels yields one of four profiles (**Table 2**).

Subsequent management in these distinct scenarios is based on the following general concepts:

- Patients with therapeutic anti-TNF drug levels have better clinical outcomes compared with those with subtherapeutic drug levels.
- Patients with subtherapeutic anti-TNF drug levels without evidence of immunogenicity benefit from dose intensification.
- Production of ADAb can be attenuated by use of concomitant immunomodulation [78], particularly if antibodies are found to be at low levels (although the understanding of what "low levels" are is limited), in which case dose intensification may also work.
- Patients who have previously responded to anti-TNF and agents who develop subtherapeutic drug levels in association with high ADAb levels often respond to alternative anti-TNF agents.
- Patients with therapeutic anti-TNF drug levels who have active disease are unlikely to respond to ongoing anti-TNF therapy with the same or a different drug, and alternative strategies should be considered.

Some of the first evidence regarding the role of TDM for anti-TNF agents came from the Mayo Clinic (Rochester, MN, USA)

in a study that reviewed the relationship between infliximab levels, ADAb, and outcomes using an ELISA [79]. Overall, 110 tests in 76 patients (49%) were performed for secondary loss of response. Patients with detectable ADAb who switched to adalimumab had superior response rates to those who underwent dose intensification with infliximab (92% vs. 17%; p<0.004). Conversely, those with subtherapeutic infliximab concentrations (defined as ≤12 μg/mL at 4 weeks after infusion or <1.4 μg/mL at trough) who underwent dose intensification were more likely to have a complete or partial clinical response compared with patients who switched to adalimumab (86% vs. 33%; p<0.02). This study supporting drug level-driven management has influenced clinical practice but it is not without weaknesses. First, it was retrospective and uncontrolled. Second, the definition of response was poorly described and was based on physician assessment of improvement in clinical symptoms. Third, of the total 177 tests performed, 47 (27%) had no bearing on clinical outcomes; in many, follow-up was incomplete or not stated. Finally, in those with subtherapeutic infliximab levels undergoing dose intensification, subsequent drug levels in responders were not reported.

In addition, a further similarly designed retrospective study from France, again using an ELISA, described contrasting results [80]. Seventy-six IBD patients with secondary loss of response to infliximab were classified according to one of four management outcomes: continuation of infliximab at current dose (n=31), infliximab dose intensification (n=39), switch to adalimumab (n=5), or surgery (n=1). Twenty-seven patients (69%) responded to dose intensification; however, there was no significant difference in mean infliximab trough levels between responders and non-responders (3.3±4.1 μg/mL vs. 2.3±2.2 μg/mL; p=0.85). Sixteen of the 76 patients (22%) had detectable ADAb, of whom 10 underwent dose intensification (six responding). Four of the six who responded and two of the four who did not respond had ADAb concentrations >200 ng/mL. Thus, in this small cohort, the success of dose escalation did not appear to be affected by antibody status. The outcomes from this paper, however, have been questioned given the differences between the assay used and other assays [45]. Moreover, the upper limit of detection of ADAb was 200 ng/mL, which, compared with that in other assays, is very low – although comparison of antibody concentrations between assays may not be valid.

Recent data may shed further light on these discrepant results. Vande Casteele et al. demonstrated that ADAb to infliximab can be transient or sustained, and that the former do not negatively influence clinical outcomes [81]. Using the new HMSA, these investigators retrospectively measured infliximab trough levels and ADAb in 1232 consecutive samples from 90 patients, approximately two-thirds of whom had previous ADAb detected with ELISA. On analysis with HMSA, amongst the 53 patients found to have ADAb, 15 (28%) had ADAb that were transient and disappeared with time whereas in 38 (72%) the ADAb persisted. Such persistent ADAb were associated with a need to discontinue infliximab compared with those with transient ADAb (RR 5.1, 95% CI 1.4–19.0; p=0.0005). Further, in patients with loss of response, those with low ADAb concentrations were more likely to respond to dose intensification than those with high ADAb concentrations (likelihood ratio of failure 3.6 if ADAb >9.1 U/mL), suggesting that dose intensification, prior to switching within class, may be worthwhile in some patients with ADAb.

Challenges to introduction to clinical practice

Many issues remain to be resolved to clarify further how drug and ADAb level testing can be used in clinical practice. First, there is a lack of clarity regarding the lower end of the therapeutic range, there being large inter-patient variability in anti-TNF trough levels. For example, in the French study discussed above, 50% of patients who underwent dose intensification for loss of response already had infliximab concentrations that were defined as therapeutic (>1.5 μg/mL) at inclusion and most of them (70%) had a clinical response [80]; it should be noted that other groups have defined a higher therapeutic concentration of 3 μg/mL [75]. Indeed, it is possible that "one size does not fit all" in defining what is a therapeutic anti-TNF drug concentration for any given patient at any single time-point, and that management based on measuring serial drug and ADAb levels requires further investigation. Furthermore, a therapeutic drug level in Crohn's disease may not necessarily equate to a therapeutic drug level in UC. Similar challenges arise in the interpretation of ADAb levels; for example, when considering "low levels" of antibodies – which may influence therapeutic strategies – a definition is lacking.

This issue is confounded further by the varying methodologies employed in the different assays used to detect drug levels and ADAb thus making direct comparison of results challenging. A round-robin experiment used three different ELISAs measuring infliximab and ADAb (two academically designed "in-house" assays and one commercially available kit) amongst 62 samples (36 from clinical patients with known varying infliximab, adalimumab, and ADAb concentrations and 26 healthy control samples spiked with known concentrations of infliximab, adalimumab, and ADAb) [45]. Although all three assays demonstrated good linear correlation for the detection of ADAb, one kit detected infliximab in 11 (18%) samples not detected by the others, including five spiked samples known to contain only ADAb and undetectable drug, although these results have subsequently been challenged [82,83]. Likewise, different units, the use of semi-quantitative results, and the measurement of free and total antibodies make interpretation of

ADAb levels even more challenging, particularly in light of recent data suggesting that not only may antibodies be transient, but also that immunomodulation may decrease antibody levels [78].

Using drug levels in IBD to treat to a target therapeutic range takes TDM a step further. Ongoing trials are examining this issue; the first such trial, TAXIT, recently reported its results. In this study, treating to target drug levels was compared with dose adjustment based on clinical parameters following an optimization phase. In this phase, 275 patients had drug and antibody levels tested, of whom 120 (44%) had levels within the target range (3–7 μg/mL) and did not require dose modification. Seventy-two (26%) had the dosing interval prolonged because of supratherapeutic trough concentrations and 83 (30%) required dose intensification to reach the target range [75]. Trough levels were inversely correlated with CRP levels. Patients were then randomized to clinically-based dose adjustment (CB) or level-based dose adjustment (LB) [84]. The primary endpoint of clinical and biochemical remission at 12 months was not different between the two treatment arms (69% CB vs. 72% LB; p=0.7). However, at 12 months, more patients in the LB arm reached target trough levels compared with the CB arm (78% vs. 56%; p<0.0001). Undetectable trough levels and ADAb were more common in the CB arm compared with the LB arm (RR 3.7, 95% CI 1.7–8.0, p<0.0001; and RR 3.3, 95% CI 1.4–7.7, p<0.01; respectively). The authors concluded that TDM allowed more efficient use of drug but did not show superiority over clinical-based judgment. Nevertheless, given the relatively short follow-up of this study and the fact that biochemical differences were seen, it may be that drug level-driven dosing proves to be superior in the long-term.

Also of importance is that in secondary loss of response, drug level-driven management-decision algorithms, rather than stepwise empiric dose intensification followed by within-class switching, may allow more cost-effective use of drug. Given the expense of anti-TNF therapy, this is certainly of relevance. Two recent studies support this hypothesis. The first, a decision analytic model in patients with Crohn's disease and secondary loss of response to infliximab, simulated the TDM algorithm first described in the study by Afif et al. [79] against an empiric intensification strategy [85]. After 12 months, clinical outcomes were not significantly different between the cohorts (remission 63% vs. 66% and response 28% vs. 26%). Quality-adjusted life-year gained was similar; however, the testing strategy was found to be less expensive (US$31 870) compared with the empiric dose intensification (US$37 266) strategy, although it should be noted this study did not incorporate the full cost of the HMSA, estimated at US$2500 [86]. Nevertheless, a randomized, controlled, single-blind, multicenter study of 69 patients with Crohn's disease and secondary loss of response to infliximab supported this mathematical model, concluding that a TDM strategy yielded similar response rates when compared with empiric dose intensification, but at a significantly lower cost [87].

Conclusion

The introduction of anti-TNF therapy has revolutionized the modern management of IBD, leading to improved patient outcomes. Nevertheless, a substantial number of patients will eventually lose response to these agents. Evidence supports a relationship between drug levels and clinical outcomes, whereas less is known regarding the impact of ADAb. TDM provides the treating physician with the tools that help guide rational, informed clinical management decisions in patients with loss of response, and recent data support the concept that acting on drug levels can improve disease activity. However, as with all new technologies, many questions remain unanswered, such that our knowledge is limited as to how drug and antibody levels should be used. These uncertainties are compounded by the number of different assays available; not only is it unclear how transferrable findings are across studies using different technologies, but also how transferrable they are between studies using different versions of the same technology. Finally, as the cost of healthcare continues to accelerate, pharmocoeconomic support for drug-level testing is required to support its long-term usefulness. This will undoubtedly be impacted by the costs of the different assays, which vary greatly. Nonetheless, it is to be hoped that the initial promising data will be supported by the results of large controlled trials such that anti-TNF drug level and ADAb testing edges IBD further forward into the age of personalized medicine.

Disclosures

Dr Irving has received fees for lecturing and acting in an advisory capacity from Abbvie and MSD. Dr Ward has no relevant interests to disclose.

References

1. Targan SR, Hanauer SB, van Deventer SJ et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;**337**:1029–35.
2. Derkx B, Taminiau J, Radema S et al. Tumour-necrosis-factor antibody treatment in Crohn's disease. *Lancet* 1993;**342**:173–4.
3. Summers RW, Switz DM, Sessions JT et al. National Cooperative Crohn's Disease Study: results of drug treatment. *Gastroenterology* 1979;**77**:847–69.
4. Malchow H, Ewe K, Brandes JW et al. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984;**86**:249–66.
5. Faubion WA, Loftus EV, Harmsen WS et al. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001;**121**:255–60.
6. Irving PM, Gearry RB, Sparrow MP et al. Review article: appropriate use of corticosteroids in Crohn's disease. *Aliment Pharmacol Ther* 2007;**26**:313–29.
7. Hanauer S, Feagan B, Lichtenstein G et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;**359**:1541–9.
8. Present DH, Rutgeerts P, Targan S et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999;**340**:1398–405.

9. Hanauer SB, Sandborn WJ, Rutgeerts P et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006;**130**:323–33; quiz 591.
10. Sandborn WJ, Hanauer SB, Rutgeerts P et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007;**56**:1232–9.
11. Colombel JF, Sandborn WJ, Rutgeerts P et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007;**132**:52–65.
12. Schreiber S, Khaliq-Kareemi M, Lawrance IC et al. Maintenance therapy with certolizumab pegol for Crohn's disease. *N Engl J Med* 2007;**357**:239–50.
13. Schreiber S, Colombel JF, Bloomfield R et al. Increased response and remission rates in short-duration Crohn's disease with subcutaneous certolizumab pegol: an analysis of PRECiSE 2 randomized maintenance trial data. *Am J Gastroenterol* 2010;**105**:1574–82.
14. Schreiber S, Rutgeerts P, Fedorak RN et al. A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. *Gastroenterology* 2005;**129**:807–18.
15. Rutgeerts P, Sandborn W, Feagan B et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005;**353**:2462–76.
16. Reinisch W, Sandborn WJ, Hommes DW et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* 2011;**60**:780–7.
17. Kamm MA, Hanauer SB, Panaccione R et al. Adalimumab sustains steroid-free remission after 3 years of therapy for Crohn's disease. *Aliment Pharmacol Ther* 2011;**34**:306–17.
18. Colombel JF, Sandborn WJ, Reinisch W et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;**362**:1383–95.
19. D'Haens G, Baert F, van Assche G et al.; Belgian Inflammatory Bowel Disease Research Group; North-Holland Gut Club. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet* 2008;**371**:660–7.
20. Rutgeerts P, Van Assche G, Sandborn WJ et al.; EXTEND Investigators. Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: data from the EXTEND trial. *Gastroenterology* 2012;**142**:1102–11.e2.
21. Baert F, Moortgat L, Van Assche G et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. *Gastroenterology* 2010;**138**:463–8.
22. Rutgeerts P, Diamond RH, Bala M et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. *Gastrointest Endosc* 2006;**63**:433–42; quiz 464.
23. Lichtenstein G, Yan S, Bala M et al. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. *Gastroenterology* 2005;**128**:862–9.
24. Bernstein CN, Loftus EV Jr, Ng SC et al.; Epidemiology and Natural History Task Force of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD). Hospitalisations and surgery in Crohn's disease. *Gut* 2012;**61**:622–9.
25. Peyrin-Biroulet L, Oussalah A, Williet N et al. Impact of azathioprine and tumour necrosis factor antagonists on the need for surgery in newly diagnosed Crohn's disease. *Gut* 2011;**60**:930–6.
26. Feagan B. The effects of infliximab maintenance therapy on health-related quality of life. *Am J Gastroenterol* 2003;**98**:2232–8.
27. Otley AR, Hyams J, Baldassano R et al. The effects of infliximab therapy on health-related quality of life in pediatric Crohn's disease. First International Symposium on Pediatric Inflammatory Bowel Disease, Rome, Italy. *J Pediatr Gastroenterol Nutr* 2006;**43**:S48–9.
28. Bodger K. Cost of illness of Crohn's disease. *Pharmacoeconomics* 2002;**20**:639–52.
29. Lindsay JO, Chipperfield R, Giles A et al.; INDIGO study investigators. A UK retrospective observational study of clinical outcomes and healthcare resource utilisation of infliximab treatment in Crohn's disease. *Aliment Pharmacol Ther* 2013;**38**:52–61.
30. Feagan BG, Sandborn WJ, Yang M et al. P-61 Adalimumab therapy reduces hospitalization and colectomy rates in patients with ulcerative colitis: data from controlled trials. *Inflamm Bowel Dis* 2011;**17**(Suppl. 2):S31.
31. Järnerot G, Hertervig E, Friis-Liby I et al. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology* 2005;**128**:1805–11.
32. Laharie D, Bourreille A, Branche J et al. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. *Lancet* 2012;**380**:1909–15.
33. Pearce CB, Lawrance IC. Careful patient selection may improve response rates to infliximab in inflammatory bowel disease. *J Gastroenterol Hepatol* 2007;**22**:1671–7.
34. Trinder MW, Lawrance IC. Efficacy of adalimumab for the management of inflammatory bowel disease in the clinical setting. *J Gastroenterol Hepatol* 2009;**24**:1252–7.
35. Hommes DW, van de Heisteeg BH, van der Spek M et al. Infliximab treatment for Crohn's disease: one-year experience in a Dutch academic hospital. *Inflamm Bowel Dis* 2002;**8**:81–6.
36. Regueiro M, Siemanowski B, Kip KE et al. Infliximab dose intensification in Crohn's disease. *Inflamm Bowel Dis* 2007;**13**:1093–9.
37. Schnitzler F, Fidder H, Ferrante M et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. *Gut* 2009;**58**:492–500.
38. Rutgeerts P, D'Haens G, Targan S et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. *Gastroenterology* 1999;**117**:761–9.
39. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009;**104**:760–7.
40. Karmiris K, Paintaud G, Noman M et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009;**137**:1628–40.
41. Sandborn WJ, Colombel JF, Schreiber S et al. Dosage adjustment during long-term adalimumab treatment for Crohn's disease: clinical efficacy and pharmacoeconomics. *Inflamm Bowel Dis* 2011;**17**:141–51.
42. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther* 2011;**33**:987–95.
43. Kopylov U, Mantzaris GJ, Katsanos KH et al. The efficacy of shortening the dosing interval to once every six weeks in Crohn's patients losing response to maintenance dose of infliximab. *Aliment Pharmacol Ther* 2010;**33**:349–57.
44. Ma C, Panaccione R, Heitman SJ et al. Systematic review: the short-term and long-term efficacy of adalimumab following discontinuation of infliximab. *Aliment Pharmacol Ther* 2009;**30**:977–86.
45. Vande Casteele N, Buurman DJ, Sturkenboom MG et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. *Aliment Pharmacol Ther* 2012;**36**:765–71.
46. Wang SL, Ohrmund L, Hauenstein S et al. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods* 2012;**382**:177–88.
47. Steenholdt C, Ainsworth MA, Tovey M et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. *Ther Drug Monit* 2013;**35**:530–8.
48. Hanauer SB, Wagner CL, Bala M et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. *Clin Gastroenterol Hepatol* 2004;**2**:542–53.
49. Vermeire S, Noman M, Van Assche G et al. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* 2007;**56**:1226–31.
50. Kopylov U, Mazor Y, Yavzori M et al. Clinical utility of antihuman lambda chain-based enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. *Inflamm Bowel Dis* 2012;**18**:1628–33.
51. Imaeda H, Andoh A, Fujiyama Y. Development of a new immunoassay for the accurate determination of anti-infliximab antibodies in inflammatory bowel disease. *J Gastroenterol* 2012;**47**:136–43.
52. Bendtzen K. Anti-TNF-α biotherapies: perspectives for evidence-based personalized medicine. *Immunotherapy* 2012;**4**:1167–79.
53. Svenson M, Geborek P, Saxne T et al. Monitoring patients treated with anti-TNF-alpha biopharmaceuticals: assessing serum infliximab and anti-infliximab antibodies. *Rheumatology (Oxford)* 2007;**46**:1828–34.
54. Bendtzen K, Geborek P, Svenson M et al. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor α inhibitor infliximab. *Arthritis Rheum* 2006;**54**:3782–9.
55. Wolbink GJ, Vis M, Lems W et al. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006;**54**:711–5.
56. Wang SL, Hauenstein S, Ohrmund L et al. Monitoring of adalimumab and antibodies-to-adalimumab levels in patient serum by the homogeneous mobility shift assay. *J Pharm Biomed Anal* 2013;**78–79**:39–44.
57. Baert F, Noman M, Vermeire S et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;**348**:601–8.
58. Maser EA, Villela R, Silverberg MS et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006;**4**:1248–54.
59. Steenholdt C, Bendtzen K, Brynskov J et al. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 2011;**46**:310–8.
60. Bortlik M, Duricova D, Malickova K et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013;**7**:736–43.
61. Chiu YL, Rubin DT, Vermeire S et al. Serum adalimumab concentration and clinical remission in patients with Crohn's disease. *Inflamm Bowel Dis* 2013;**19**:1112–22.
62. Bodini G, Savarino V, Fazio V et al. Sa2045 Relationship between drug serum concentration and clinical activity in patients with Crohn disease who achieved remission with adalimumab – a prospective study. *Gastroenterology* 2012;**142**(5 Suppl. 1):S-388.
63. Seow CH, Newman A, Irwin SP et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut* 2010;**59**:49–54.
64. Murthy S, Kevans D, Seow CH et al. Sa2047 Association of serum infliximab and antibodies to infliximab to long-term clinical outcome in acute ulcerative colitis. *Gastroenterology* 2012;**142**(5 Suppl. 1):S-388.
65. Reinisch W, Feagan BG, Rutgeerts PJ et al. 566 Infliximab concentration and clinical outcome in patients with ulcerative colitis. *Gastroenterology* 2012;**142**(5 Suppl. 1):S-114.

66. Mostafa NM, Eckert D, Pradhan RS et al. Sa1195 Exposure–efficacy relationship (ER) for adalimumab during induction phase of treatment of adult patients with moderate to severe ulcerative colitis. *Gastroenterology* 2013;**144**(5 Suppl. 1):S-225–6.
67. Paul S, Del Tedesco E, Marotte H et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013;**19**:2568–76.
68. Imaeda H, Bamba S, Takahashi K et al. Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. *J Gastroenterol* 2013 May 11; Epub ahead of print.
69. Roblin X, Marotte H, Rinaudo M et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2013 Jul 23; Epub ahead of print.
70. van der Woude CJ, Deuring JJ, West R et al. Mo1062 Adalimumab trough levels in a prospective cohort of Crohn's disease patients. *Gastroenterology* 2013;**144**(5 Suppl. 1):S-567.
71. Van Assche G, Magdelaine-Beuzelin C, D'Haens G et al. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology* 2008;**134**:1861–8.
72. Sandborn WJ, Rutgeerts P, Enns R et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. *Ann Intern Med* 2007;**146**:829–38.
73. Brandse JF, Wildenberg M, de Bruyn JR et al. 157 Fecal loss of infliximab as a cause of lack of response in severe inflammatory bowel disease. *Gastroenterology* 2013;**144**(5 Suppl. 1):S-36.
74. Fasanmade AA, Adedokun OJ, Blank M et al. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 Phase III clinical trials. *Clin Ther* 2011;**33**:946–64.
75. Casteele NV, Compernolle G, Ballet V et al. 1159 Results on the optimisation phase of the prospective controlled Trough Level Adapted Infliximab Treatment (TAXIT) Trial. *Gastroenterology* 2012;**142**(5 Suppl. 1):S-211–2.
76. Lee LY, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. *Eur J Gastroenterol Hepatol* 2012;**24**:1078–85.
77. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. *Am J Gastroenterol* 2013;**108**:40–7; quiz 48.
78. Ben-Horin S, Waterman M, Kopylov U et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2013;**11**:444–7.
79. Afif W, Loftus EV Jr, Faubion WA et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010;**105**:1133–9.
80. Pariente B, Pineton de Chambrun G, Krzysiek R et al. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012;**18**:1199–206.
81. Vande Casteele N, Gils A, Singh S et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am J Gastroenterol* 2013;**108**:962–71.
82. Parussini E. Letter: detection of infliximab levels and anti-infliximab antibodies – comparison of three different assays. *Aliment Pharmacol Ther* 2013;**37**:281.
83. Buurman DJ, Vande Casteele N, Sturkenboom MG et al. Letter: detection of infliximab levels and anti-infliximab antibodies – comparison of three different assays; authors' reply. *Aliment Pharmacol Ther* 2013;**37**:282.
84. Vande Casteele N, Gils A, Ballet V et al. OP001 Randomised controlled trial of drug level versus clinically based dosing of infliximab maintenance therapy in IBD: final results of the TAXIT Study. UEG Week 2013 Oral Presentations.
85. Velayos FS, Kahn JG, Sandborn WJ et al. A test-based strategy is more cost effective than empiric dose escalation for patients with Crohn's disease who lose responsiveness to infliximab. *Clin Gastroenterol Hepatol* 2013;**11**:654–66.
86. Moss AC. True costs of infliximab testing and administration. *Clin Gastroenterol Hepatol* 2013 Jun 28; Epub ahead of print.
87. Steenholdt C, Brynskov J, Thomsen OO et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. *Gut* 2013 Jul 22; Epub ahead of print.

CHAPTER 6:

Analytical perspectives: Inter-kit comparison of ELISAs for therapeutic drug monitoring of infliximab and adalimumab in Crohn's disease

TITLE PAGE

Inter-kit comparison of ELISAs for therapeutic drug monitoring of infliximab and adalimumab in Crohn's disease

Short title: Infliximab and adalimumab ELISA comparison

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Abbreviations:
IFX = infliximab
ADA = adalimumab
ADAb= Anti-drug antibody
TDM = therapeutic drug monitoring
LT = Lisa-Tracker assay
IM = IDKmonitor assay
PRO = Promonitor assay
RIDA = RIDAscreen assay
ELISA = enzyme-linked immunosorbent assay
HMSA = homogenous mobility shift assay
TNF = tumor necrosis factor
ICC = intra-class coefficient

Abstract

Background: Infliximab (IFX) and adalimumab (ADA) drug levels and anti-drug antibodies (ADAb) guide management in inflammatory bowel disease (IBD). Data comparing enzyme-linked immunosorbent assays (ELISAs) is limited. Inter-kit variation may influence clinical outcomes.

Aims: To compare IFX and ADA drug levels and ADAb between four different ELISAs and to determine misclassification rates for drug levels, assay bias and concordance between kits.

Methods: Samples from Crohn's disease patients receiving maintenance IFX (n = 105) and ADA (n = 98) were analysed using LISA-TRACKER (LT), IDK*monitor*® (IM), Promonitor (PRO) and RIDAscreen (RIDA) assays. Levels < 2 (IFX) or < 4.9 μg/mL (ADA) were considered sub-therapeutic.

Results: IFX drug levels (μg/mL) were highest with RIDA showing average positive bias against LT (2.7), IM (3.1) and PRO (2.0). Degree of absolute bias between RIDA and LT was concentration dependent but proportional whereas bias against PRO and IM was variable. LT ADA drug levels showed systematic negative bias (-5.0 and -4.8 μg/mL) against IM and PRO respectively. ADAb were more frequently seen with IM (22% IFX and 6% ADA) reflecting methodological differences between assays. Applying therapeutic cut-off concentrations using LT as the reference resulted in a misclassification rate > 6% (IFX) and > 19% (ADA) with other assays.

Conclusion: Variable bias in IFX was observed between ELISAs whereas bias in ADA was consistent. This results in misclassification into therapeutic categories when kit specific cut- offs are not used. In the absence of assay standardisation, use of method-specific cut-offs is essential in managing patients with IBD.

Keywords: drug level, Crohn's disease, therapeutic drug monitoring

INTRODUCTION

A growing body of data supports the clinical utility of therapeutic drug monitoring (TDM) of infliximab (IFX) and adalimumab (ADA) in the management of Crohn's disease and ulcerative colitis. Studies have consistently reported an association between low IFX and ADA drug levels and

higher rates of secondary loss of response[1-3] and lower rates of mucosal healing[4,5]. Less evidence exists on the relevance and importance of anti-drug antibodies due to a number of factors. First, assays detect antibodies in different ways and report the results using different units thus comparisons across different assays are difficult.[6]. Second, some anti-drug antibodies are transient which limits the value of a single result[7]. Third, differences in assay design and sample processing mean that some assays detect the presence of free antibodies, in other words only detecting antibodies in the absence of detectable drug, while others detect total antibody concentrations [6,8]. Despite this, recent meta-analyses have demonstrated higher rates of clinical loss of response in patients with detectable anti-drug antibodies with both IFX and ADA.[9,10]

Several different assays are in use, many of which are available for commercial use and some of which have been developed 'in-house'. These can broadly be summarised into three distinct platforms with key methodological differences; ELISA, fluid phase radio-immunoassay and homogenous mobility-shift assay (HMSA). To date, the majority of data has arisen from studies employing ELISA based assays[10,12]. This is likely a reflection of their widespread uptake in practice relative to other platforms, due to lower cost, familiarity with the laboratory technique and wide availability. A key issue in interpreting this literature is a lack of high quality published data directly comparing individual ELISAs.[8,13,14]

Such comparisons are important; in the case of drug levels a sample analysed using one ELISA assay may not give the same result if performed on another. Reported therapeutic cut-offs may, therefore, not be applicable on different assays, which may, in theory, influence clinical outcomes should patients be misclassified as having therapeutic or sub-therapeutic drug levels. Further, the variation in antibody detection methods as well as the development of drug tolerant assays for antibody detection has complicated matters further.[15] The clinical relevance of antibodies, particularly transient antibodies and those seen in the presence of detectable drug, is uncertain. Such gaps in our understanding of the role of TDM in IBD have been highlighted in a recent statement by NICE

(National Institute for Health and Care Excellence), recommending that further inter-kit comparative data are needed before TDM can be implemented into everyday practice.[16]

Accordingly, the aim of the current study was to perform an inter-kit comparison of four ELISAs used for IFX and ADA TDM in Crohn's disease and explore whether variation between kits resulted in misclassification of drug level status.

MATERIALS AND METHODS

Study design

We performed a cross-sectional observational study comparing ELISAs used to measure IFX and ADA drug levels and anti-drug antibodies on samples collected from the outpatient clinics of Guy's and St. Thomas' NHS Foundation Trust and Addenbrookes' Hospital, United Kingdom between October 2013 and April 2014.

Patient population and TDM platform

Peripheral blood was collected from adult patients with Crohn's disease established on maintenance IFX or ADA (> 14 weeks of therapy) at doses of 5 mg/kg 8 or 6 weekly or 10 mg/kg 8-weekly (IFX) or 40 mg every other week, each week or every 10 days (ADA). IFX samples were collected at trough, defined as prior to the next scheduled infusion. ADA samples were collected at any time point in a treatment cycle. IFX drug levels were compared using four commercially available ELISAs: LISA-TRACKER (LT) Duo (Theradiag, France), IDK*monitor*® (IM) (Immundiagnostik, Germany), Promonitor (PRO) Progenika Biopharma, Spain) and RIDASCREEN (RIDA) IFX (RS, R-Biopharm AG / KU Leuven). ADA drug levels were compared using LT, IM and PRO assays. Anti-drug antibodies were measured on LT, IM and PRO platforms with LT and PRO assays measuring "free" anti-drug antibody and IM measuring "total" anti-drug antibody. Drug levels are reported in µg/mL and anti-drug antibodies in ng/mL (LT) and AU/mL (IM and PRO).

Laboratory Methods

Serum samples were collected in serum separator tubes (SST) and centrifuged at 3000 rpm for ten minutes prior to storage at -20ºC. Frozen samples were thawed on a roller mixer and re-centrifuged prior to analysis. Thawed samples were stored at 2-8ºC for a maximum of five days during parallel analysis using different ELISA kits.

All ELISA assays were automated on the eRobot^2 (LT) or Triturus® (Grifols International, Barcelona, Spain) (PRO, IM and RS). Assay parameters were programmed in accordance with manufacturer instructions incorporating all necessary validation criteria for assay acceptance including quality control. Data presented in this manuscript is based on the first version of the IM IDKmonitor IFX kit which utilised a recombinant TNFα coated microtitre plate. All samples analysed using this version of the assay were subsequently repeated using the new format (which utilised a monoclonal anti-IFX antibody coated microtitre plate) to eliminate this modification in kit format as a cause of variation.

Infliximab Drug Levels

- *LT:* Assays were automated on the eRobot^2 platform. Pre-diluted (1 in 100) patient samples, calibrators and control were added to microwell plates coated with TNFα and incubated at room temperature for 60 minutes. Following three wash cycles, biotinylated anti-human IgG1 antibody was added to each well and incubated at room temperature for 60 minutes. Following further wash cycles, streptavidin-horseradish peroxidase (HRP) conjugate was added to wells and incubated for 30 minutes at room temperature. After a final wash cycle, enzyme substrate (3,3',5,5' tetramethylbenzidine, TMB) was added to the wells and incubated for 15 minutes forming a blue colour. The enzymatic reaction was stopped by addition of sulphuric acid (0.25M) giving rise to a yellow colour, the optical density (OD) of which was read at 450 nm (620 nm reference filter) using an on-board plate reader. Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated. Patient samples with results above the measuring range were re-analysed on dilution with wash buffer (1 in 3 or greater as required).

- *PRO:* Assays were automated on the Grifols Triturus platform. Pre-diluted (1 in 10 and 1 in 200) patient samples, calibrators and controls were added to microwell plates coated with TNFα bound to monoclonal anti-TNFα antibody and incubated at room temperature for 60 minutes. Following three wash cycles, horseradish peroxidase (HRP) conjugated anti-IFX antibody was added to wells and incubated for 60 minutes at room temperature. After a final wash cycle, enzyme substrate (3,3',5,5' tetramethylbenzidine, TMB) was added to the wells and incubated for 15 minutes forming a blue colour. The enzymatic reaction was stopped by addition of stop solution giving rise to a yellow

colour, the optical density (OD) of which was read at 450 nm (620 nm reference filter) using an on-board plate reader. Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated. Patient samples with results above the measuring range were re-analysed on dilution with wash buffer (manual 1 in 8 dilution: x80 / x1600 final dilution factors).

- *IM:* Assays were automated on the Grifols Triturus platform. Pre-diluted (1 in 200) patient samples, calibrators and controls were added to microwell plates coated with monoclonal anti-IFX antibody and incubated at room temperature for 60 minutes on a horizontal shaker. Following five wash cycles, horseradish peroxidase (HRP) conjugated anti-IFX antibody was added to wells and incubated for 60 minutes at room temperature on a horizontal shaker. After a final wash cycle, enzyme substrate (3,3',5,5' tetramethylbenzidine, TMB) was added to the wells and incubated for 15 minutes forming a blue colour. The enzymatic reaction was stopped by addition of stop solution giving rise to a yellow colour, the optical density (OD) of which was read at 450 nm (620 nm reference filter) using an on-board plate reader. Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated.

- *RS:* Assays were automated on the Grifols Triturus platform. Pre-diluted (1 in 100) patient samples, calibrators and controls were added to microwell plates coated with monoclonal anti-IFX antibody and incubated at 37ºC for 60 minutes. Following five wash cycles, horseradish peroxidase (HRP) conjugated anti-IFX antibody (MA-IFX6B&, KU Leuven) was added to wells and incubated for 30 minutes at 37ºC. After a final wash cycle, enzyme substrate (3,3',5,5' tetramethylbenzidine, TMB) was added to the wells and incubated for 10 minutes at 37ºC forming a blue colour. The enzymatic reaction was stopped by addition of 0.5 M sulphuric acid giving rise to a yellow colour, the optical density (OD) of which was read at 450 nm (620 nm reference filter) using an on-board plate reader. Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated. Patient samples with results above the measurement range were re-analysed on dilution with sample diluent (manual 1 in 4 dilution: x400 final dilution factor).

Anti-Infliximab antibody

- *LT:* Free anti-Infliximab antibody was measured in parallel to Infliximab drug levels using the Duo ELISA kit. Pre-diluted patient samples (1 in 2), calibrators and control were added to Infliximab-coated wells. All incubations and wash steps were identical to those stated for Infliximab. Biotinylated Infliximab was used as the primary conjugate (bridging ELISA). Calibration standard OD's were automatically plotted using a quadratic curve fit from which patient results were extrapolated.
- *PRO:* Free anti-Infliximab antibody was measured in parallel to Infliximab drug levels. Neat and pre-diluted patient samples (1 in 10), calibrators and controls were added to Infliximab-coated wells. Incubations and wash steps were identical to those stated for Infliximab with the exception that TMB substrate was incubated for 30 minutes. HRP-conjugated Infliximab was used as the primary conjugate (bridging ELISA). Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated.
- *IM:* Measurement of total anti-Infliximab antibody performed by dissociating anti-Infliximab antibody from Infliximab. Patient samples and controls were diluted 1 in 10 in assay buffer to dissociate anti-Infliximab antibody / Infliximab complexes. Samples and controls were incubated for 20 minutes on a horizontal shaker. A tracer / conjugate solution containing biotinylated IFX and HRP-conjugated IFX was added to all samples and incubated for 1 hour with shaking. Streptavidin coated plate was washed five times on the Grifols Triturus prior to addition of samples and controls and incubation for 1.5 hours with shaking. After a final wash cycle, TMB substrate was added and incubated for 15 minutes. After addition of stop solution, optical densities were read using an on-board plate reader at 450 nm (620 reference filter). Optical densities obtained for patient samples were divided by the OD for the cut-off control and multiplied by the assigned value (10 AU/mL) to provide semi-quantitative results. Samples with ODs less than the cut-off control were regarded as negative for total anti-Infliximab antibody.

Adalimumab Drug Levels

- *LT:* Adalimumab drug levels were measured in exactly the same way as Infliximab drug levels using the LISA-TRACKER Duo ELISA kit automated on the eRobot^2.

- *PRO:* Assays were automated on the Grifols Triturus platform. Pre-diluted (1 in 10 and 1 in 200) patient samples, calibrators and controls were added to microwell plates coated with monoclonal anti-Adalimumab antibody and incubated at room temperature for 60 minutes. Following three wash cycles, horseradish peroxidase (HRP) conjugated anti-ADA antibody was added to wells and incubated for 60 minutes at room temperature. After a final wash cycle, enzyme substrate (3,3',5,5' tetramethylbenzidine, TMB) was added to the wells and incubated for 30 minutes forming a blue colour. The enzymatic reaction was stopped by addition of stop solution giving rise to a yellow colour, the optical density (OD) of which was read at 450 nm (620 nm reference filter) using an on-board plate reader. Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated. Patient samples with results above the measuring range were re-analysed on dilution with wash buffer (manual 1 in 8 dilution: x80 / x1600 final dilution factors).

- *IM:* Adalimumab drug levels were measured on the Grifols Triturus in exactly the same way as previously described for Infliximab drug levels with the use of anti-ADA coated plates and HRP-conjugated anti-ADA primary conjugate.

Anti-Adalimumab antibody

- *LT:* Free anti-Adalimumab antibody was measured in parallel to Adalimumab drug levels using the Duo ELISA kit on the eRobot^2. Pre-diluted patient samples (1 in 2), calibrators and control were added to Adalimumab-coated wells. All incubations and wash steps were identical to those stated for Adalimumab. Biotinylated Adalimumab was used as the primary conjugate (bridging ELISA). Calibration standard OD's were automatically plotted using a quadratic curve fit from which patient results were extrapolated.

- *PRO:* Free anti-Adalimumab antibody was measured in parallel to Adalimumab drug levels on the Grifols Triturus. Neat and pre-diluted patient samples (1 in 10), calibrators and controls were added to Adalimumab-coated wells. Incubations and wash steps were identical to those stated for Adalimumab. HRP-conjugated Adalimumab was used as the primary conjugate (bridging ELISA). Calibration standard OD's were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated.
- *IM:* Total anti-Adalimumab antibody was measured by dissociation of anti-Adalimumab antibody / Adalimumab complexes with manual sample pre-treatment and subsequent analysis on the Grifols Triturus as previously described for total anti-Infliximab antibody.

Qualitative impact of inter-kit variation in drug levels

We investigated how frequently differences in reported drug levels between kits would translate into qualitative differences in drug level status. In this study, the LT assay was considered the reference assay to which other assays were compared, given it is in routine use at our institution. IFX drug levels < 2 μg/mL were defined as sub-therapeutic (as per our practice and in line with a recent meta-analysis[12]) and ADA drug levels < 4.9 μg/mL sub-therapeutic[17,18]. Drug levels (therapeutic or sub-therapeutic) using LT were then compared to paired IM, PRO and RIDA samples for agreement and misclassification rate. This was conducted in a sub-group of samples (IFX = 96, ADA = 95) from individual patients (given the additional samples reported elsewhere were samples taken from the same patient, but at different time intervals.

Statistical Analysis

SPSS Version 21 (IBM Inc, Chicago, IL) and Prism Version 6.0 (Graphpad Software, San Diego, CA) were used for statistical analyses and generation of graphs. Method comparisons were performed by means of difference plots and Passing Bablok regression analysis[19] using Analyse-It Version 2.11 (Analyse-it Software, Ltd. http://www.analyse-it.com/; 2009) on Microsoft Excel Version 15.24 (XP professional edition, Microsoft Corp, Redmond, WA). Between group comparisons were performed

using Kruskal-Wallis or Mann-Whitney U tests, as appropriate. Drug levels from kits were compared for linear correlation using Spearman rho. Intra-class coefficient (ICC) values (reported as absolute agreement using a two-way mixed model single measures test) were interpreted as follows: 0 – 0.3 lack of agreement, 0.31 – 0.5 weak agreement, 0.51 – 0.7 moderate agreement, 0.71 – 0.9 strong agreement and > 0.91 very strong agreement.[20] Agreement in drug level status between other assays compared to LT was performed using the method described by Fleiss[21] and expressed as the positive and negative percent agreement (correlating with therapeutic and sub-therapeutic classification, respectively). Coefficient of agreement was reported using Cohen's kappa (K) with 95% confidence interval (CI) calculated as K±1.96(standard error K); and classified as almost perfect (above 0.9), strong (0.8 – 0.9), moderate (0.6 – 0.79), weak (0.4 – 0.59), minimal (0.21 – 0.39), and none (0 – 0.2).[22] All reported p-values were 2-sided; $p \leq 0.05$ were considered statistically significant.

Ethical considerations

As the samples were collected as part of routine clinical care, the study was considered a service evaluation and ethical approval was not required, according to the guidelines of the UK Health Research Authority. All authors had access to the study data, and reviewed and approved the final manuscript.

RESULTS

Infliximab

IFX drug levels were measured in 100 samples with LT, IM and PRO assays and in 99 samples with RIDA assay (1/100 samples were of insufficient volume). 4/100 (4%), 6/100 (6%), 4/100 (4%) and 3/99 (3%) samples were below the lower limit of quantification with LT, IM, PRO and RIDA and were considered as 0 μg/mL. Drug levels according to assay are shown (Fig 1). There was a significant difference in median drug levels between assays (Kruskal-Wallis $p = 0.0049$) as shown in table (1). There were no significant differences in drug levels between LT, IM and PRO. Linear correlation between IFX drug levels and assays was determined using Spearman rho (Table 1). Correlation of drug levels between all assays was acceptable with the closest correlation observed between LT and RIDA (rho = 0.98, 95% CI: 0.97 – 0.99, $p < 0.0001$). However, significant variation in bias (including direction of bias and scatter of results) was observed between RIDA and LT against PRO and IM as shown in Figures 2-7.

Agreement between assays was expressed using an intra-class coefficient (ICC); the closest agreement was seen between PRO and RIDA (ICC = 0.93, 95% CI: 0.73 - 0.97, $p < 0.0001$). The remaining assays were in strong agreement, although this was comparably weaker between IM and RIDA (ICC = 0.74, 95% CI: 0.36 – 0.87, $p < 0.0001$) and IM and PRO (ICC = 0.75, 95% CI: 0.63 - 0.84, $p < 0.0001$) (Table 2).

Anti-drug antibodies were present in 3/96 (3.2%) of LT samples; (titre >200, 149 and 12) 2 of these were also detected with IM (titre 78 and 29) and PRO (titre 7 and 5). Anti-drug antibodies were detected more frequently with the IM assay (which measures total anti-drug antibody) found in 21/96 (21.9%) samples, (median titre 35, IQR (17 – 58). Drug levels were similar between samples with undetectable and detectable anti-drug antibodies using the IM assay (3.9 vs 3.3 μg/mL, $p = 0.107$), (Fig 11).

Adalimumab

ADA drug levels measured in 98 samples according to assay are shown in Fig 1. Drug levels were significantly different between assays (Kruskal-Wallis $p < 0.0001$). The median drug level (IQR) with LT was significantly lower compared to IM and PRO (6.1 μg/mL (4.9 – 7.9) vs 11 μg/mL (8.4 – 14.1) and μg/mL (8.1 – 14.1), $p < 0.0001$) respectively. No difference in drug levels was observed between IM and PRO ($p = 0.65$). All three showed good linear correlation (LT vs IM rho = 0.92, $p < 0.0001$, LT vs PRO rho = 0.75, $p < 0.0001$ and IM vs PRO rho = 0.84, $p < 0.0001$, Table 1).

Agreement according to ICC was strong between IM and PRO (ICC = 0.86, 95% CI: 0.79 – 0.90; $p < 0.0001$) however weak between LT and PRO (ICC = 0.45, 95% CI: -0.09 – 0.75; $p < 0.0001$) and only just moderate between LT and IM (ICC = 0.51, 95% CI: -0.09 – 0.81; $p < 0.0001$). According to Bias plots (Fig 8-10), IM and PRO were in close agreement, where the mean difference between the two was 0.18 μg/mL (95% LOA: -5.88 – 5.53). LT showed a systematic negative mean bias (-5.1 and -4.8 μg/mL) against IM and PRO respectively.

Anti-drug antibodies were present in 1/98 (1%) of LT samples (titre 39 ng/mL), 2/96 (2.1%) of PRO samples (2 samples of insufficient volume to perform analysis, titre 56 AU/mL and 132 AU/mL) and in 6/98 (6.1%) of IM samples (titre 12 AU/mL in n = 3, 10 AU/mL n = 1, titre 299 AU/mL and 1175 AU/mL in remaining). The sample with detectable anti-drug antibody using LT was also detected on PRO and IM. 1/2 samples with detectable anti-drug antibodies using PRO were not detected using LT or IM. A trend towards lower drug levels amongst samples with detectable anti-drug antibodies, compared with no anti-drug antibodies was observed using IM assay (5.5 vs 11 μg/mL, $p = 0.055$), (Fig. 10).

Clinical impact of difference in drug levels between kits

Infliximab

Qualitative agreement in drug level status (therapeutic or sub-therapeutic) was strong between LT and PRO (K = 0.84 ($p < 0.001$), 95% CI: 0.72 - 0.96) and moderate between LT and IM (K = 0.78 ($p < 0.001$), 95% CI: 0.64 - 0.92) and LT and RIDA (K = 0.76 ($p < 0.001$), 95% CI: 0.60 - 0.92). 8/98 (8%) drug levels using IM were misclassified as therapeutic (3/24) and sub-therapeutic (5/72) compared to LT (negative and positive agreement 88 and 93%, respectively) (Table 2). 6/96 (6%) drug levels with PRO were misclassified as therapeutic (1/24) and sub-therapeutic (5/72) compared to LT, (negative and positive agreement 96 and 93%, respectively). 8/96 (8%) drug levels with RIDA were misclassified as therapeutic (7/24) and sub-therapeutic (1/72) compared to LT, (negative and positive agreement 71 and 99%, respectively).

Adalimumab

Agreement in drug level status was almost perfect between IM and PRO kits (K = 0.90 ($p < 0.001$), 95% CI: 0.7 - 1.0) and minimal between LT and IM (K = 0.32 ($p < 0.001$), 95% CI: 0.1 - 0.5) and between LT and PRO (K = 0.27 ($p < 0.001$), 95% CI: 0.5 – 0.7). 19/25 (76%) of IM and 20/25 (80%) of PRO drug levels classified as therapeutic with these assays were sub-therapeutic with LT (Table 3). 1/98 (1%) of PRO drug levels classified as therapeutic were sub-therapeutic with IM. The positive percentage agreement for therapeutic drug levels was 100% for IM and PRO compared with LT.

DISCUSSION

Our study demonstrates significant inter-kit variability between ELISAs used for TDM.

IFX drug levels were highest with RIDA showing average positive bias against LT (2.7 μg/mL), IM (3.1 μg/mL) and PRO (2.0 μg/mL). However, assessing agreement between assays on the basis of mean bias alone is misleading. This is evident when looking at the direction and distribution of bias observed between LT and RIDA vs. IM and PRO. The degree of absolute bias between RIDA and LT was concentration dependent but proportional whereas bias against PRO and IM was variable making test interpretation extremely difficult. The change in direction of bias observed could not be explained by modification in the assay format for IM during this study as confirmed by repeat analysis of samples using the new format.

For ADA, drug levels were significantly lower with LT compared with PRO and IM Qualitatively, this correlated to a substantial proportion of samples which would be misclassified as therapeutic or sub-therapeutic, depending on the assay employed which may have clinical implications. In the absence of method-specific therapeutic cut-offs, the constant bias observed between LT against PRO and IM may allow extrapolation of therapeutic ranges however when monitoring patients and modifying therapy based on drug levels, results are not be interchangeable between methods.

Using IM, anti-drug antibodies were seen more frequently with IFX than with ADA, although qualitative outcomes between other assays was comparable.

TDM has become an important tool in the armamentarium of clinicians managing patients with inflammatory bowel disease. A large body of data has shown an inverse relationship between IFX and ADA drug levels and clinical outcomes[10,12], and, to a lesser extent, that the development of sustained detectable anti-drug antibodies is associated with subsequent loss of response[9,10] and an increased risk of infusion reactions[23]. In the setting of secondary loss of response to these therapies, sub-therapeutic drug levels can select which patients will be more likely to respond to dose intensification[24,25].

Conversely the finding of adequate drug levels can identify those who would benefit from a switch out-of-class to an alternative agent[24]. In the case of detectable anti-drug antibodies changing therapy within class, expectant management[24] or the introduction of concomitant immunomodulation can lead to recapture of response[26,27]. Further, evidence suggests that the results of TDM obtained soon after initiating an anti-TNF[2,28] can predict long term outcomes before an anticipated loss of response occurs, and that early, proactive management may circumvent such issues. Management algorithms which incorporate TDM have been shown to be more cost effective than reactive empirical strategies[29,30]. Taken together, TDM has a broad range of clinical roles and is now established as an integral component in the management of inflammatory bowel disease.

Decisions based on the results of TDM rely in part on the definition, or more specifically the cut-off, that defines a therapeutic drug level. In this regard data pertaining to target concentrations in the literature vary widely due to factors which include the TDM platform used, population studied, design of the study and the end-point against which drug levels are measured. In a retrospective observational study of 255 patients with Crohn's disease treated with maintenance IFX, Van Moerkercke et al identified an association between higher IFX trough levels and mucosal healing (5.8 vs 0.95 μg/mL, $p = 0.013$).[31] In ulcerative colitis, using the HMSA technology, an IFX drug level > 2 μg/mL was associated with improved rates of steroid-free clinical remission (69 vs 16%).[32]. In Crohn's disease using ELISA, a similar therapeutic cut-off after week 14 for IFX (> 3 μg/mL) was found to predict long term clinical remission.[28] A meta-analysis of 5 studies reporting on 459 patients receiving maintenance ADA found that a trough level 4.85 – 5.9 μg/mL was associated with improved rates of clinical remission (OR 2.6; 95% CI: 1.79 – 3.77, $p < 0.0001$)[10]. Recent studies have reported that higher drug levels may be required to completely neutralise TNFa levels in order to achieve deep remission; in a retrospective study of 66 patients with inflammatory bowel disease, Yarur et al observed higher ADA drug levels in patients with endoscopic and histologic remission (13.3 μg/mL) compared to those with active disease (9.2 μg/mL, $p = 0.02$) using HMSA.[33]

Despite a large number of studies investigating the utility of TDM, data directly comparing ELISAs is surprisingly lacking. A round-robin experiment of 62 serum samples and spiked controls performed on two academic in-house and one commercially available drug sensitive ELISAs (including LISA-TRACKER, reported in our study) demonstrated good inter-kit linear correlation, however qualitatively one assay detected IFX in 11/62 (18%) samples not detected with others. These findings were subsequently challenged by the manufacturer of this assay[34]. Whilst agreement between inter-assay agreement was good, differences in mean drug levels and hence the potential clinical impact, was not reported.

We demonstrated good correlation between some assays but not others. For example, when measuring IFX levels the distribution of bias between methods was variable (-6.7 to +87.8%) with PRO and ID showing scattered, bimodal distributions of percentage bias. A consistent, proportional relationship however was observed between the LT assay and the RS assay. The magnitude of this bias (+50.4%) is in agreement with the lower end of the therapeutic range for the LT assay (>2.0 ug/mL) and that which is used for the RIDA assay (3.0 ug/mL)[35]. It is, therefore, possible to extrapolate the therapeutic range for the RS assay (3-7ug/ml) to an approximated therapeutic range for the LT assay (2-5 μg/mL). Unfortunately, given the much greater variation between the RIDA and LT assays and the other assays, interpretation of results between these platforms is not possible. Whilst there is no gold standard for the measurement of anti-TNF drug levels, the RIDA kit has been used extensively in studies performed in Leuven and is, therefore, of some value when making clinical decisions.

In keeping with this we demonstrated good linear correlation between all assays for both IFX and ADA ($rs \geq 0.85$, $p < 0.0001$). This result can be misleading; despite a high correlation coefficient implying a strong linear relationship, individual values can deviate significantly from one another. Accordingly, we assessed the degree of agreement in drug levels between assays using Bland-Altman plots and interclass coefficients. Appling these statistical methods we found that IFX drug levels were, on average, 3.7, 4.2 and 1.8 µg/mL higher using RIDA compared with LT, IM and PRO, respectively. Considering ADA, drug levels using LT were 5.0 and 4.8 µg/mL lower than with IM

and PRO respectively. Agreement was excellent between IM and PRO for ADA (mean bias = 0.17 μg/mL).

Given the similarity between infliximab and adalimumab, the degree of variation observed between ELISA kits was not consistent. The assays for ADA, PRO and IM share similar designs, whereas LT is different. This may explain the close correlation between PRO and IM and the constant negative bias for LT. However, for IFX, there is a higher degree of variation between assays which can probably be attributed to differences in microplate coatings and antibody conjugates used in assay design.

For ADA drug levels, agreement was minimal between LT and both IM (K = 0.32) or PRO (K = 0.27), resulting in a misclassification rate in approximately 20% with sub-therapeutic LT drug levels defined as therapeutic using these other assays. This has clear clinical implications. Current treatment algorithms for loss of response propose dose intensification in the setting of sub-therapeutic drug levels and out-of-class switching when therapeutic drug levels are found. Hence management decisions based around a therapeutic cut-off need to be made on assay-specific values.

Using LT and PRO, free anti-drug antibodies were detected in < 4% of samples which is comparable to that reported elsewhere (0.9 – 43% and 2.8 – 9.2% during IFX and ADA maintenance therapy, respectively).[36] As expected, anti-drug antibodies were found more frequently with the IM assay in 22% of IFX and 6% of ADA samples as it measures both free and drug-bound ADAb rather than free ADAb alone. Data on the clinical significance of total, rather than free anti-drug antibody is scarce. In this cohort, there was no difference in IFX drug levels in patients with and without total anti-drug antibodies. In order to investigate this further, we retrospectively reviewed the outcomes of 21 patients treated with IFX who had detectable total ADAb and undetectable free ADAb using the IM assay.[37] Of the 3/21 (14%) who went on to develop free anti-drug antibodies, all developed sub-therapeutic drug levels and required a switch in anti-TNF therapy due to loss of response. None of the

remaining 18 patients developed undetectable drug levels, however 6% required a switch in anti-TNF therapy, 22% a clinical flare and 17% required corticosteroids. Total anti-drug antibodies did not accurately predict the development of free anti-drug antibodies, and sub-therapeutic drug levels, nor were they associated with worse clinical outcomes.

Conclusion

In this study of TDM performed on four different ELISA kits, we found good linear correlation in drug levels but significant differences in agreement. This was most marked when comparing IFX drug levels between RIDA and LT against IM and PRO and ADA drug levels using LT compared with PRO and IM. This equated to misclassifying samples as therapeutic or sub-therapeutic in a substantial proportion of samples with PRO and IM compared with the reference method, LT. Qualitatively, anti-drug antibody detection was comparable between LT and PRO and seen more frequently with IM, explained by different methodology. In the absence of assay standardisation, the use of method-specific therapeutic cut-offs is essential in the interpretation of TDM results and subsequent clinical decision making. As such, clinicians should be aware of differences in ELISA assays when making management decisions on the basis of the results of TDM.

Funding: The study received no specific funding. Manufacturers provided assays at no charge.

Acknowledgements: We are grateful to the manufacturers for providing LT, IM and PRO kits, and to University Hospitals Leuven for providing RS kits, at no cost. We acknowledge support from the Department of Health via the NIHR comprehensive Biomedical Research Centre awards to Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London and to Addenbrooke's Hospital in partnership with the University of Cambridge.

Conflict of Interest: MGW has served as a speaker for Janssen, Abbvie, Ferring and Takeda and has received research funding from Abbvie NU, BW, SWC, SS, JDS,MP and ZA have no conflicts of interest.PMI has received lecture fees from Abbvie, Warner Chilcott, Ferring, Falk Pharma, Takeda, MSD, Johnson and Johnson, Shire and financial support for research from MSD and Takeda and advisory fees from Abbvie, Warner Chilcott, Takeda, MSD, Vifor Pharma, Pharmacosmos, Topivert, Genentech, Hospira and Samsung Bioepis.

Author Contributions: MGW: study conception and design, statistical analysis and interpretation of the data, drafting article and manuscript writing. NU, ZA: design of study, acquisition of data, analysis and interpretation of the data, manuscript critical revision. BW: analysis and interpretation of the data and manuscript critical revision. SWC, SS: acquisition of data and manuscript critical revision. JDS, MP, PMI: conception and design of study, interpretation of data and manuscript critical revision. All authors approved the final version of the manuscript at submission.

REFERENCES

1. Roblin X, Marotte H, Leclerc M, et al. Combination of C-reactive Protein, Infliximab Trough Levels, and Stable but Not Transient Antibodies to Infliximab Are Associated With Loss of Response to Infliximab in Inflammatory Bowel Disease. Journal of Crohn's and Colitis 2015;9(7):525–31.

2. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut 2014;63(11):1721–7.

3. Mazor Y, Almog R, Kopylov U, et al. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. Aliment Pharmacol Ther 2014;40(6):620–8.

4. Zittan E, Kabakchiev B, Milgrom R, et al. Higher Adalimumab Drug Levels are Associated with Mucosal Healing in Patients with Crohn's Disease. Journal of Crohn's and Colitis 2016;10(5):510–5.

5. Ungar B, Levy I, Yavne Y, et al. Optimizing Anti-TNF-α Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol 2016;14(4):550–2.

6. Steenholdt C, Ainsworth MA, Tovey M, et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. Ther Drug Monit 2013;35(4):530–8.

7. Vande Casteele N, Gils A, Singh S, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. Am J Gastroenterol 2013;108(6):962–71.

8. Vande Casteele N, Buurman DJ, Sturkenboom MGG, et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. Aliment Pharmacol Ther 2012;36(8):765–71.

9. Nanda KS, Cheifetz AS, Moss AC. Impact of Antibodies to Infliximab on Clinical Outcomes and Serum Infliximab Levels in Patients With Inflammatory Bowel Disease (IBD): A Meta-Analysis. Am J Gastroenterol 2012;108(1):40–7.

10. Paul S, Moreau AC, Del Tedesco E, et al. Pharmacokinetics of Adalimumab in Inflammatory Bowel Diseases. Inflamm Bowel Dis 2014;20(7):1288–95.

11. Louis E, Mary J-Y, Vernier-Massouille G, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. Gastroenterology 2012;142(1):63–70.

12. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. Journal of Crohn's and Colitis 2016;10(5):619–25.

13. Guiotto C, Daperno M, Frigerio F, et al. Clinical relevance and inter-test reliability of anti-infliximab antibodies and infliximab trough levels in patients with inflammatory bowel disease. Dig Liver Dis 2016;48(2):138–43.

14. Schmitz EMH, van de Kerkhof D, Hamann D, et al. Therapeutic drug monitoring of infliximab: performance evaluation of three commercial ELISA kits. Clin Chem Lab Med 2016;54(7):1211–9.

15. Imaeda H, Takahashi K, Fujimoto T, et al. Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn's disease. J Gastroenterol 2013;

16. Therapeutic monitoring of TNF-alpha inhibitors in Crohn's disease (LISA- TRACKER ELISA kits, IDKmonitor ELISA kits, and Promonitor ELISA kits). https://www.nice.org.uk/guidance/dg22/resources/therapeutic-monitoring-of-tnfalpha-inhibitors-in-crohns-disease-lisatracker-elisa-kits-idkmonitor-elisa-kits-and-promonitor-elisa-kits-1053686896837. accessed online July 2016.

17. Roblin X, Marotte H, Rinaudo M, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol 2014;12(1):80–2.

18. Ward MG, Kariyawasam VC, Arkir Z, et al. Clinical utility of measuring adalimumab trough levels and antibodies to adalimumab in patients with inflammatory bowel diseases. J Gastroenterol Hepatol 2013;28(Suppl.2):100.

19. Passing H, Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem 1983;21(11):709–20.

20. LeBreton JM, Senter JL. Answers to 20 Questions About Interrater Reliability and Interrater Agreement. Organizational Research Methods 2007;11(4):815–52.

21. Fleiss JL, Levin B, Paik MC. Statistical Methods for Rates and Proportions, 3rd Edition. John Wiley Sons 2016;

22. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22(3):276–82.

23. Lee LYW, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. Eur J Gastroenterol Hepatol 2012;24(9):1078–85.

24. Yanai H, Lichtenstein L, Assa A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. Clin Gastroenterol Hepatol 2015;13(3):522–2.

25. Melmed GY, Irving PM, Jones J, et al. Appropriateness of Testing for Anti-Tumor Necrosis Factor Agent and Antibody Concentrations, and Interpretation of Results. Clin Gastroenterol Hepatol 2016;

26. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2013;11(4):444–7.

27. Papamichael K, Karatzas P, Mantzaris GJ. Addition of an immunomodulator as a rescue therapy for loss of response to adalimumab dose escalation in patients with Crohn's

disease. Journal of Crohn's and Colitis 2015;9(7):589–90.

28. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. Journal of Crohn's and Colitis 2013;7(9):736–43.

29. Velayos FS, Kahn JG, Sandborn WJ, Feagan BG. A Test-based Strategy Is More Cost Effective Than Empiric Dose Escalation for Patients With Crohn's Disease Who Lose Responsiveness to Infliximab. Clin Gastroenterol Hepatol 2013;11(6):654–66.

30. Steenholdt C, Brynskov J, Thomsen OØ, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. Gut 2014;63(6):919–27.

31. Van Moerkercke W, Ackaert C, Compernolle G, et al. 405 High Infliximab Trough Levels are Associated With Mucosal Healing in Crohn's Disease. Gastroenterology 2010;138(5):S–60–60.

32. Murthy S, Kevans D, Seow CH, et al. Sa2047 Association of Serum Infliximab and Antibodies to Infliximab to Long-Term Clinical Outcome in Acute Ulcerative Colitis. Gastroenterology 2012;142(5):S–388.

33. Yarur AJ, Jain A, Hauenstein SI, et al. Higher Adalimumab Levels Are Associated with Histologic and Endoscopic Remission in Patients with Crohn's Disease and Ulcerative Colitis. Inflamm Bowel Dis 2016;22(2):409–15.

34. Parussini E. Letter: detection of infliximab levels and anti-infliximab antibodies - comparison of three different assays. Aliment Pharmacol Ther 2012;37(2):281–1.

35. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148(7):1320–3.

36. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. J Clin Pharmacol 2015;55 Suppl 3:S39–50.

37. Samaan MA, Unsworth N, Ward MG, et al. The Presence of total anti-drug antibodies to biologic drugs does not adversely affect long-term outcomes in Crohn's disease patients with adequate drug levels and absent free anti-drug antibodies. Gut 2016;7(1):A1–A310.

TABLES

Table 1. Median (IQR) drug levels according to assay

Drug	ELISA Assay	Median Drug Level (μg/mL)	Inter-quartile range (μg/mL)	p value vs. RS
Infliximab	LT	4.7	2.3 – 6.4	0.007
	IM	3.7	1.9 – 6.5	0.0007
	PRO	4.0	1.9 – 8.7	0.014
	RIDA	6.0	3.4 – 9.8	-
Adalimumab	LT	6.1	4.9 – 7.9	-
	IM	11.0	8.4 – 14.1	<0.0001
	PRO	10.1	8.1 – 14.1	<0.0001

Table 2. Correlation and agreement of infliximab and adalimumab drug levels between assays

	INFLIXIMAB		ADALIMUMAB	
Assay Comparison	Correlation, rs (95% CI)	Intra-class coefficient, ICC (95% CI)	Correlation, rs (95% CI)	Intra-class coefficient, ICC (95% CI)
LT vs IM	0.85 (0.78 to 0.90)[a]	0.84 (0.77 to 0.89)[a]	0.92 (0.88 to 0.95)[a]	0.51 (-0.09 to 0.81) [a]
LT vs PRO	0.92 (0.89 to 0.95)[a]	0.85 (0.77 to 0.90)[a]	0.75 (0.64 to 0.82)[a]	0.45 (-0.09 to 0.75) [a]
LT vs RS	0.98 (0.97 to 0.99)[a]	0.80 (0.40 to 0.91)[a]		
IM vs PRO	0.87 (0.81 to 0.91)[a]	0.75 (0.63 to 0.84)[a]	0.84 (0.76 to 0.89)[a]	0.86 (0.79 to 0.90)[a]
IM vs RS	0.88 (0.83 to 0.92)[a]	0.74 (0.36 to 0.87)[a]		
PRO vs RS	0.91 (0.87 to 0.94)[a]	0.93 (0.73 to 0.97)[a]		

rs = Spearman's correlation coefficient, CI = confidence interval, LT = Lisa-Tracker, IM = IDKmonitor, PRO = Promonitor, RS = RIDAscreen

[a]$p < 0.0001$.

Table 3. Qualitative agreement in IFX drug levels between LT and other assays

		LT assay			
		Sub-therapeutic	Therapeutic	Total	Percentage agreement
IM assay	Sub-therapeutic	21	5	26	Negative agreement = 88%
	Therapeutic	3	67	70	Positive agreement = 93%
	Total	24	72	96	Total agreement = 92%
		LT assay			
		Sub-therapeutic	Therapeutic	Total	Percentage agreement
PRO assay	Sub-therapeutic	23	5	28	Negative agreement = 96%
	Therapeutic	1	67	68	Positive agreement = 93%
	Total	24	72	96	Total agreement = 94%
		LT assay			
		Sub-therapeutic	Therapeutic	Total	Percentage Agreement
RS assay	Sub-therapeutic	17	1	18	Negative agreement =71%
	Therapeutic	7	71	78	Positive agreement = 99%
	Total	24	72	96	Total agreement = 92%

IFX = infliximab, LT = Lisa-Tracker, IM = IDKmonitor, PRO = promonitor, RS = RIDAscreen

Table 4. Qualitative agreement in ADA drug levels between LT and other assays

		LT assay			
		Sub-therapeutic	Therapeutic	Total	Percentage agreement
IM assay	Sub-therapeutic	6	0	6	Negative agreement = 24%
	Therapeutic	19	73	92	Positive agreement = 100%
	Total	25	73	98	Total agreement = 81%
		LT assay			
		Sub-therapeutic	Therapeutic	Total	Percentage agreement
PRO assay	Sub-therapeutic	5	0	5	Negative agreement = 20%
	Therapeutic	20	73	93	Positive agreement = 100%
	Total	25	73	98	Total agreement = 80%

ADA = adalimumab, LT = Lisa-Tracker, IM = IDKmonitor, PRO = promonitor

FIGURES

Figure 1: Drug levels for infliximab (IFX) and adalimumab (ADA) according to assay.
Data represented as box-whisker plots with middle band representing median drug level, outer box limits defining interquartile range and long bars range. Stars and crosses represent outliers

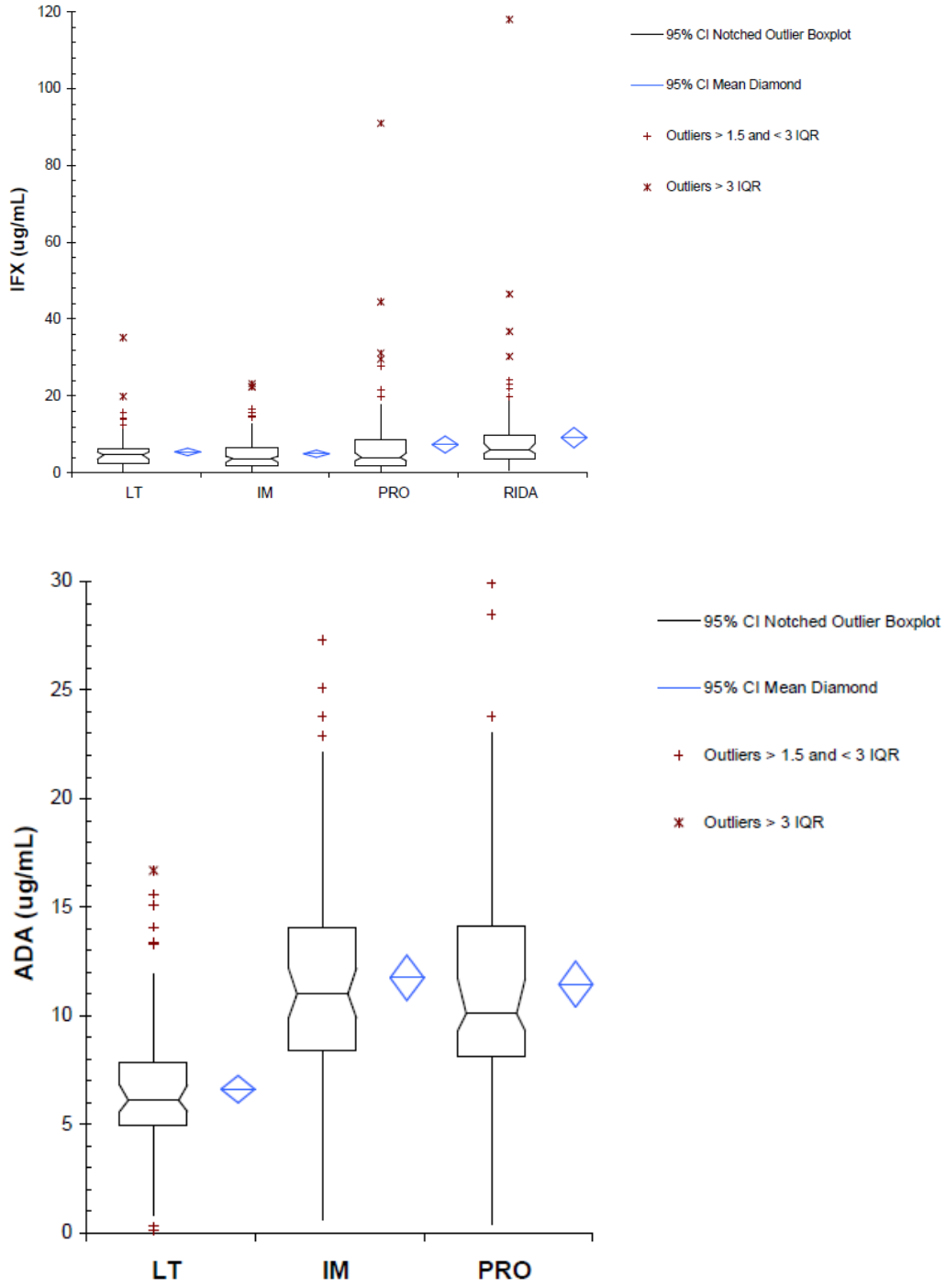

Figure 2a LT IFX vs RIDA IFX bias plot (absolute values)

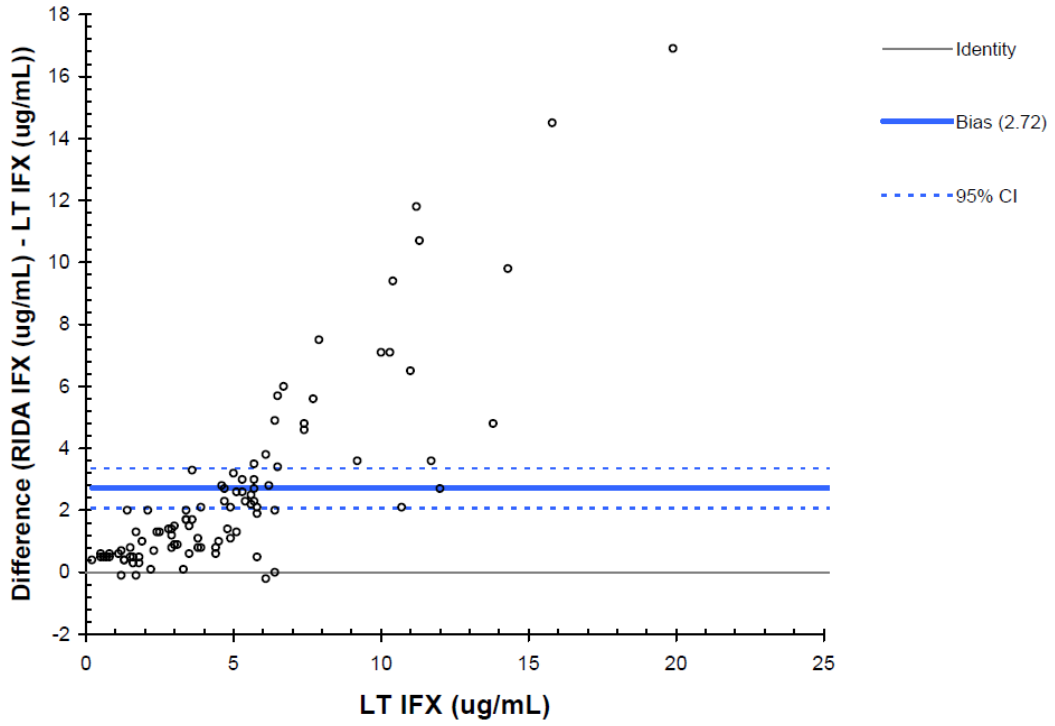

Figure 2b: LT IFX vs RIDA Passing Bablok plot

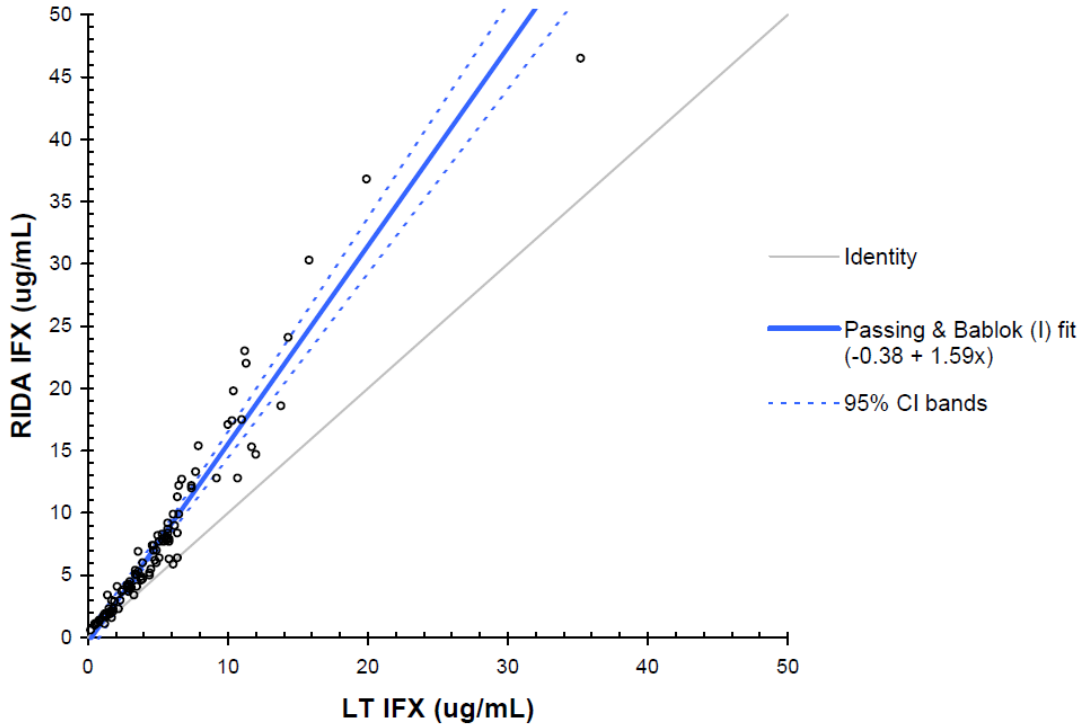

Figure 3a: LT IFX vs PRO IFX Bias plot (absolute values)

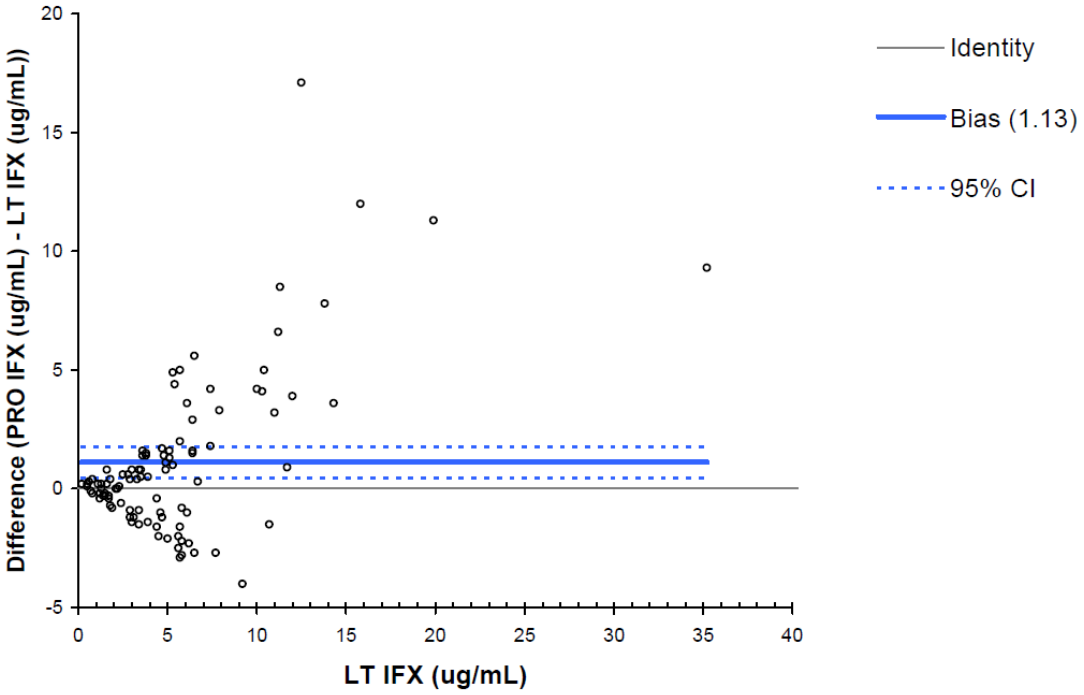

Figure 3b: LT IFX vs PRO IFX Passing Bablok plot

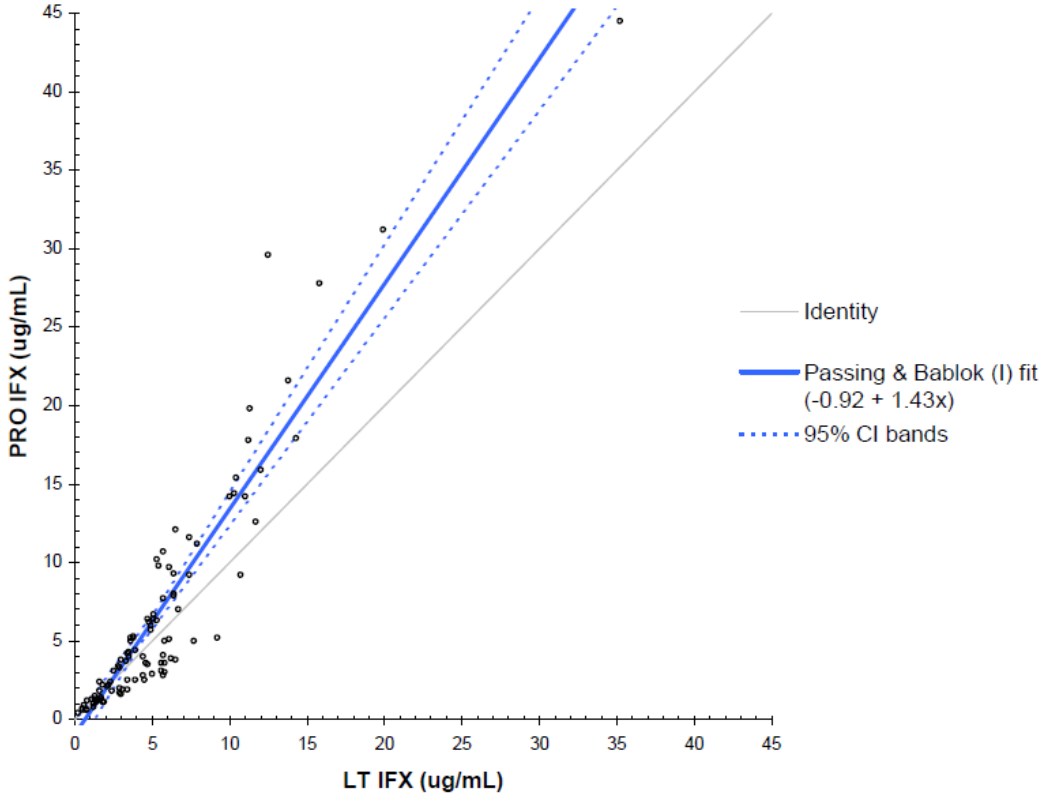

Figure 4a: LT IFX vs IM IFX Bia plot (absolute values)

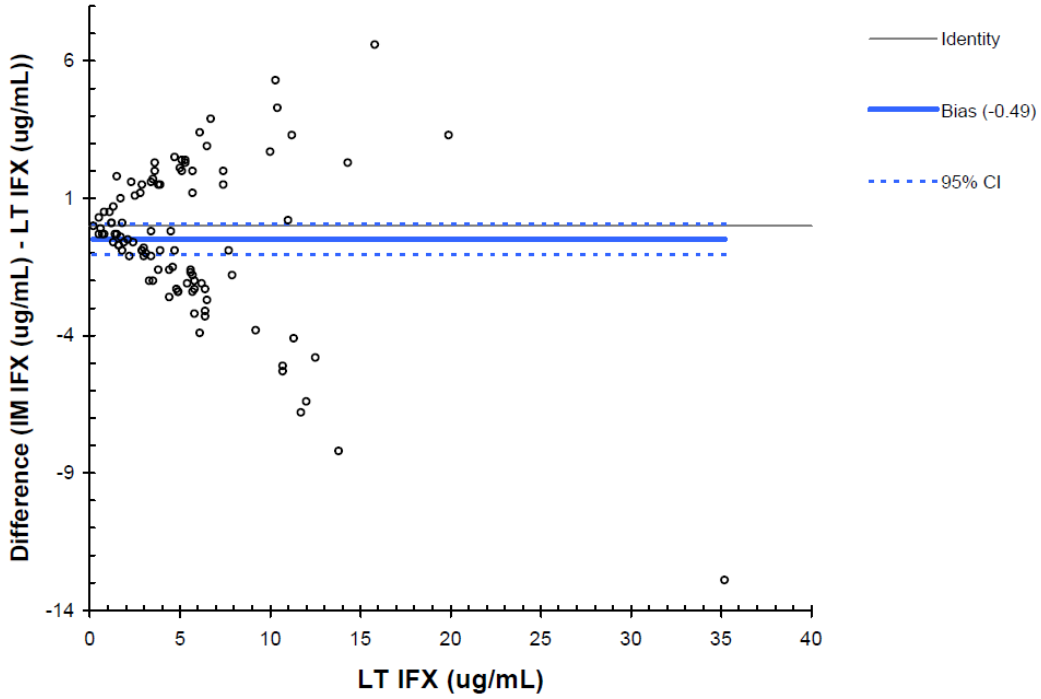

Figure 4b: LT IFX vs PRO IFX Passing Bablok plot

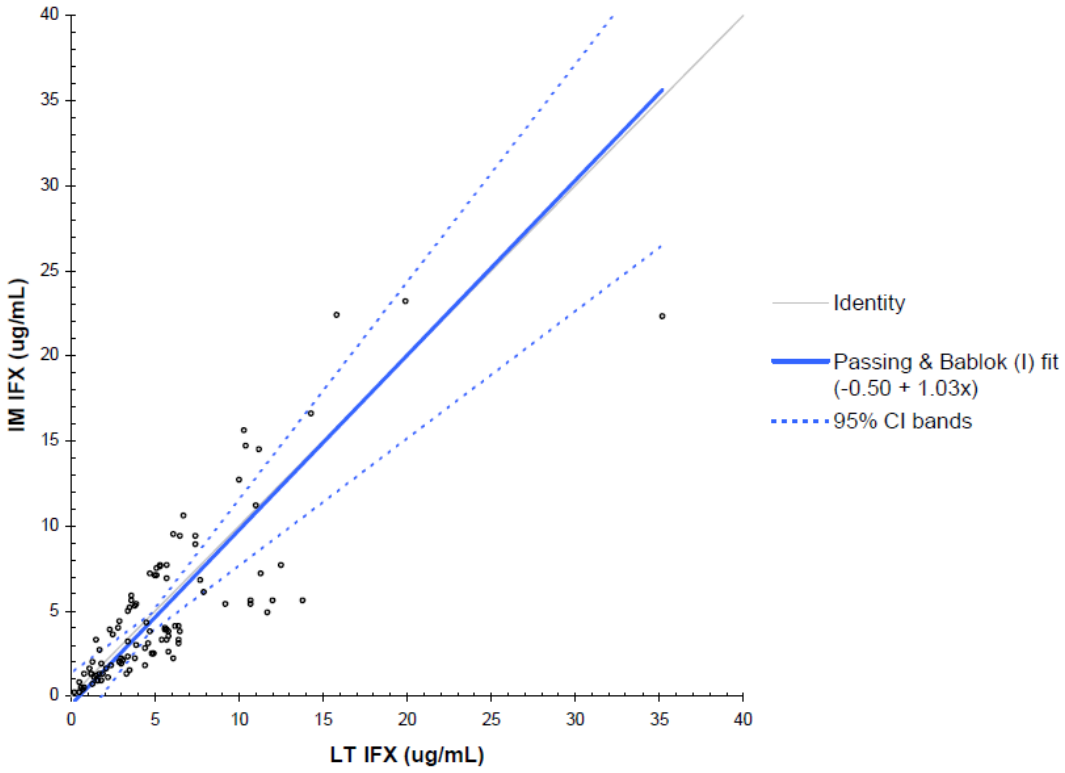

Figure 5a IM IFX vs RIDA IFX Bias plot (absolute values)

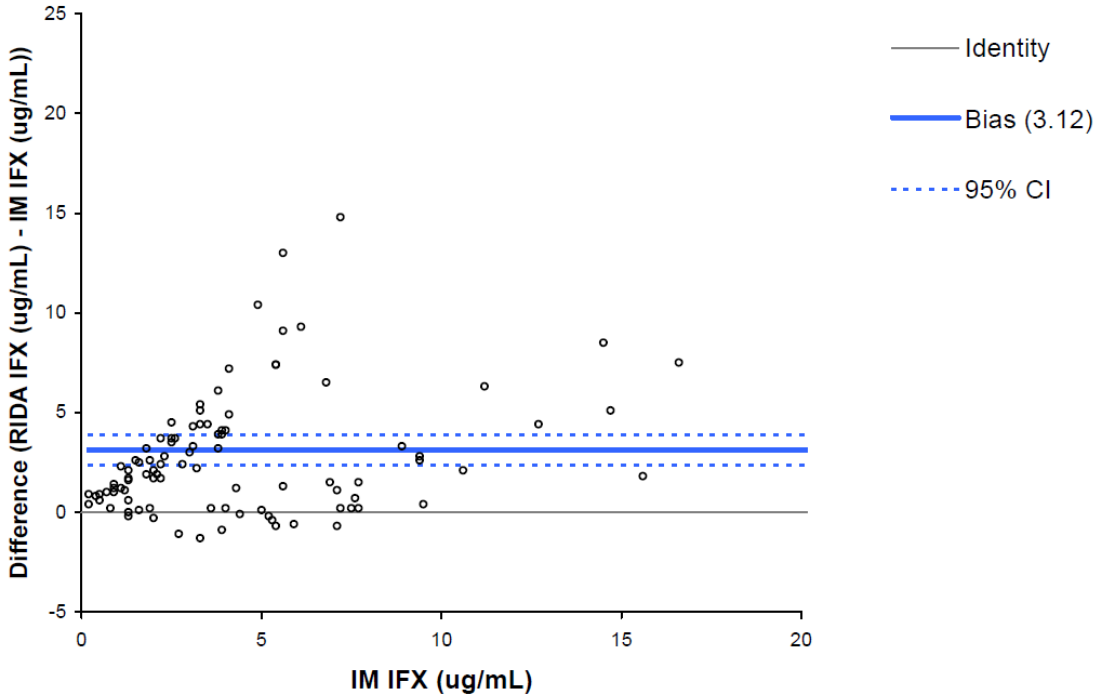

Figure 5b: IM IFX vs RIDA IFX Passing Bablok plot

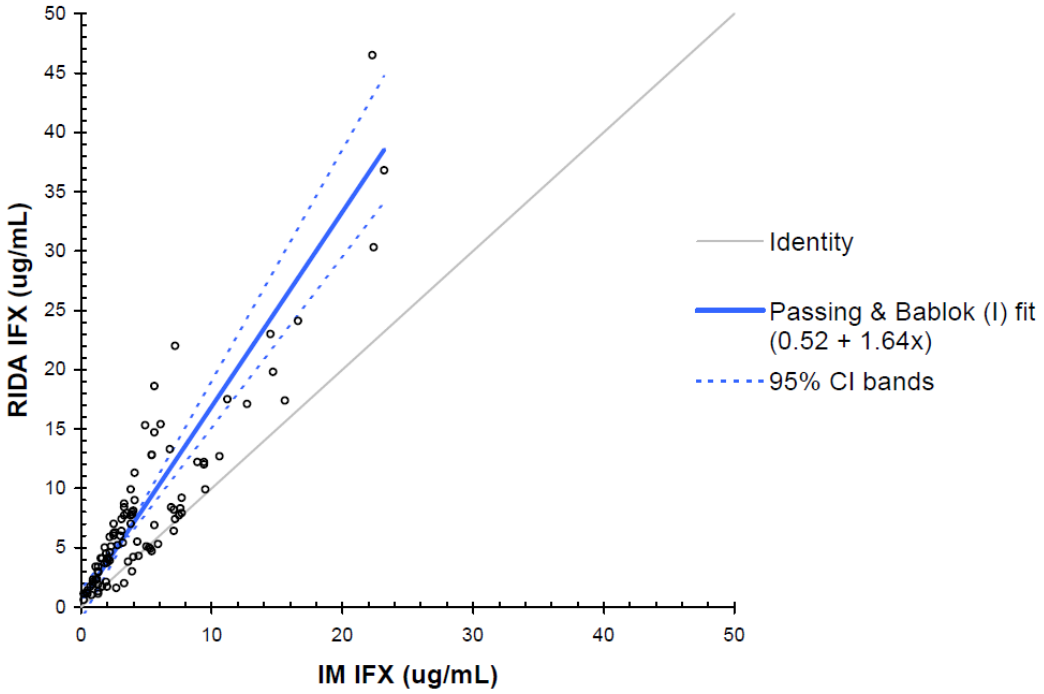

Figure 6a: IM IFX vs PRO IFX Bia plot (absolute values)

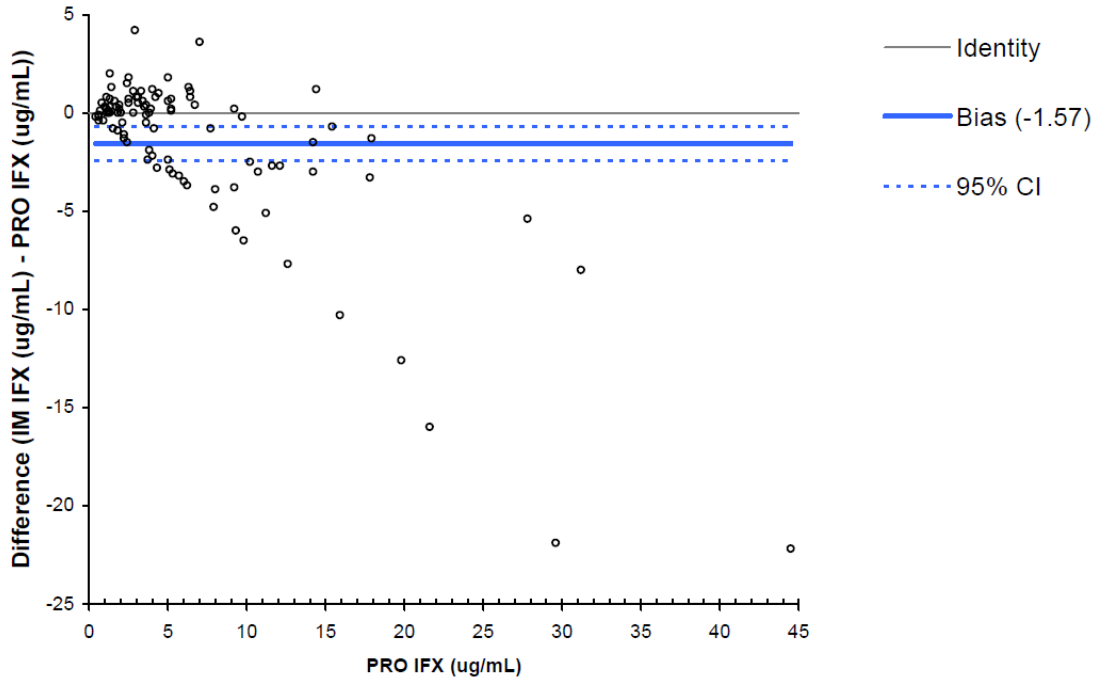

Figure 6b: IM IFX vs PRO IFX Passing Bablok Plot

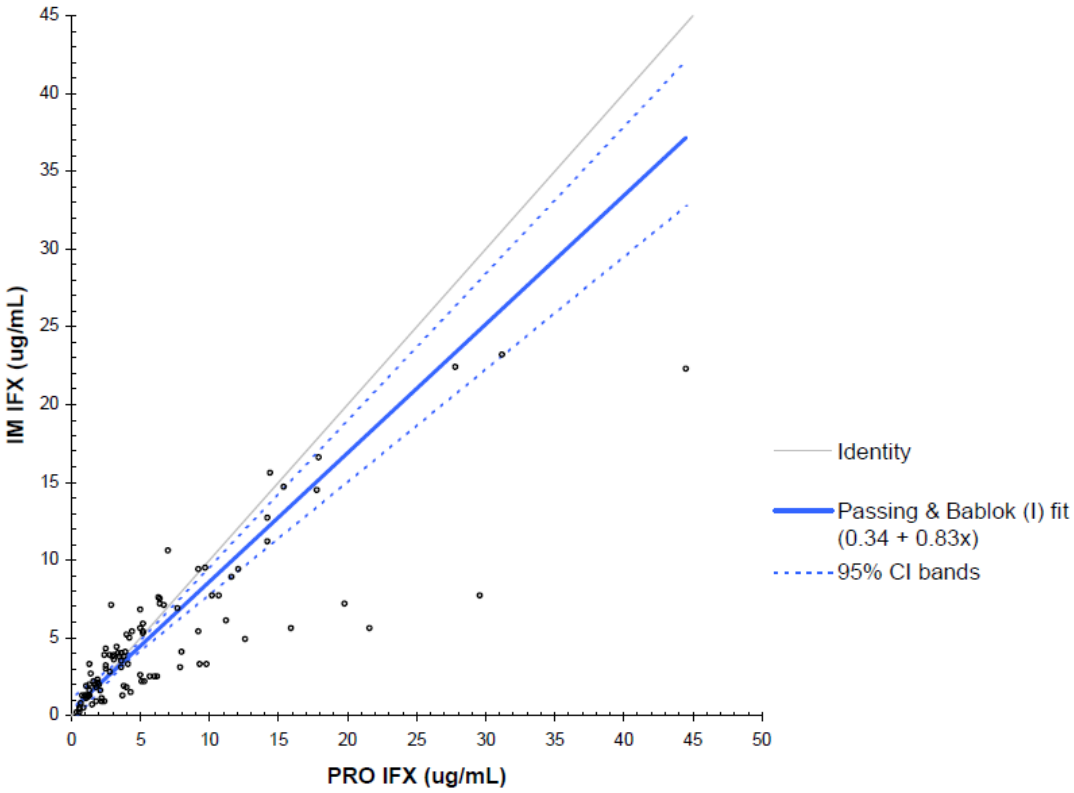

Figure 7a: PRO IFX vs RIDA IFX Bias plot (absolute values)

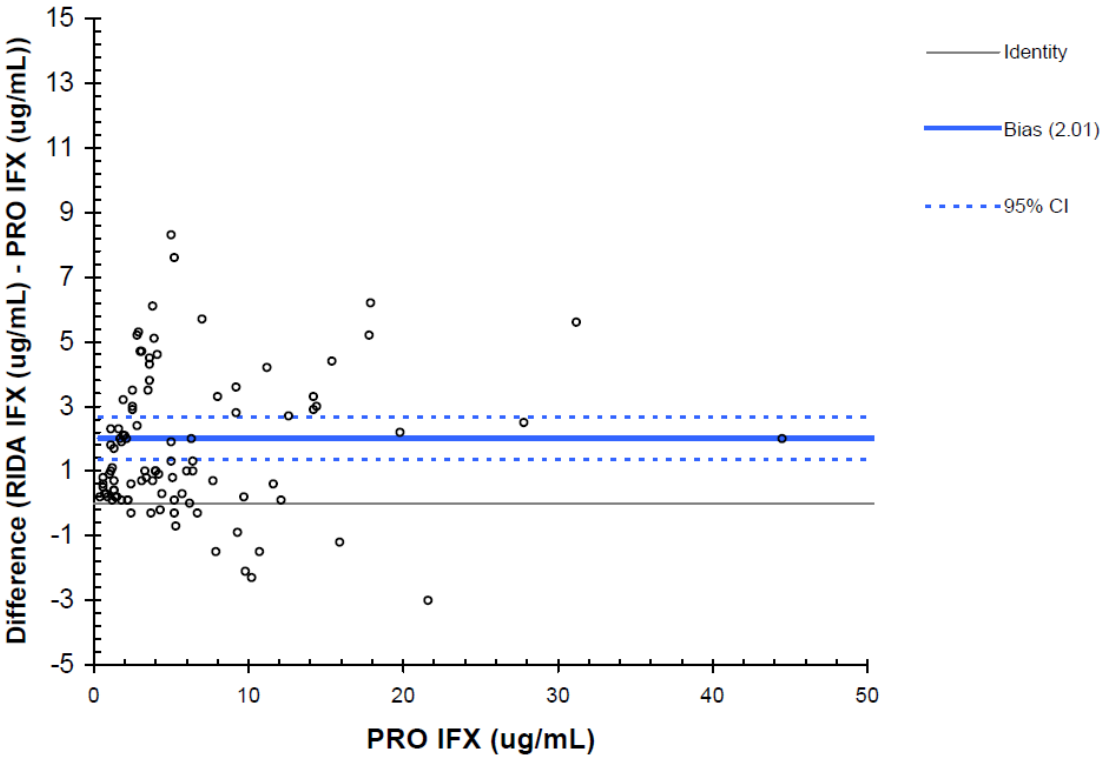

Figure 7b: PRO IFX vs RIDA IFX Passing Bablok Plot

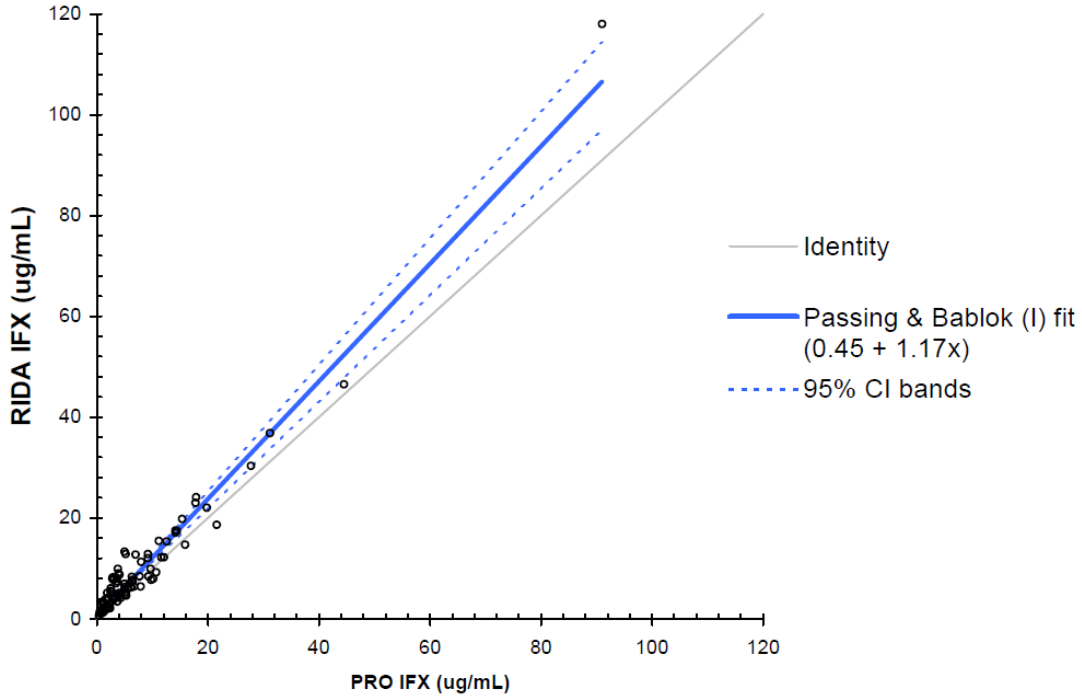

Figure 8a: LT ADA vs IM Bias plot (absolute values)

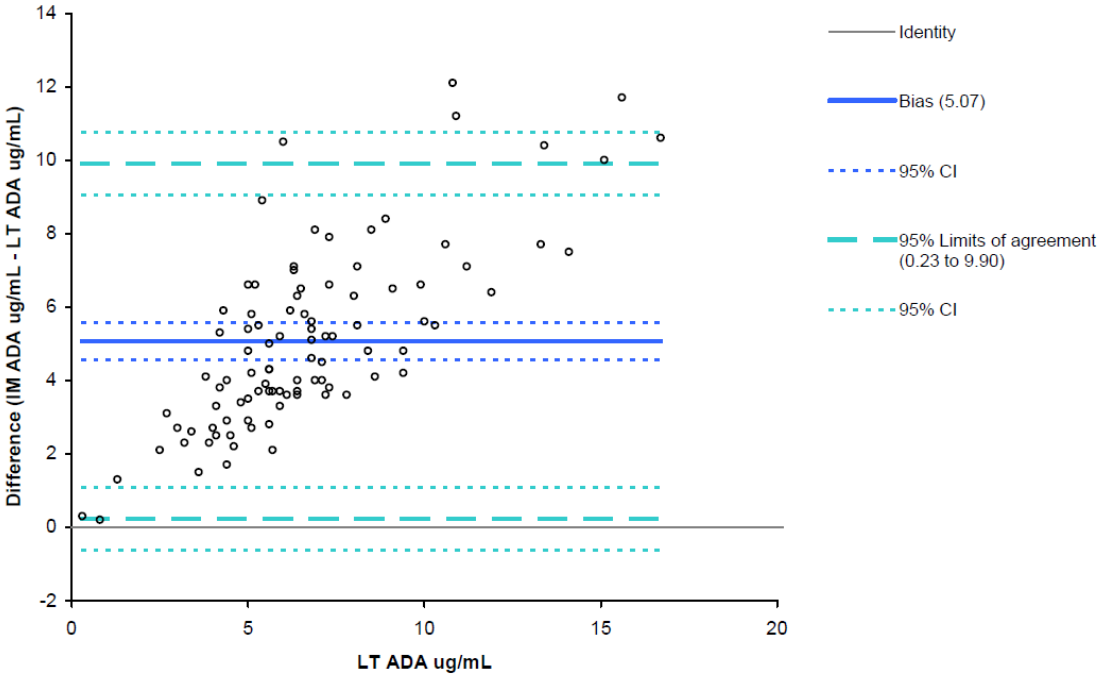

Figure 8b: LT ADA vs IM Passing Bablok Plot

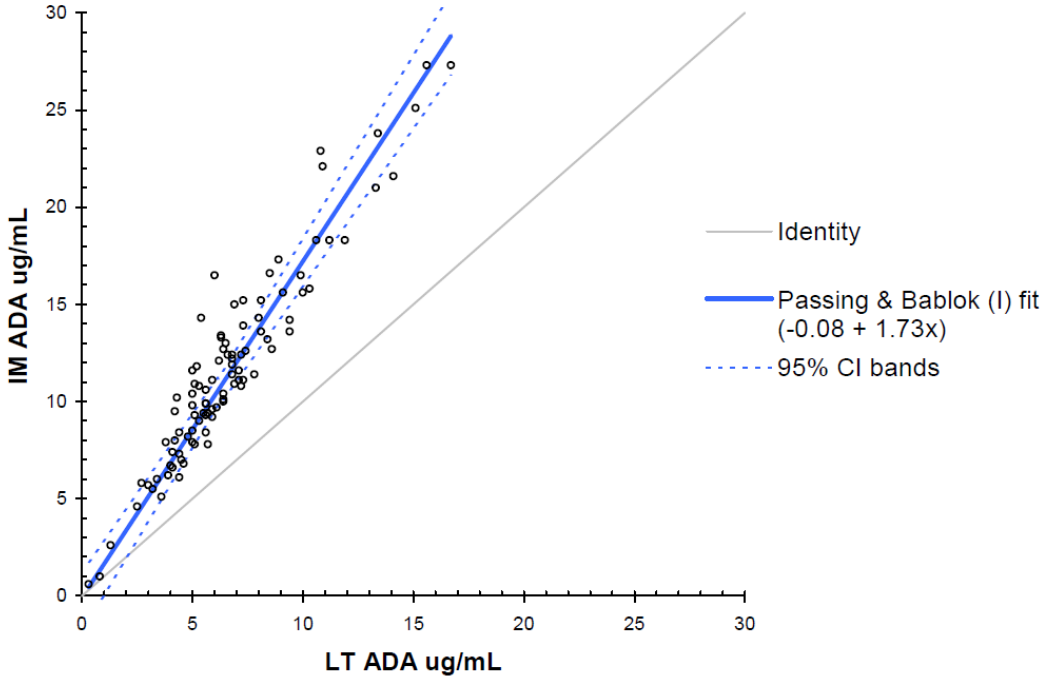

Figure 9a: LT ADA vs PRO Bias plot (absolute values)

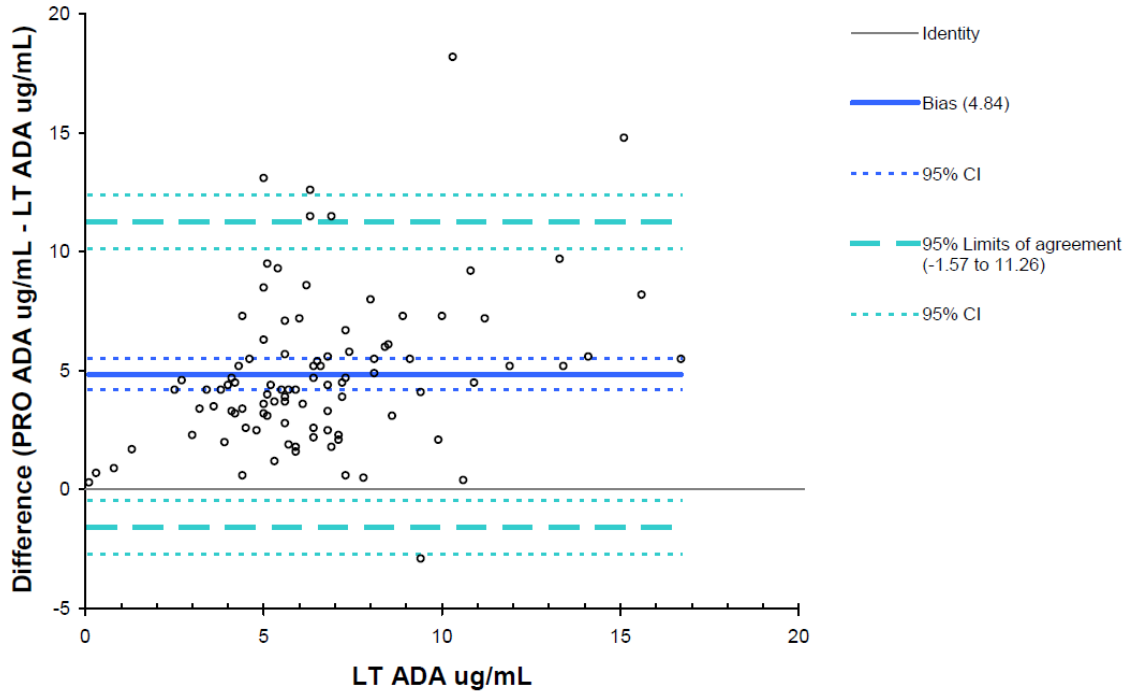

Figure 9b: LT ADA vs PRO Passing Bablok Plot

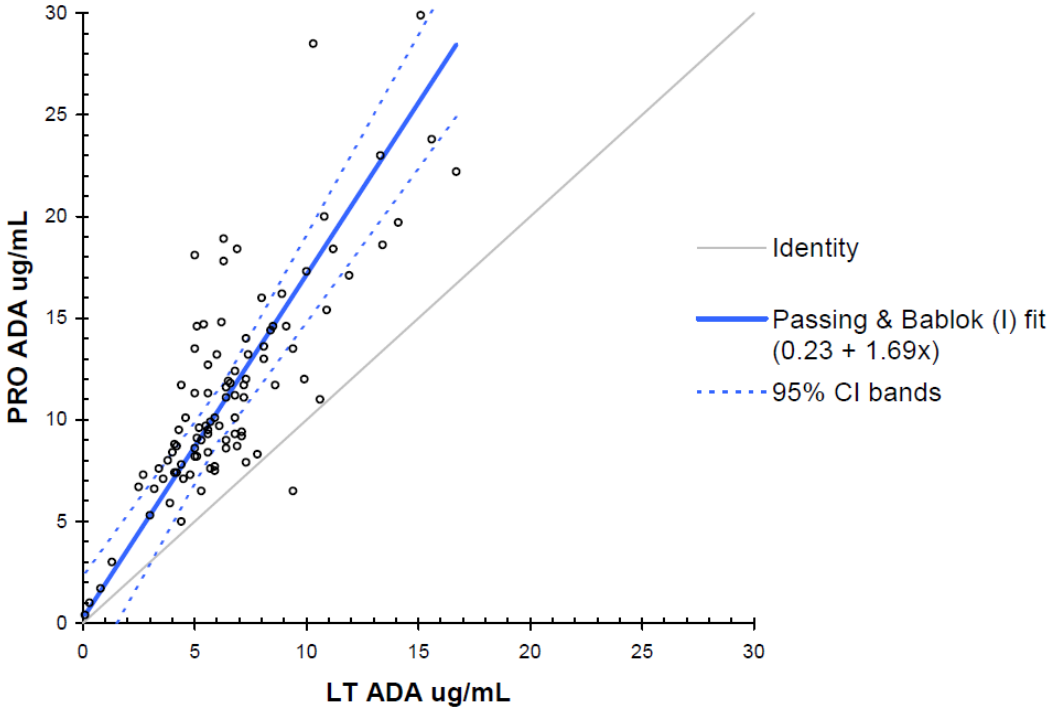

Figure 10a: IM ADA vs PRO Bias plot (Absolute values)

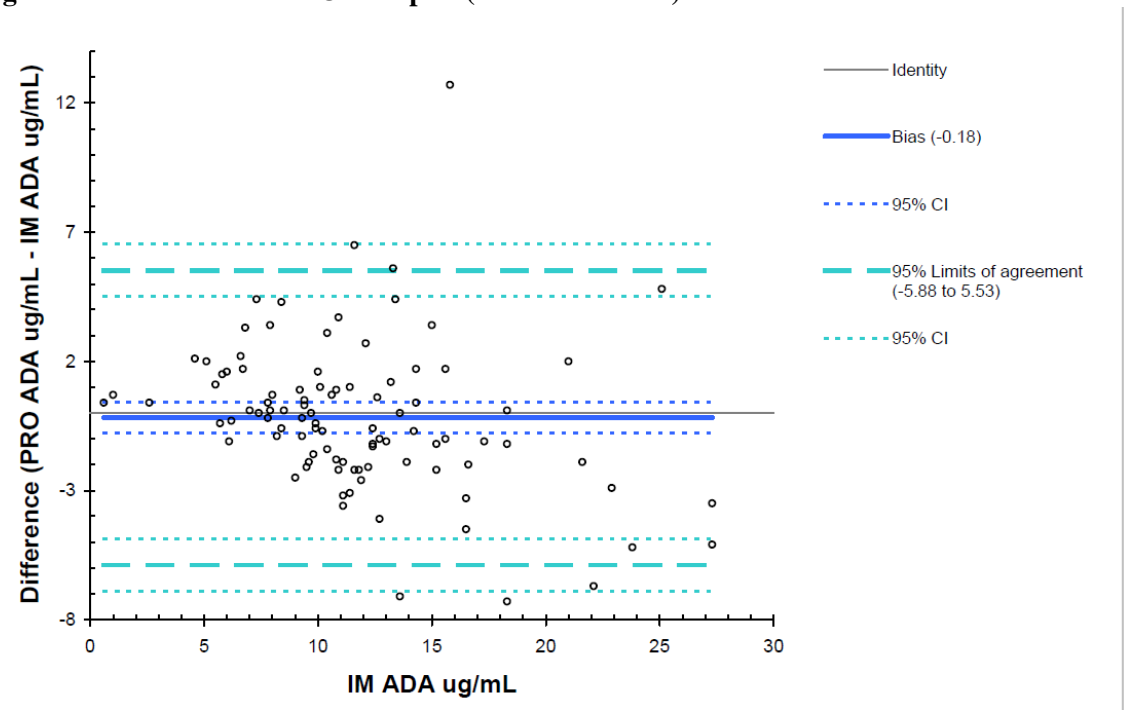

Figure 10b: IM ADA vs PRO Passing Bablok Plot

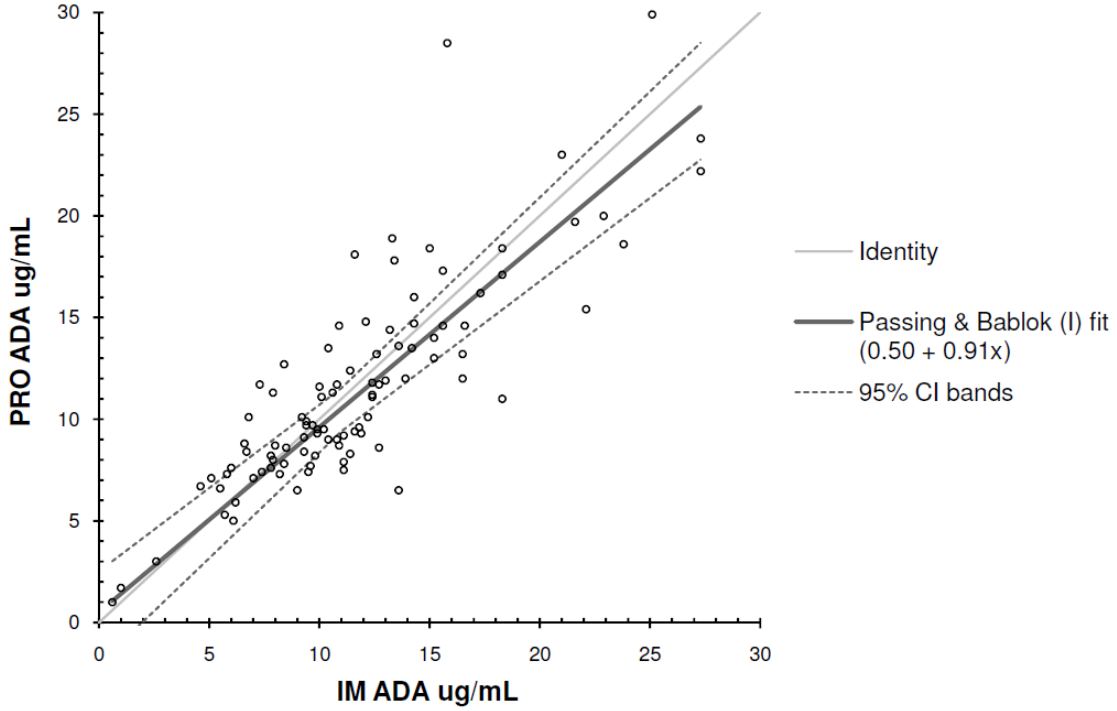

Figure 11. Infliximab (a) and adalimumab (b) drug levels according to anti-drug antibody status using IM assay. Significant differences in median drug levels were observed for adalimumab but not for infliximab. Box and Whisker plot demonstrating median, interquartile range and range. IFX = infliximab, ADA = adalimumab

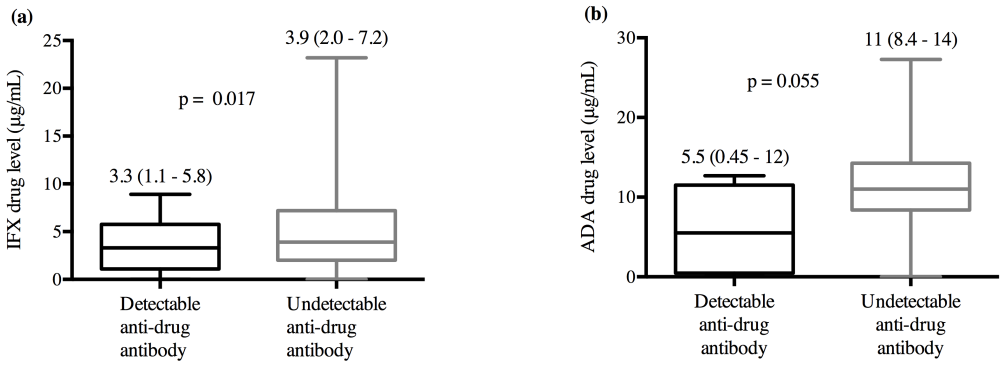

Figure 4

CHAPTER 7:

Clinical perspectives: Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors

TITLE PAGE

Infliximab and adalimumab drug levels in Crohn's disease: contrasting associations with disease activity and influencing factors

Short title: Anti-TNF therapeutic drug monitoring in Crohn's

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Peter M Irving: [redacted]

Abbreviations:
IFX = infliximab, ADA = adalimumab, CD = Crohn's disease, TGN = 6-thioguanine nucleotide, ROC = receiver operated curve, TNF = tumour necrosis factor, TDM = therapeutic drug monitoring, HBI = Harvey-Bradshaw index, RBC = red blood cell, CRP = c-reactive protein, ELISA = enzyme-linked immunosorbent assay, FCP = faecal calprotectin

ABSTRACT

Background: For therapeutic drug monitoring of infliximab (IFX) and adalimumab (ADA) in Crohn's disease (CD), discriminative drug level thresholds for disease end-points have been consistently demonstrated with IFX but not ADA.

Aims: To identify threshold concentrations for IFX and ADA in CD according to different disease endpoints and identify factors that influence drug levels.

Methods: We performed a cross-sectional service evaluation of patients receiving maintenance IFX or ADA for CD. Therapeutic drug monitoring was performed at trough for IFX and at any time point for ADA. Endpoints included Harvey-Bradshaw index, C-reactive protein and faecal calprotectin. 6-thioguanine nucleotide concentrations (TGNs) were measured in patients treated with thiopurines.

Results: 191 patients (96 IFX, 95 ADA) were included. Differences in IFX levels were observed for clinical ($p = 0.081$) and biochemical remission ($p = 0.003$) and faecal calprotectin normalisation ($p < 0.0001$) with corresponding thresholds identified on ROC analysis of >1.5, >3.4 and >5.7 μg/mL. ADA levels were similar between active disease and remission regardless of the endpoint assessed. Modelling identified that higher IFX dose, body mass index and colonic disease accounted for 31% of the variation in IFX levels, and weekly ADA, albumin and weight for 23% of ADA level variation. TGNs did not correlate with drug levels.

Conclusions: Therapeutic drug monitoring of IFX in CD is useful, however its utility with ADA is less clear. Higher IFX thresholds are associated with 'deeper' levels of remission. More data is needed to explain the variation in drug levels.

Keywords: Crohn's disease, drug monitoring, infliximab

INTRODUCTION

Infliximab (IFX) and adalimumab (ADA) are effective in luminal and fistulising Crohn's disease (CD),[1-4] but 10-40% of patients lose response within 12 months[5-8], and a further 10-20% annually thereafter.[9] In this setting a change in management may recapture response; a proportion respond to dose intensification[10] whilst in others the addition of an immunomodulator is of benefit.[11] Changing within class or out-of-class are alternative strategies.[12,13]

In order to assist in making therapeutic decision in the setting of primary non-response or secondary loss of response, therapeutic drug monitoring of IFX and ADA has been proposed on the basis that drug levels directly relate to pharmacodynamics. If this is correct, then maintaining circulating drug levels above a therapeutic threshold would be associated with better control of intestinal inflammation. There is a growing evidence base supporting this concept for IFX, both in cross-sectional and longitudinal/interventional studies.[14-17] Drug levels above 2-3 μg/mL predict a higher chance of clinical remission[18] whereas levels over 5 μg/mL are associated with mucosal healing.[19] In contrast, published data for ADA are more variable; an association between low drug levels and worse outcomes has been reported by some authors,[20-22] but not by others.[23] A threshold of 4.9-5.9 μg/mL has been identified for clinical remission and > 7μg/mL for mucosal healing.[19,24]

The major reason for inter-patient variability in drug levels is due to differences in drug clearance and distribution in the body. Dose, schedule and route of administration account for some of the variation in pharmacokinetics between anti-TNF therapies; IFX displays high peak concentrations with low troughs compared with a more uniform concentration-time profile with ADA.[25] Anti-drug antibodies and other non-immune mechanisms increase drug clearance.[26,27] Other factors, such as weight and serum albumin have also been implicated.[28] Further data will help us to understand better the factors that influence anti-TNF drug levels in patients with CD

For IFX, the use of combination therapy with a thiopurine has been shown to be superior to IFX monotherapy.[29] This is, in part, explained by a beneficial effect of thiopurines on IFX pharmacokinetics by increasing drug levels and reducing immunogenicity. A recent study suggested that the level of the major therapeutic metabolite, 6-thioguanine (TGN), required to augment IFX levels was nearly 50% lower than that required for clinical efficacy.[30] This is important because, if replicated in subsequent studies, a lower dose of concomitant immunomodulation may be sufficient to confer optimal benefit whilst minimising toxicity.

We aimed to utilise data from a large cohort of patients with CD in order to address the following key issues in the application of therapeutic drug monitoring. First, the association of drug levels with the achievement of clinical targets from clinical to deep remission for ADA were compared with those for IFX and cut-off concentrations that might predict those therapeutic targets from clinical to deep remission were explored. Secondly, patient and disease factors that might influence drug levels were investigated. Thirdly, the association of TGN levels with IFX and ADA levels was addressed in patients treated with combination therapy.

METHODS

Patients and design

We performed a cross-sectional service evaluation of therapeutic drug monitoring amongst adult patients with CD attending the outpatient clinics of two tertiary centres in the UK between October 2013 and April 2014. The diagnosis of CD was based on standard criteria[31] and was confirmed after review of the patient's medical record. Patients were included if they were established on IFX or ADA (> 14 weeks of treatment) at doses of 5 mg/kg 8-weekly, 5 mg/kg 6-weekly or 10 mg/kg 8-weekly (for IFX) or 40 mg every other week, each week or every 10 days (for ADA). Clinical disease activity was recorded prospectively as part of routine care using the Harvey Bradshaw Index (HBI)[32]; an HBI ≤ 4 was considered remission. Systemic inflammation was assessed by serum concentrations of C-reactive protein (CRP), a value < 5 mg/L being classified as remission. Faecal calprotectin measurements were used as a surrogate marker of intestinal inflammation. Values < 59 μg/g were considered normal.

IFX levels were performed in serum taken at trough, i.e., just prior to infusions, whereas ADA levels were taken at any time point within a treatment cycle; trough was defined as day 13 or 14 for every-other-week, day 6 or 7 for every-week and day 9 or 10 for 10-daily dosing. In patients co-treated with azathioprine or mercaptopurine, TGN concentrations were assessed when patients had been on stable doses for at least 6 weeks. TGN concentrations > 235 pmol/8x10^8 RBC were considered therapeutic.[33] In addition, the TGN cut-off value proposed by Yarur et al[30] (< 125 pmol/8x10^8 RBC) was compared to 125-235 and > 235 in examining the association with IFX and ADA drug levels.

Laboratory Methods

Therapeutic drug monitoring was performed on serum samples by ELISA (Lisa-Tracker, Theradiag, Marne la Valée, France) as per the manufacturer's instructions. The average of duplicate samples was expressed as μg/mL. TGNs were analysed by ultra-high-performance liquid chromatography as described

elsewhere and reported as pmol/8x10^{8}RBC.[34] Faecal calprotectin was measured in duplicate on extracts of 50 mg of homogenised stool by ELISA (B€uhlmann Laboratories, Basel, Switzerland) as per manufacturer's instructions. The results were reported as lg/g faeces.

Statistical Analysis

SPSS Version 21 (IBM Inc, Chicago, IL) and Prism Version 6.0 (Graphpad Software, San Diego, CA) were used for statistical analyses and generation of graphs. Descriptive statistics are presented as number with percentage or median with inter-quartile range. For categorical values, between-group comparisons were performed with chi-squared tests and, for continuous data, independent-sample t-tests, Mann-Whitney-U or Kruskal-Wallis tests were used where appropriate. Univariate and multivariate regression analysis was performed to explore the relationship between patient and disease factors (independent values) and drug levels (dependent value). A stepwise regression procedure, based on t-tests for adding or dropping terms from the linear regression models, was used to find a parsimonious best model. Receiver operating characteristic (ROC) curves, created using Prism, were used to determine drug concentrations associated with specific disease endpoints. The Youden Index was calculated to identify the optimal cut-off concentration. Correlations between drug levels and TGNs, and, between days-between-last-dose of ADA and drug level sampling were investigated using Spearman rank correlation. All reported p-values were 2-sided and $p \leq 0.05$ was considered statistically significant.

Ethical considerations

As the data collected were part of routine clinical care, the study was considered a review of clinical practice and ethical approval was not required, according to the guidelines of the UK Health Research Authority.[35] All authors had access to the study data, and reviewed and approved the final manuscript.

RESULTS

Drug levels and outcomes

Patient characteristics

96 patients treated with IFX were available for analysis. Of 98 patients treated with ADA, 3 were excluded due to inconsistent adherence to scheduled treatment. Thus, data from 95 patients were included. Patient characteristics are shown in Table 1. Patients in each group were well-matched with respect to gender, phenotype, smoking status, weight and body mass index (BMI). Concomitant immunomodulation was more common in patients treated with IFX than with ADA (90 vs 79%, p = 0.043). IFX was dosed 5 mg/kg/q8 in 74 (76%), 5 mg/kg/q6 in 11 (12%) and 10 mg/kg/q8 in 11 (12%) whilst ADA was dosed every other week in 72 (76%), weekly in 20 (21%) and every 10 days in 3 (3%). Therapeutic drug monitoring was performed at trough in all patients treated with IFX and in 20/95 (21%) of those treated with ADA.

Infliximab drug levels and disease activity

Median IFX drug levels were 4.45 (IQR: 1.95-6.40) μg/mL. There were no significant differences in drug levels according to dosing regimens of IFX (p = 0.98) or between those treated with IFX monotherapy compared to combination therapy with an immunomodulator (p = 0.93). As shown in Figure 1, significant differences in median IFX drug levels were observed between patients with, and without, biochemical remission and calprotectin normalisation (p = 0.003 and p < 0.0001, respectively). Further, IFX drug levels in patients with calprotectin normalisation were significantly higher than those observed for biochemical and clinical remission (p = 0.048, Kruskal-Wallis test). IFX drug levels were also higher in composite endpoints of clinical and biochemical remission (5 vs 2.9 μg/mL 9, p = 0.005), biochemical remission and calprotectin normalisation (6.2 vs 3 μg/mL, p < 0.0001) and ‘deep remission’, defined as normal HBI, CRP and calprotectin (6.2 vs 3.2 μg/mL, p < 0.0001). (Supplementary Table 1).

ROC analysis was performed to identify optimal thresholds that best discriminated disease activity according to outcomes (Fig. 2). A drug level > 5.7 μg/mL predicted calprotectin normalisation (AUC 0.77, $p < 0.0001$, sensitivity = 61%, specificity = 88%) with a positive predictive value (PPV) of 83% and negative predictive value (NPV) of 71%. (Table 2). For biochemical remission, levels > 3.4 predicted absence of systemic inflammation (AUC 0.71, $p = 0.003$, sensitivity 74%, specificity 73%, PPV 46%, NPV 90%) and, for clinical remission, > 1.5 μg/mL was identified (AUC 0.67, sensitivity 86%, specificity 50%, PPV 94%, NPV 33%).

Adalimumab drug level and disease activity

Median (IQR) ADA drug levels were 6.2 (5-8) μg/mL. Drug levels were significantly higher in patients dosed weekly compared with those dosed every-other-week (7.3 vs 5.8 μg/mL, $p = 0.002$). There was no difference in drug levels between those on combination therapy compared to ADA monotherapy ($p = 0.46$). Drug levels collected at trough were not different to those collected at earlier time points in a therapeutic cycle (6.1 vs 6.3 μg/mL, $p = 0.43$). Although drug levels decreased with increasing time since last dose, correlation was poor, (rho = -0.27, 95% CI: -0.45 to -0.07); this relationship was seen in patients dosed every other week (rho = -0.23, 95% CI: -0.44 to 0.01) as well as in those on weekly therapy (rho = -0.18, 95% CI: -0.57 to 0.29).

Median drug levels were no different between patients with active disease compared with those in remission, regardless of the definition employed ($p > 0.15$ for all, Fig. 1). Sub-group analysis of drug levels stratified according to dosing regimen failed to demonstrate any difference (data not shown). On ROC analysis thresholds of > 5.1 (AUC 0.61), > 8.5 (AUC 0.49) and > 7.2 (AUC 0.54) μg/mL were identified that predicted clinical and biochemical remission, and calprotectin normalisation, respectively, however the discriminative power was poor ($p > 0.15$) (Table 2 and Fig. 3).

Relationship between patient and disease factors and drug levels

Linear regression was performed to identify factors that influenced drug levels for IFX and ADA. On univariate analysis (Table 3), active mucosal inflammation was negatively associated with IFX trough levels ($p < 0.001$). Predictive models were then constructed using multivariate analysis to determine the influence of such factors on the variation in IFX trough levels. In a four-factor model, decreases in IFX trough levels were independently predicted by elevated faecal calprotectin ($\beta = -4.008$, $p < 0.001$) and elevated CRP ($\beta = -4.364$, $p = 0.001$) and higher IFX trough levels were predicted by IFX dosed at 10 mg/kg/q8 ($\beta = 6.600$, $p = < 0.001$) and BMI ($\beta = 0.161$, $p = 0.043$) ($R^2 = 31\%$). Colonic disease phenotype was significantly associated with higher IFX trough levels ($\beta = 2.811$, $p = 0.041$) but addition of the factor Montreal location to the four-factor model did not improve the goodness of fit ($p = 0.123$). Other covariates, including weight, serum albumin and combination therapy did not influence trough levels. As anti-drug antibodies were only detected in 3 of 96 serum samples, they were not considered in the analysis.

For ADA, patient weight and BMI ($p = 0.053$ and $p = 0.035$) were inversely associated with ADA drug levels on univariate analysis (Table 4). Colonic disease, serum albumin and weekly dosing were positively associated with higher drug levels ($p = 0.007$, $p = 0.005$ and $p < 0.001$, respectively). For each additional day between last dose of ADA and performing drug monitoring, drug levels decreased by an average of 0.24 μg/mL ($p = 0.002$). In multivariate regression analysis increases in ADA drug levels were independently predicted by higher serum albumin ($\beta = 0.147$, $p = 0.004$) and weekly dosing ($\beta = 2.680$, $p < 0.001$), but lower ADA levels were predicted by higher weight ($\beta = -0.038$, $p = 0.032$) ($R^2 = 23\%$). A similar model adding in days between last dose and therapeutic drug monitoring ($p = 0.065$) increased the R^2 to 25%. Anti-drug antibodies were detected in only 1 of the 95 patients.

Relationship between TGN and drug levels

TGNs were assessed in 70/71 (99%) and 63/65 (94%) of patients treated with IFX and ADA, respectively; 26% were sub-therapeutic. TGN levels were no different for the IFX cohort (median 272; IQR: 194 – 412) compared with the ADA cohort (283 (179-388), p = 0.94). No correlation between levels of TGN and drug levels for IFX (Spearman rho = 0.1, p = 0.39) or ADA (rho = 0.1, p = 0.41) were observed. Correlation improved marginally when considering only those on 5 mg/kg 8 weekly (rho = 0.23, p = 0.098) and ADA weekly (rho = 0.21, p = 0.47). No significant differences in median drug levels for IFX or ADA were observed when classifying patients according to the threshold proposed by Yarur et al (Fig. 4.)

DISCUSSION

Management decisions based on therapeutic drug monitoring for IFX and ADA rely upon drug level thresholds that discriminate patients with active disease from those without. The determination of such levels is influenced by a complex pharmacokinetic-pharmacodynamic relationship. This retrospective study of 191 well-characterised patients with CD addresses these issues with several key findings. First, significant differences in IFX drug levels were consistently observed between patients with active disease compared with clinical remission, biochemical remission and calprotectin normalisation. Second, these differences permitted the identification of target thresholds. Third, such thresholds varied according to different indices of disease activity; higher cut-offs were needed to achieve deeper levels of disease control. Fourth, no such relationship was observed for ADA. Fifth, patient and disease factors, namely higher doses of IFX or ADA and, in the case of IFX, active systemic and mucosal inflammation and BMI, and for ADA, weight and albumin, significantly influenced drug levels. However, this accounted for a relatively small amount of the variation in drug levels. Finally, no correlation was observed between TGNs and drug levels in patients treated with combination therapy with thiopurines and drug levels were similar across different TGN cut-offs.

Our findings that IFX drug levels differ according to disease activity status is in keeping with the literature.[14,29,36,37] Earlier studies reported a threshold of 2-3 μg/mL above which clinical remission was more likely.[18] However, the end-point of clinical remission is no longer viewed as the principle goal for treatment and a strategy targeting tighter disease control by normalisation of C-reactive protein and mucosal healing has been suggested.[38] This is a particularly important concept as there is a poor correlation between symptoms (as reflected in disease activity indices such as the HBI) and mucosal healing,[39,40] an end-point which is being shown to be associated with improved outcomes. In parallel with this new treatment paradigm, higher thresholds are reported to be needed to neutralise inflammatory activity and to achieve mucosal healing. For example, a cross-sectional study in 145 patients with CD and

ulcerative colitis identified thresholds of IFX levels of 5.0 μg/mL that best predicted mucosal healing and 6.8 μg/mL for normalisation of CRP.[19] Similar findings have been reported by others.[24,41] In keeping with these observations, we identified target thresholds for IFX of 1.5, 3.4 and 5.7μg/mL that predicted clinical and biochemical remission and calprotectin normalisation, respectively. Thus, the current study confirms that there is clear relationship between pharmacokinetics and pharmacodynamics for IFX in patients with CD.

The situation for ADA seems quite different. We found no relationship at all between levels of ADA and any of the indices of disease activity. Compared with IFX, there are fewer data supporting the utility of therapeutic drug monitoring with ADA.[42] An early study showed a large difference in outcome between patients with undetectable compared with readily detectable levels of ADA.[43] However, defining a threshold above which predicts remission has proven more troublesome. In a post-hoc analysis of CLASSIC I and II, Chiu et al demonstrated differences in drug levels according to clinical disease status at week 4 and 24 but not at week 56.[23] Further, despite applying complex statistical methods, no thresholds could be found due to significant overlap in drug levels between patients with and without remission. In contrast, thresholds of ADA levels of 7.1 μg/mL that best predicted mucosal healing and 6.6 μg/mL for normalisation of CRP have more recently been reported.[19]

Reasons for the marked contrast between the correlation of IFX and ADA levels with different measures of disease activity are not clear. It seems unlikely that in this study this was due to lack of patient numbers or methodology since the cohorts were large and patients treated with IFX and ADA were examined in identical fashion. The timing of drug level measurement was different in that, for IFX, it was always at trough while, for ADA, it was at different times during the treatment cycle. A relatively small number of ADA levels were sampled at trough (21%), when drug levels have reached their nadir. Due to the relatively flat concentration-time profile seen with ADA, some experts, and limited data, have suggested that

therapeutic drug monitoring can be performed at any time in a treatment cycle.[24,25,44] Despite finding no difference in median drug levels at trough compared with earlier time-points in a treatment cycle (6.1 vs 6.3 μg/mL, p = 0.43), we observed a trend on multivariate regression analysis towards lower drug levels with increasing days between last dose and sampling (β = -0.135, p = 0.065). Further studies that incorporate intensive pharmacokinetic sampling are required to address this issue. The results do indicate, however, that pharmacodynamics and, presumably, levels of ADA at the point of its action in the intestinal tissue do not have a close relationship to the circulating drug levels. A proof-of-concept study has addressed this issue by comparing serum and mucosal tissue levels of IFX and ADA in CD.[45] A significant correlation was seen in patients on IFX (r = 0.51, p = 0.017) but not with ADA (r = 0.23, p = 0.17). Further, in areas of severe inflammation, the ratio of tissue TNF to anti-TNF was elevated, compared to non-inflamed tissue, and those with active mucosal disease had a higher rate of serum-to-tissue mismatch compared to those in remission (p = 0.03). This implies that in active disease, high serum drug levels may not equate to high tissue levels and provides an explanation as to why some patients with a 'therapeutic' drug level have persisting disease.

We sought to identify patient and disease factors that could be used to construct models that predict drug levels. First, as might be expected, we identified that the drug dose was an important factor. Thus, higher doses of IFX (10 mg/kg/q8 rather than 5 mg/kg/q6) and ADA (weekly vs less frequent dosing) were independently associated with higher drug levels. Few data exist as to the ideal dose intensification strategy in the situation of secondary loss of response. In a retrospective study of 168 CD patients a higher response rate with doubling the dose to 10 mg/kg/q8 compared to halving the interval to 5 mg/kg/q4 was observed (77 vs 66%) although this was not statistically significant (p = 0.14).[46] Moreover, drug levels were not measured in this study. Whether dose escalation or reduced frequency is more effective for IFX warrants further study given the pharmacoeconomic benefits that would be expected if infusions are performed less frequently. Second, colonic disease phenotype was significantly associated with higher IFX drug levels (β

= 2.811, p = 0.041) and a similar trend was observed with ADA (β = 1.669, p = 0.086). Third, elevated CRP (p = 0.001) and mucosal inflammation (p < 0.001) were significant predictors of lower IFX levels. Systemic inflammation can accelerate drug clearance via metabolism in the reticuloendothelial system[47] and has been shown to be negatively associated with IFX drug levels by others.[48] Recent data in ulcerative colitis has demonstrated that active mucosal inflammation leads to faecal loss of IFX.[27] Fourth, patient factors, (weight and serum albumin) were identified as independent predictors for ADA, but not for IFX, levels on multivariate analysis. This supports the hypothesis that individualised weight-based dosing may be worthy of investigation with ADA. For every increasing kilogram of body weight, ADA drug levels decreased by 0.038 μg/mL, (p = 0.018) suggesting that heavier patients may need higher doses of ADA to achieve drug levels similar to lighter patients. ADA pharmacokinetic data for CD is relatively sparse. Lie et al found an inverse relationship between BMI and ADA drug levels.[44] Similar findings have been reported in RA.[49] The finding that increasing BMI is associated with higher IFX drug levels is interesting. Higher weight will result in higher doses of IFX but has been shown to be associated with increased clearance of IFX in a non-linear fashion, as well as a higher volume of distribution. The relationship is, therefore, complex and requires further investigation to identify whether it is real and to understand it more fully. Of note, the impact of adding BMI to the model was modest, with an increase in R^2 from 28.3 to 30.8%. Low serum albumin has been associated with lower IFX drug levels in both CD[50] and acute severe ulcerative colitis,[51] but to our knowledge, not with ADA. Ideal predictive models accounted for only 23-31% of the variation in drug levels which highlights the complex pharmacokinetic-dynamic interplay of monoclonal antibodies operating within biological systems.

Finally, we found no correlation between TGNs and drug levels. We did not replicate the findings by Yarur et al, whereby TGN concentrations above a threshold of 125 pmol/$8x10^8$ RBC best predicted higher IFX drug levels, but acknowledge there were few patients with levels below this threshold. Nevertheless, drug levels in the TGN range 125-235 were similar to > 235, suggesting no pharmacokinetic advantage in

dosing thiopurines to a 'therapeutic' range when used in combination with IFX or ADA. These results should be interpreted in the context that no difference in drug levels between those treated with combination therapy compared to monotherapy was observed ($p = 0.86$). Some studies have found higher drug levels in combination therapy compared with monotherapy,[52,53] whereas others have not.[22] Given our cross-sectional study design, we cannot exclude that patients previously on monotherapy with low levels may have subsequently been escalated to combination therapy. Alternatively this may relate to the duration of combination therapy at the time of drug level sampling, as anti-drug antibodies, which have been shown to increase drug clearance and are reduced with co-therapy with immunomodulators, occur early, generally within the first 12 months of therapy.[54] In this regard the median duration of combination therapy in our cohort was 22 months, and the proportion of patients with detectable anti-drug antibodies was low. Future prospective studies randomising patients to different TGN thresholds in combination therapy compared with monotherapy are needed.

Several limitations of the study are acknowledged. First, the cross-sectional design meant samples were measured at a single point in time and patients were not followed to assess subsequent outcomes. Second, it is not possible to assume that the relationships we identified are necessarily causal. Studies are therefore required to show that interventions that correct sub-therapeutic drug levels are associated with better outcome (as was seen in patients with CD in the pre-optimisation phase of TAXIT[55]) Third, only 20/95 (21%) of ADA samples were collected at trough which may in part explain why drug levels did not discriminate between outcomes. The relatively flat peak-trough concentration pharmacokinetics seen with ADA have lead some experts to suggest ADA therapeutic drug monitoring can be performed at any time point in a treatment cycle.[25,56] Our own data (submitted for publication) has shown that ADA drug levels remain stable during the first 9 days in a treatment cycle but then decline towards the end of a two week cycle. Fourth, we did not find a difference in drug levels between patients on combination therapy compared with anti-TNF monotherapy, in contrast to what has been reported elsewhere.[30,48] This may be

explained by the relatively small proportion of patients treated with monotherapy in this cohort (10% of IFX, 20% of ADA). Fifth we used faecal calprotectin as a surrogate of mucosal healing, rather than endoscopy. Although studies have shown good correlation between calprotectin and mucosal inflammation at endoscopy,[57] the accuracy in isolated small bowel CD is questionable.[58] Finally, we were unable to examine the impact of anti-drug antibodies given numbers were small.

Conclusions

IFX, but not ADA drug levels were associated with indices of disease activity in this retrospective cross-sectional study of 191 patients with CD. Optimal IFX thresholds that predicted mucosal healing were higher than for CRP normalisation which were higher again then for clinical remission. No correlation was found between TGNs and drug levels and drug levels were similar across different TGN thresholds. Prospective randomised controlled trials are needed that explore the utility of treating to target drug levels and to investigate the relationship between TGNs and therapeutic drug monitoring of anti-TNF in CD.

Acknowledgements

We acknowledge support from the Department of Health via the National Institute for Health Insurance (NIHR) comprehensive Biomedical Research Centre awards to Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London and to Addenbrooke's Hospital in partnership with the University of Cambridge.

Guarantor of study: MGW

Author contributions: MGW conceived the study design, participated in data acquisition and analysis and drafted the manuscript. BW, CB, SWC and SS participated in data acquisition and revised the manuscript. NU and ZA performed laboratory analysis and revised the manuscript. JR participated in study design, performed statistical analysis and revised the manuscript. JDS, MP, PRG and PMI participated in study design and analysis and revised the manuscript. All authors approved the final version of the manuscript.

Declaration of funding interests: None.

Declaration of personal interests: MGW has served as a speaker for Janssen, AbbVie, Ferring and Takeda and has received research funding from AbbVie**.** BW, CB, NU, SS, SWC, JDS and ZA have no disclosures. MP has served on advisory boards for Pfizer and Takeda and is paid an honorarium by Actavis for chairing a regional meeting. JR holds shares in Novartis. PRG has served as consultant or advisory board member for AbbVie, Ferring, Janssen, Merck, Nestle Health Science, Danone, Allergan and Takeda. His institution has received speaking honoraria from AbbVie, Janssen, Ferring, Takeda, Fresenius Kabi, Mylan and Pfizer. He has received research grants for investigator-driven studies from AbbVie, Janssen, Falk Pharma, Danone and A2 Milk Company. His Department financially benefits from the sales of a digital application and booklets on the low FODMAP diet. He has published an educational/recipe book on diet. PMI has received lecture fees from Abbvie, Warner Chilcott, Ferring, Falk Pharma, Takeda, MSD, Johnson and Johnson, Shire and financial support for research from MSD and Takeda and advisory fees from Abbvie, Warner Chilcott, Takeda, MSD, Vifor Pharma, Pharmacosmos, Topivert, Genentech, Hospira and Samsung Bioepis.

REFERENCES

1. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;**359**:1541–9.

2. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004;**350**:876–85.

3. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006;**130**:323–33.

4. Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. *Gut* 2007;**56**:1232–9.

5. Baert F, Glorieus E, Reenaers C, et al. Adalimumab dose escalation and dose de-escalation success rate and predictors in a large national cohort of Crohn's patients. *J Crohns Colitis* 2013;**7**:154–60.

6. Molnar T, Farkas K, Nyári T, et al. Frequency and predictors of loss of response to infliximab or adalimumab in Crohn's disease after one-year treatment period - a single center experience. *J Gastrointestin Liver Dis* 2012;**21**:265–9.

7. Rutgeerts P, Van Assche G, Sandborn WJ, et al. Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: data from the EXTEND trial. *Gastroenterology* 2012;**142**:1102–2.

8. Kiss LS, Szamosi T, Molnar T, et al. Early clinical remission and normalisation of CRP are the strongest predictors of efficacy, mucosal healing and dose escalation during the first year of adalimumab therapy in Crohn's disease. *Aliment Pharmacol Ther* 2011;**34**:911–22.

9. Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in Crohn's disease: a systematic review. *Am J Gastroenterol* 2011;**106**:674–84.

10. Ma C, Huang V, Fedorak DK, et al. Adalimumab dose escalation is effective for managing secondary loss of response in Crohn's disease. *Aliment Pharmacol Ther* 2014;**40**:1044–55.

11. Papamichael K, Karatzas P, Mantzaris GJ. Addition of an immunomodulator as a rescue therapy for loss of response to adalimumab dose escalation in patients with Crohn's disease. *J Crohns Colitis* 2015;**9**:589–90.

12. Frederiksen MT, Ainsworth MA, Brynskov J, et al. Antibodies against infliximab are associated with de novo development of antibodies to adalimumab and therapeutic failure in infliximab-to-adalimumab switchers with IBD. *Inflamm Bowel Dis* 2014;**20**:1714–21.

13. Yanai H, Lichtenstein L, Assa A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin Gastroenterol Hepatol* 2015;**13**:522–2.

14. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006;**4**:1248–54.

15. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013;**7**:736–43.

16. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014;**63**:1721–7.

17. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013;**19**:2568–76.

18. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. *J Crohns Colitis* 2016;**10**:619–25.

19. Ungar B, Levy I, Yavne Y, et al. Optimizing Anti-TNF-α Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2016;**14**:550–2.

20. Roblin X, Marotte H, Rinaudo M, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014;**12**:80–2.

21. Imaeda H, Takahashi K, Fujimoto T, et al. Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn's disease. *J Gastroenterol* 2014;**49**:100–09.

22. Mazor Y, Almog R, Kopylov U, et al. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. *Aliment Pharmacol Ther* 2014;**40**:620–8.

23. Chiu Y-L, Rubin DT, Vermeire S, et al. Serum Adalimumab Concentration and Clinical Remission in Patients with Crohn's Disease. *Inflamm Bowel Dis* 2013;**19**:1112–22.

24. Zittan E, Kabakchiev B, Milgrom R, et al. Higher Adalimumab Drug Levels are Associated with Mucosal Healing in Patients with Crohn's Disease. *J Crohns Colitis* 2016;**10**:510–5.

25. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. *J Clin Pharmacol* 2015;**55 Suppl 3**:S39–50.

26. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;**348**:601–8.

27. Brandse JF, van den Brink GR, Wildenberg ME, et al. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. *Gastroenterology* 2015;**149**:350–2.

28. Fasanmade AA, Adedokun OJ, Blank M, et al. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. *Clin Ther* 2011;**33**:946–64.

29. Colombel J-F, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy

for Crohn's disease. *N Engl J Med* 2010;**362**:1383–95.

30. Yarur AJ, Kubiliun MJ, Czul F, et al. Concentrations of 6-Thioguanine Nucleotide Correlate With Trough Levels of Infliximab in Patients With Inflammatory Bowel Disease on Combination Therapy. *Clin Gastroenterol Hepatol* 2015;**13**:1118–1124

31. Van Assche G, Dignass A, Panés J, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010;**4**:7-27

32. Harvey RF, Bradshaw MJ Measuring Crohn's disease activity. *Lancet* 1980;**1**:1134–5.

33. Moreau AC, Paul S, Del Tedesco E, et al. Association between 6-thioguanine nucleotides levels and clinical remission in inflammatory disease: a meta-analysis. *Inflamm Bowel Dis* 2014;**20**:464–71.

34. Dervieux T, Boulieu R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 1998;**44**:551–5.

35. http://www.hra.nhs.uk/documents/2016/06/defining-research.pdf Accessed - August 2016

36. Steenholdt C, Bendtzen K, Brynskov J, et al. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 2011;**46**:310–8.

37. Imaeda H, Bamba S, Takahashi K, et al. Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. *J Gastroenterol* 2014;**49**:674–82.

38. Bouguen G, Levesque BG, Feagan BG, et al. Treat to target: a proposed new paradigm for the management of Crohn's disease. *Clin Gastroenterol Hepatol* 2015;**13**:1042–2.

39. Peyrin-Biroulet L, Reinisch W, Colombel J-F, et al. Clinical disease activity, C-reactive protein normalisation and mucosal healing in Crohn's disease in the SONIC trial. *Gut* 2014;**63**:88–95.

40. Falvey JD, Hoskin T, Meijer B, et al. Disease activity assessment in IBD: clinical indices and biomarkers fail to predict endoscopic remission. *Inflamm Bowel Dis* 2015;**21**:824–31.

41. Yarur AJ, Jain A, Hauenstein SI, et al. Higher Adalimumab Levels Are Associated with Histologic and Endoscopic Remission in Patients with Crohn's Disease and Ulcerative Colitis. *Inflamm Bowel Dis* 2016;**22**:409–15.

42. Paul S, Moreau AC, Del Tedesco E, et al. Pharmacokinetics of Adalimumab in Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2014;**20**:1288–95.

43. Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009;**137**:1628–40.

44. Lie MRKL, Peppelenbosch MP, West RL, et al. Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment. *Aliment Pharmacol Ther* 2014;**40**:1202–8.

45. Yarur AJ, Jain A, Sussman DA, et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. *Gut* 2016;**65**:249–55.

46. Katz L, Gisbert JP, Manoogian B, et al. Doubling the infliximab dose versus halving the infusion intervals in Crohn's disease patients with loss of response. *Inflamm Bowel Dis* 2012;**18**:2026–33.

47. Ordás I, Mould DR, Feagan BG, et al. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther* 2012;**91**:635–46.

48. Papamichael K, Van Stappen T, Vande Casteele N, et al. Infliximab Concentration Thresholds During Induction Therapy Are Associated With Short-term Mucosal Healing in Patients With Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2016;**14**:543–9.

49. Ternant D, Ducourau E, Fuzibet P, et al. Pharmacokinetics and concentration-effect relationship of adalimumab in rheumatoid arthritis. *British Journal of Clinical Pharmacology* 2015;**79**:286–97.

50. Dotan I, Ron Y, Yanai H, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis* 2014;**20**:2247–59.

51. Brandse JF, Mathôt RA, van der Kleij D, et al. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2016;**14**:251–2.

52. Colombel J-F, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of biologics for inflammatory bowel disease. *Inflamm Bowel Dis* 2012;**18**:349–58.

53. van Schaik T, Maljaars JPW, Roopram RK, et al. Influence of combination therapy with immune modulators on anti-TNF trough levels and antibodies in patients with IBD. *Inflamm Bowel Dis* 2014;**20**:2292–8.

54. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014;**63**:1258–64.

55. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015;**148**:1320–3.

56. Vaughn BP, Sandborn WJ, Cheifetz AS. Biologic Concentration Testing in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2015:1–8.

57. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal Calprotectin Correlates More Closely With the Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, Blood Leukocytes, and the CDAI. *Am J Gastroenterol* 2009;**105**:162–9.

58. Stawczyk-Eder K, Eder P, Lykowska-Szuber L, et al. Is faecal calprotectin equally useful in all Crohn's disease locations? A prospective, comparative study. *Arch Med Sci* 2015;**11**:353–61.

FIGURES

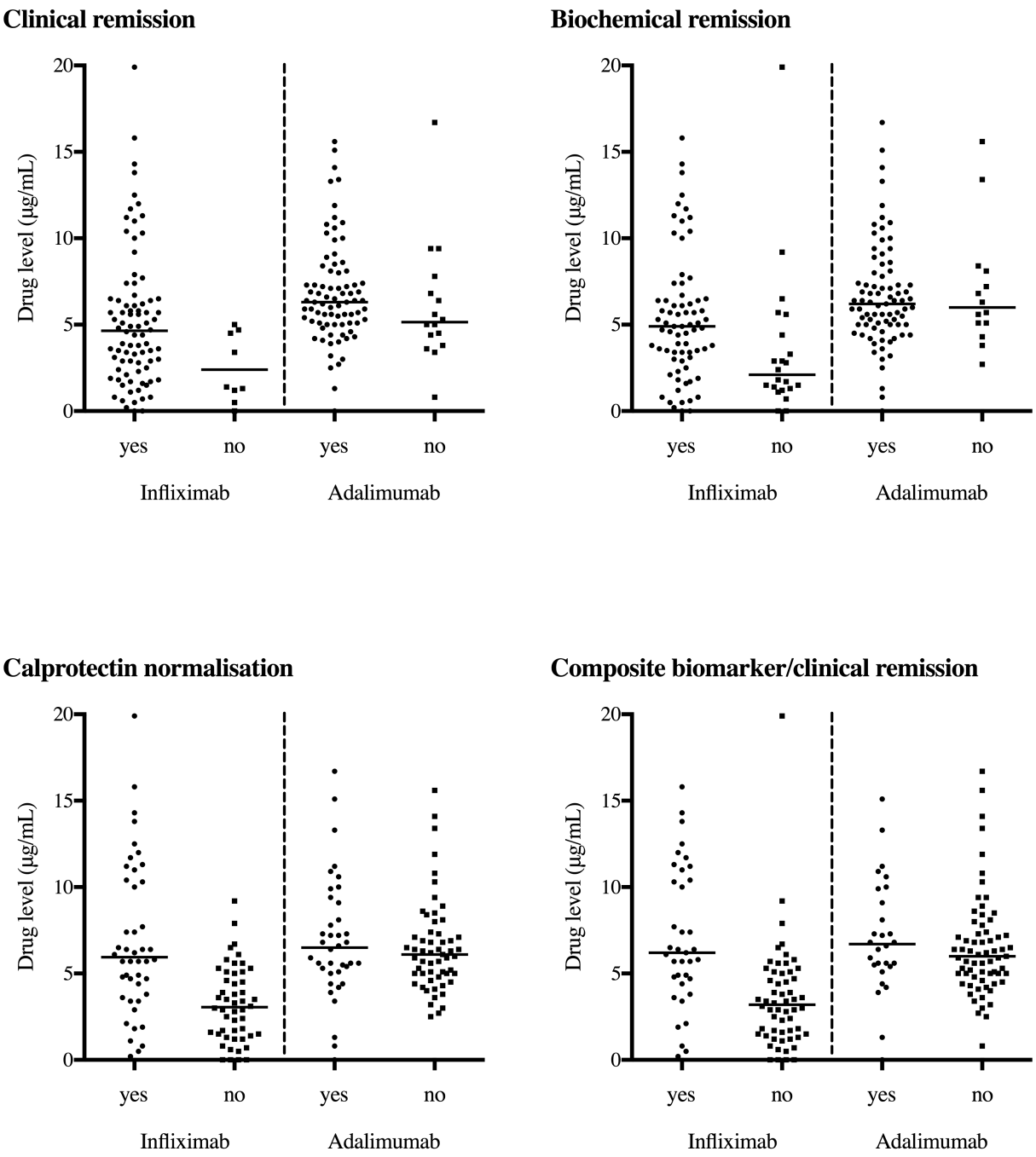

Figure 1. Scatterplots of relationship between IFX (n = 96) and ADA (n = 95) drug levels and disease indices. Significant differences in IFX levels were observed for biochemical remission (4.9 vs 2.1 µg/mL, $p = 0.003$), calprotectin normalisation (6.0 vs 3.1, $p < 0.0001$) and deep remission (6.2 vs 3.2 µg/mL $p <$

0.0001). No difference in ADA drug levels was observed for any endpoint ($p > 0.15$), (Mann-Whitney test). Horizontal bars represent median drug levels. One outlier with IFX drug level = 35.2 µg/mL not shown. HBI not calculated in 2 patients and deep remission not assessed in 1 patient due to stoma. IFX = infliximab, ADA = adalimumab, HBI = Harvey-Bradshaw index.

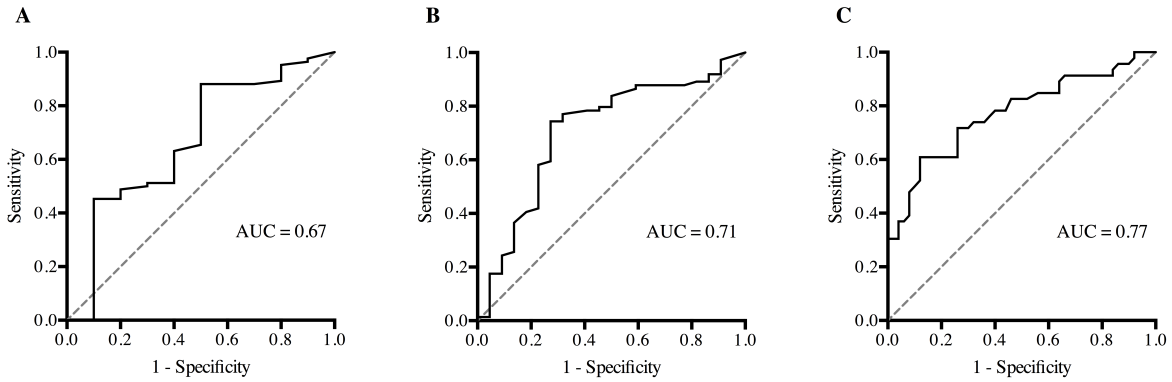

Figure 2. ROC analysis for infliximab trough levels stratifying patients with and without (A) clinical remission, (B) biochemical remission and (C) calprotectin normalisation. AUC = area under curve

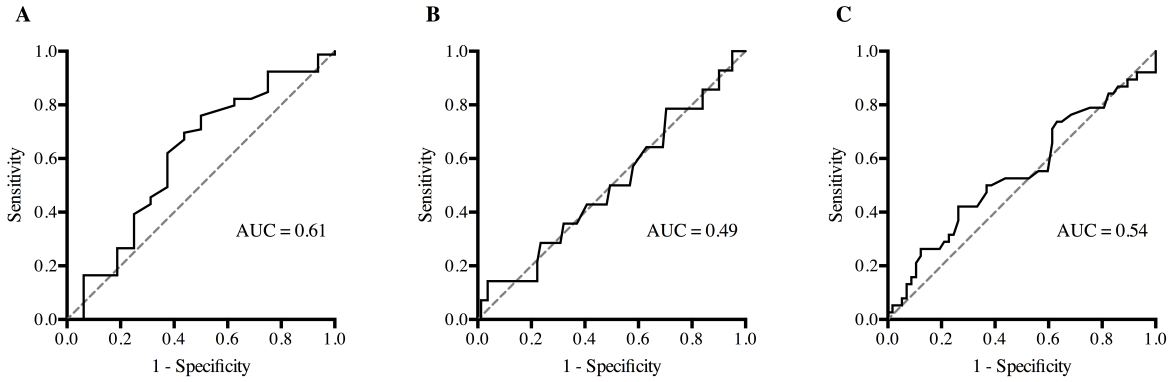

Figure 3. ROC analysis for adalimumab drug levels stratifying patients with and without (A) clinical remission, (B) biochemical remission and (C) calprotectin normalisation. AUC = area under curve

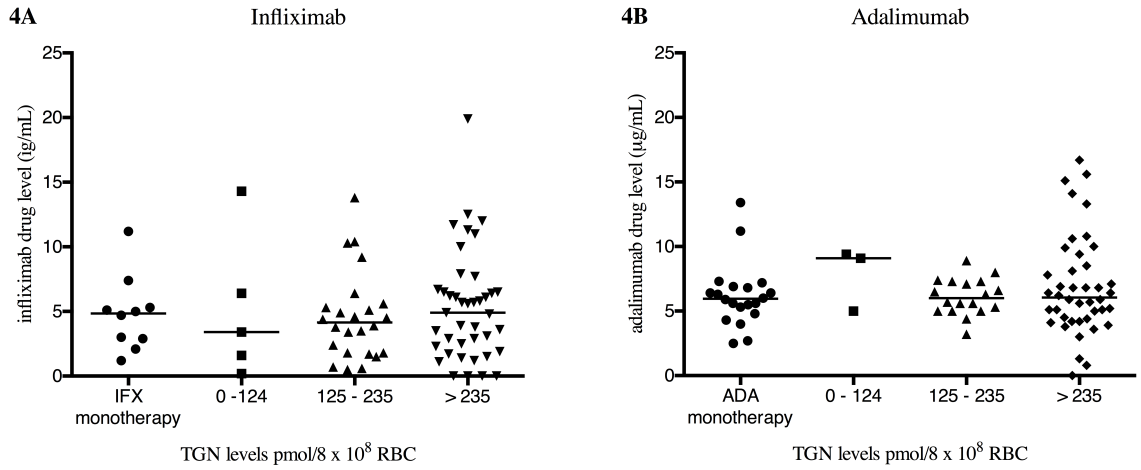

Figure 4A and B. Scatterplot of IFX (A) and ADA (B) drug levels stratified by TGNs in patients treated with combination therapy and by monotherapy. Horizontal bars represent median drug levels. No significant difference in drug levels was seen between TGN cut-offs of 0-124, 125-235, >235 and monotherapy ($p = 0.89$ IFX, $p = 0.80$ ADA, Kruskal-Wallis test). IFX = infliximab, ADA = adalimumab, TGN = 6-thioguanine nucleotides.

TABLES

Table 1. Patient characteristics (n = 191)

Characteristic		IFX (n=96)	ADA (n=95)	p value
Male		48/96 (50%)	52/95 (54.7%)	0.512
Age years, median (IQR)		34 (28 – 44)	37 (31 – 47)	0.094
Disease duration years, median (IQR)		9.5 (3 – 17)	11 (5 – 18)	0.218
Montreal classification	A1	15 (15.6%)	9 (9.5%)	0.384
	A2	71 (74%)	73 (76.8%)	
	A3	10 (10.4%)	13 (13.7%)	
	B1	56 (58.3%)	54 (56.8%)	0.165
	B2	24 (25%)	16 (16.8%)	
	B3	16 (16.7%)	25 (26.3%)	
	L1	18 (18.8%)	11 (11.6%)	0.336
	L2	23 (24%)	28 (29.5%)	
	L3	55 (57.3%)	56 (58.9%)	
Current smoker		14 (14.6%)	10 (10.5%)	0.398
Weight kg, mean (±SD)		76.1 (±18.7)	73.5 (±16.1)	0.305
BMI kg/m^2, mean (±SD)		25.9 (±5.9)[a]	24.9 (±4.8)	0.217
Proportion with active disease	HBI ≥ 5	10 (10.6%)[b]	16 (16.8%)	0.216
	CRP ≥ 5 mg/L	22 (22.9%)	14 (14.7%)	0.148
	FCP ≥ 59 µg/g	50 (52.1%)	57 (60%)	0.270
Serum albumin g/L, mean (±SD)		44.8 (±3.2)	42.5 (±5.8)	0.001
Concurrent immunomodulator use	Any	86 (89.6%)	75 (78.9%)	0.043
	Thiopurines	71 (74%)	65 (68.4%)	0.113
	Methotrexate	10 (10.4%)	8 (8.4%)	
	Thioguanine	5 (5.2%)	1 (1.1%)	
	Mycophenolate mofetil	0 (0%)	1 (1.1%)	
TGN pmol/8x10^8 RBC, median (IQR)		271.5 (193.5-412.5)	283 (179-388)	0.939
Proportion with therapeutic TGN		41/70 (58.6%)	42/63 (66.7%)	0.336
Dosing		5mg/kg/q8 74/96 (77.1%)	EOW 72/95 (75.8%)	
		5mg/kg/q6 11 (11.5%)	EW 20/95 (21.1%)	
		10mg/kg/q8 11 (11.5%)	Every 10 days 3/95 (3.2%)	
	Duration at dose months, median (IQR)	15 (6 – 32)	18 (9 – 34)	0.405
Therapeutic drug monitoring performed at trough		Every other week	13/72 (18.1%)	EOW
		Weekly	6/20 (30%)	EW

	Every 10 days	1/3 (33.3%)	Every 10 days

[a] available in 94/95, [b] HBI not calculated in two patients with a stoma BMI, body mass index; CRP, c-reactive protein; HBI, Harvey Bradshaw Index; FCP, faecal calprotectin; RBC, red blood cell

Table 2. Results from ROC curve analysis of infliximab and adalimumab drug levels associated with remission according to endpoint.

Drug	Remission type	AUC [95% CI]	p value	Cut-off	Sensitivity	Specificity	PPV	NPV
Infliximab	Clinical	0.67 [0.48, 0.86]	0.081	> 1.5	85.7	50.7	93.7	33.3
	Biochemical	0.71 [0.58, 0.84]	0.003	> 3.4	74.3	72.7	90.2	45.7
	calprotectin normalisation	0.77 [0.68, 0.87]	< 0.0001	> 5.7	60.9	88.0	88.2	71.0
Adalimumab	Clinical	0.61 [0.44, 0.77]	0.157	> 5.1	75.6	50.0	88.2	29.6
	Biochemical	0.49 [0.32, 0.67]	0.971	> 8.5	21.2	85.7	90.0	16.0
	calprotectin normalisation	0.54 [0.42, 0.66]	0.436	> 7.2	40.1	73.7	51.6	65.6

Cut-off thresholds reported in µg/mL and calculated using Youden Index. AUC = Area under curve, CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value

Table 3. Linear regression analysis of relationship between patient and disease factors and trough infliximab drug levels.

Modulating factors		Univariate analysis			Multivariate analyses (4-factor model with FCP, IFX dose, CRP and BMI, and, estimates for a 5th added factor)[a]		
Variable	Factor level or units	Estimate of beta	SE	Wald test p-value	Estimate of beta	SE	Wald test p-value
Gender (ref = male)	Female	0.200	1.009	0.843	0.300	0.866	[0.730]
Age at drug level sampling	Years	0.020	0.044	0.642	0.024	0.040	[0.550]
Disease duration	Years	-0.001	0.056	0.984	-0.022	0.048	[0.655]
Montreal A (ref A1)	A2	-1.100	1.380	0.428	0.485	1.267	[0.703]
Montreal A (ref A1)	A3	2.213	1.983	0.267	2.191	1.839	[0.237]
Montreal B (ref B1)	B2	-1.934	1.191	0.108	-0.787	1.131	[0.488]
Montreal B (ref B1)	B3	-1.690	1.384	0.225	-0.627	1.226	[0.610]
Montreal L (ref L1)	**L2**	2.330	1.541	0.134	**2.811**	**1.357**	**[0.041][b]**
Montreal L (ref L1)	L3	0.566	1.329	0.671	1.448	1.130	[0.204]
Smoker (ref = No)	Yes	0.885	1.427	0.536	-0.102	1.272	[0.936]
Weight	kg	0.029	0.027	0.284	0.008	0.046	[0.855]
BMI	**kg/m^2**	0.072	0.087	0.406	**0.161**	**0.078**	**0.043**
albumin	g/L	0.174	0.160	0.279	0.038	0.147	[0.799]
Combo vs mono (ref = mono)	Combination therapy	0.510	1.650	0.760	-0.918	1.410	[0.517]
IFX dose (ref = 5mg/kg/q8	**10mg/kg/q8**	2.960	1.580	0.064	**6.600**	**1.576**	**<0.001**
IFX dose (ref = 5mg/kg/q8	**5mg/kg/q6**	0.460	1.580	0.773	**1.375**	**1.370**	**0.318**
HBI (ref = remission)	active disease	0.484	1.660	0.771	1.337	1.440	[0.356]
CRP (ref = remission)	**active disease**	-2.215	1.179	0.063	**-4.364**	**1.310**	**0.001**
FCP (ref = remission)	**active disease**	-4.223	0.911	<0.001	**-4.008**	**0.889**	**< 0.001**

Montreal classification: A = age, B = behaviour, L = location, BMI = body mass index, Combo = combination therapy, mono = IFX monotherapy IFX = infliximab, HBI = Harvey-Bradshaw index, CRP = c-reactive protein, FCP = faecal calprotectin. SE = standard error. P-values in square brackets refer to the t-test for adding the associated extra term to the model. [a]*P-values in square brackets refer to the t-test for adding the associated extra term to the four-factor model.* [b]*Wald Test p=0.123 for the MonL factor in the five-factor model.*

Table 4. Linear regression analysis of relationship between patient and disease factors and adalimumab drug levels.

Modulating factors		Univariate analysis			Multivariate analyses (3-factor model with ADA dose, weight and albumin, and, estimates for a 4th added factor)[a]		
Variable	Factor level or units	Estimate of beta	SE	Wald test p-value	Estimate of beta	SE	Wald test p-value
Gender (reference level = male)	Female	0.593	0.630	0.349	0.149	0.644	[0.817]
Age at drug level sampling	Years	-0.011	0.029	0.711	0.020	0.027	[0.460]
Disease duration	Years	-0.018	0.035	0.615	-0.030	0.031	[0.339]
Montreal A (ref A1)	A2	1.683	1.076	0.121	1.754	0.959	[0.071]
Montreal A (ref A1)	A3	1.209	1.321	0.362	2.027	1.214	[0.099]
Montreal B (ref B1)	B2	0.227	0.877	0.797	-0.684	0.787	[0.387]
Montreal B (ref B1)	B3	-0.436	0.745	0.560	0.498	0.691	[0.473]
Montreal L (ref L1)	L2	2.901	1.048	0.007	1.669	0.961	[0.086]
Montreal L (ref L1)	L3	1.331	0.972	0.174	0.060	0.916	[0.948]
Smoker (reference = No)	Yes	-1.311	1.018	0.201	-1.069	0.920	[0.248]
Weight	**kg**	-0.038	0.019	0.053	**-0.038**	**0.018**	**0.032**
BMI	kg/m2	-0.139	0.065	0.035	-0.031	0.120	[0.796]
Albumin	**g/L**	0.153	0.053	0.005	**0.147**	**0.050**	**0.004**
Combo vs mono (ref = mono)	Combination therapy	0.631	0.770	0.415	-0.271	0.708	[0.703]
Days between dose and TDM	Days	-0.235	0.073	0.002	-0.135	0.072	[0.065]
ADA dose (ref = EOW)	**Weekly**	3.033	0.714	<0.001	**2.680**	**0.684**	**<0.001**
ADA dose (ref = EOW)	**Every 10 days**	0.725	1.664	0.664	2.231	1.635	[0.176]
HBI (ref = remission)	active disease	-0.703	0.839	0.404	-0.804	0.739	[0.279]
CRP (ref = remission)	active disease	0.357	0.888	0.689	-0.036	0.811	[0.965]
FCP (ref = remission)	active disease	-0.438	0.642	0.497	-0.417	0.598	[0.488]

Montreal classification: A = age, B = behaviour, L = location, BMI = body mass index, Combo = combination therapy, mono = ADA monotherapy ADA = adalimumab, HBI = Harvey-Bradshaw index, CRP = c-reactive protein, FCP = faecal calprotectin. SE = standard error. P-values in square brackets refer to the t-test for adding the associated extra term to the model. [a]*P-values in square brackets refer to the t-test for adding the associated extra term to the three-factor model.*

Supplementary Table 1: Infliximab drug levels according to composite end-points of remission

Endpoint	Active disease, number (%)	Drug level, median (IQR)	Remission, number (%)	Drug level, median (IQR)	p value
HBI/CRP remission[a]	28/94 (29.8)	2.9 (1.3 – 4.9)	66/94 (70.2)	5 (3.1 – 6.9)	0.005
CRP/FCP	55/96 (57.3)	3 (1.5 – 5.1)	41/96 (42.7)	6.2 (4.6 – 10.7)	< 0.0001
HBI/CRP/FCP[b]	58/95 (61.1)	3.2 (1.5 – 5.1)	37/95 (38.9)	6.2 (4.6 – 10.7)	< 0.0001

Drug levels reported in µg/mL. Differences in median drug levels calculated using Mann-Whitney test. HBI not calculated in [a]2 and [b]1 due to stoma. HBI = Harvey-Bradshaw index, CRP = c-reactive protein, FCP = faecal calprotectin, IQR = inter-quartile range

CHAPTER 8:

Pharmacokinetic perspectives: Intra-patient Variability in Adalimumab Drug Levels Within and Across Cycles in Crohn's Disease

Intra-patient Variability in Adalimumab Drug Levels Within and Across Cycles in Crohn's Disease

Short title: Variability in adalimumab drug levels

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Abbreviations:

IFX = infliximab, ADA = adalimumab, CD = Crohn's disease, IBD = inflammatory bowel disease, HBI = Harvey-Bradshaw Index, CRP = C-reactive protein, FC = faecal calprotectin, TNF = tumour necrosis factor, ELISA = enzyme-linked immunosorbent assay, DDD = drug delivery device.

ABSTRACT

Background: Therapeutic drug monitoring with infliximab is performed at trough, but whether this is necessary for adalimumab (ADA) has not been defined. The aim was to determine intra-patient ADA drug level variation and to identify modulating patient and disease factors.

Methods: In this prospective observational study, adult patients with Crohn's disease established on maintenance ADA underwent pharmacokinetic-pharmacodynamic evaluations according to pre-defined schedules (visit 1: day 4-6, visit 2: day 7-9, trough: day 13-14) across two consecutive fortnightly cycles. ADA drug levels and disease activity were assessed. Trough levels ≥4.9 μg/mL were considered therapeutic.

Results: 19 patients underwent 111 evaluations. Intra-patient drug levels from paired visits across subsequent cycles did not differ significantly (p=0.542). Drug levels were stable over the first 9 days, but declined to trough by a mean 1.06 and 0.89 μg/mL between visit 1 or 2, respectively (p<0.001). Models using non-temporal factors (smoking, syringe-delivery device) and drug levels at previous visits accounted for 66-80% of the variance in trough levels. On ROC analysis, thresholds identified in the first 9 days that predicted a therapeutic trough level were similar to the trough threshold itself, with high sensitivity but modest specificity.

Conclusions: Intra-patient drug levels do not change between subsequent cycles and remain stable during the first 9 days, but then decline to trough. Drug levels within the first 9 days accurately predict trough levels using defined models. The trough cut-off value can be applied to the earlier time points in predicting a therapeutic trough drug level with reasonable confidence.

Keywords: adalimumab, Crohn's disease, therapeutic drug monitoring

INTRODUCTION

The monoclonal anti-tumour necrosis factor (TNF) inhibitors, infliximab (IFX) and (ADA) are effective agents for the induction and maintenance of remission in luminal and fistulising Crohn's disease[1-4] and ulcerative colitis.[5,6] Despite their effectiveness, 5-10% fail to respond to induction and a further 15% to 54% of patients subsequently lose response by 12 months, depending on the definition employed.[7] Mechanisms underpinning primary non-response and secondary loss of response include immunogenicity due to the development of anti-drug antibodies[8,9] and other non-immune mechanisms that increase drug clearance.[10] A shift of disease away from a predominant TNF-α pathway to involve other mediators has also been implicated.[7] Managing and preventing loss of response is a key issue in inflammatory bowel disease because few alternative efficacious agents exist, unlike in other chronic autoimmune diseases where a raft of monoclonal antibodies are available.

In this regard, there is a growing body of evidence supporting the use of therapeutic drug monitoring of IFX and ADA. Studies have consistently demonstrated that undetectable or low IFX trough levels (taken immediately before the scheduled dose of IFX) are associated with worse clinical outcomes[11-14] and that the therapeutic range associated with clinical remission using ELISA based assays is between 3-7 μg/mL.[15-18] Although some have demonstrated a similar relationship with ADA,[1,19-21] others have found no association between drug levels and clinical outcomes.[22] There is also a paucity of data identifying what is a 'therapeutic cut-off'.[23] Differences between assays used, the sample timing and the pharmacokinetics of IFX and ADA may explain these discrepant results. Moreover, it is likely that more data exist for IFX simply because it is easier to sample drug levels at trough when the patient presents for their scheduled infusion, rather than recruit patients treated with ADA who self-administer the drug at home.

ADA is administered subcutaneously at a dose of 160 mg and 80 mg fortnightly during induction, and then continued at 40 mg every other week during maintenance. The subcutaneous route limits the volume of drug that can be administered and, in comparison with the intravenous route, potentially

leads to inconsistent bioavailability due to variations in absorption and subsequent lymphatic clearance prior to reaching the systemic circulation. Absorption is relatively slow, with the maximum plasma concentration being achieved in 5.46 ± 2.3 days in patients with Crohn's disease.[24] Clearance is generally linear, exhibiting dose-proportional behavior, and is influenced by body weight, inflammatory burden and the presence of circulating ADA antibodies. The half-life of ADA in patients with Crohn's disease is 10-20 (mean 14) days.[25] There appears no overall difference in the bioavailability of ADA between the delivery device (pen or syringe) or the injection site (abdomen vs thigh), although high quality data are lacking.[26] Differences in the loading doses and pharmacokinetics between IFX and ADA lead to contrasting concentration-time profiles; IFX yields high peak concentrations and low trough levels whereas ADA exhibits more uniform concentration-time profiles at steady state.

Before therapeutic drug monitoring of ADA can be integrated into the standard of care, the clinician must have confidence in the results of a single 'spot' drug level. This depends first on demonstrating minimal intra-individual variation from one treatment cycle to the next given hypothetically, differences in bioavailability from one injection to the next may occur. Secondly, any variability in timing of blood sampling within a cycle may be important and should be evaluated. Given the uniform concentration-time pharmacokinetic profile of ADA, it is possible that drug-level sampling can be performed at any time point during a fortnightly cycle, rather than at trough, but the validity of this approach has yet to be demonstrated in a well-designed study.[27]

Hence, the aims of this study were to address the hypothesis that there are minimal variations of ADA drug levels between and within a cycle, by assessing and comparing intra-individual ADA drug levels at multiple time-points during and between fortnightly dosing regimens amongst patients with Crohn's disease, and to examine potential modulating factors thereof.

METHODS

Patients

Eligible patients, 18 years of age or greater, with Crohn's disease were recruited between July 2014 and August 2015 from the inflammatory bowel disease outpatient clinics of the Alfred Hospital and Eastern Health, Melbourne, Australia. The diagnosis of Crohn's disease was based on standard endoscopic, histopathologic, and radiological criteria.[28] Patients were established on maintenance ADA 40 mg every other week (defined as >14 weeks of treatment). Where prescribed, concomitant immunomodulators (azathioprine, mercaptopurine or methotrexate) were maintained at a stable dose for at least 12 weeks prior to enrolment and continued throughout the study. No patients received concurrent corticosteroids. All patients provided written informed consent. The study was approved by the institutional ethics review committees of the participating centres.

Study Design

Patients attended at each time-point of days 4-6 ('visit 1'), days 7-9 ('visit 2') and days 13-14 ('trough') across two consecutive 14-day ADA treatment cycles (cycle 1 and 2), where day 1 was the first day after the last ADA dose. At each study visit, clinical disease activity was assessed using the Harvey-Bradshaw Index (HBI)[29] with an HBI ≥ 5 deemed to represent active disease and systemic inflammation was assessed by measuring serum C-reactive protein (CRP) with concentration >3 mg/L being defined as active. Faecal calprotectin, a surrogate of mucosal healing, was performed once in each ADA fortnightly cycle, with ≥150 μg/g considered to be active disease. Patient demographics, disease phenotype by the Montreal classification[30], weight, body mass index (BMI), injection method (device: pen vs syringe, site: abdomen vs thigh) and smoking status were documented. Peripheral blood was taken at each study visit (i.e., six samples were taken) for ADA drug levels. Serum was stored at -20 ºC until assayed.

Laboratory Methods

ADA serum levels were measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) (Shikari Q-ADA, Matriks Biotek, Turkey) as per manufacturer's instructions. All samples were measured in duplicate and the average reported in μg/mL. The samples were diluted with the kit assay buffer at either 1:20, 1:10 or 1:4 as required and the concentration determined from the standard curve multiplied by the dilution factor. The upper limit of the assay was 20 μg/ml. The lower limit of quantification was 0.1 μg/mL. An ADA level <4.9 μg/mL was defined as sub-therapeutic.[20,21] CRP serum levels were measured using an in-vitro diagnostic assay on Architect ci16200 analyser (Abbott Laboratories, Abbott Park, IL, USA). Faecal calprotectin was measured in duplicate on extracts of 50 mg of homogenised stool by ELISA (Bühlmann Laboratories, Switzerland) as per manufacturer's instructions. The results were reported as μg/g faeces.

Statistical Analyses

Categorical variables are presented as number and percentage, and quantitative data as mean with standard deviation or median with interquartile range (IQR). Comparisons between patient groups were carried out using Pearson χ^2, independent sample t-test or Mann-Whitney U-test, as appropriate. Linear mixed models for drug levels were fitted to investigate inter and intra-patient variation and to enable F-tests for significant differences between cycles, visits and their two-way interaction. Univariate and multivariate linear regression models for trough drug level and logistic regression models for the achievement of a therapeutic drug level at trough were evaluated for the following factors and covariates: gender, cycle (1st or 2nd), smoking status, delivery device (DDD), weight at study entry, body mass index (BMI), use of concomitant immunomodulation, drug level at visits 1 and 2, serum albumin, and indices of disease activity (HBI, CRP and faecal calprotectin). A stepwise regression procedure, based on t-tests for adding or dropping terms from the linear regression models, was used to find a parsimonious best model. A similar stepwise procedure, based on Wald Test p-values, was used in the exploration of the logistic regression models. Models were fitted using the GenStat statistical package version 17 (VSN International Ltd, Hemel Hempstead, UK) and receiver-operator characteristic (ROC) curves for logistic regression models were produced using SAS software version 9.4 (SAS Institute.,

Cary, NC, USA).

RESULTS

Patient characteristics

Nineteen patients (11 female, 58%) underwent 111 evaluations; one patient did not attend for blood testing during the second cycle. Mean age was 39.2 (SD 9.5) y and median disease duration was 11 (IQR, 6-18) y. 7/19 (37%) were smokers and 14/19 (74%) were co-treated with an immunomodulator. Pen delivery device was used in 16/19 (84%) and all patients administered ADA into the abdomen. Patient demographics are shown in Table 1.

ADA drug levels

Drug levels at all time points in individual patients are shown (Fig 1). Summary data are shown in Table 2. Variation in drug levels was predominantly between patients (between-patient variance component = 4.13, within-patient variance component = 1.05, intra-class coefficient = 0.798). At trough, 23/37 (62%) were sub-therapeutic.

Between-cycle differences: Drug levels did not differ significantly between cycles ($F = 0.38$ with 1 and 87 df, $p = 0.542$) and the differences between visits did not differ between cycles ($F = 0.51$ with 2 and 87 df, $p = 0.604$). In only one of the 18 patients did the qualitative assessment of therapeutic vs sub-therapeutic (<4.9 μg/mL) at trough change across cycles (cycle 1: 2.49, cycle 2: 5.12 μg/mL).

Between-visit differences: Drug levels were similar between visit 1 and 2, with means (SEM) of 5.01 (0.37) and 4.84 μg/mL (0.40), respectively ($p = 0.49$). The levels declined significantly from both visits 1 and 2 to the trough level of 3.95 μg/mL (0.35) ($p<0.001$ for both). This equated to a mean fall of 0.17 (3%) from visit 1 (day 4-6) to visit 2 (day 7-9), 0.89 (18%) from visit 2 (day 7-9) to trough (day 13-14), and 1.06 μg/mL (21%) from visit 1 (day 4-6) to trough (day 13-14). (Fig 2). The declines in drug levels over the visits in each cycle were similar (visit 1 to 2: Pearson's $r = 0.869$; 1 to 3: $r = 0.765$; and 2 to 3: $r = 0.860$).

Relationship between non-trough drug levels, covariates of interest and trough drug levels

Predictive models that included drug levels at visit 1 or 2 and other potentially relevant covariates were constructed using univariate and multivariate linear regression. As shown in Table 3, factors predictive of trough drug levels via univariate analysis included drug levels at visit 1 and 2 ($p < 0.001$), smoking ($p = 0.04$) and syringe delivery device used ($p = 0.036$).

In multiple regression analysis, increases in trough drug levels were independently predicted by increases in levels at visit 1 ($\beta = 0.625$, $p < 0.001$), and an increase with syringe delivery ($\beta = 1.795$, $p = 0.005$), but lower trough levels were predicted by smoking ($\beta = -1.038$, $p = 0.034$) ($R^2 = 65.9\%$). In a similar model, increases in trough drug levels were predicted by increases in levels at visit 2 ($\beta = 0.681$, $p < 0.001$) and an increase with syringe delivery ($\beta = 1.602$, $p = 0.001$) but a decline in trough levels was predicted by smoking ($\beta = -0.864$, $p = 0.022$), ($R^2 = 80.0$). In these multivariate regression models, indices of active disease (CRP, faecal calprotectin and HBI) were not significantly associated with trough levels, although a trend was observed for lower level with active mucosal inflammation on faecal calprotectin. No relationship was observed between patient weight or BMI and trough drug level.

Predictors of therapeutic drug levels

Logistic regression analysis was also performed to identify pharmacokinetic and pharmacodynamic factors associated with achieving a therapeutic adalimumab trough level (>4.9 μg/mL). As shown in Table 4, levels at visit 1 and 2 were significant predictors of a therapeutic trough level. The corresponding ROC curves and threshold concentrations for visit 1: AUC = 0.851, Youden Index = 4.93 μg/mL, (sensitivity = 100%, specificity = 65.2%), Optimal Cut-off = 5.07 μg/mL (sensitivity = 92.9%, specificity = 69.6%) and for visit 2: AUC = 0.866, Youden Index = Optimal Cut-off = 4.72 μg/mL (sensitivity = 100%, specificity = 69.6%), are displayed in Figures 3A and C, respectively.

Logistic regression curves in which the probability of achieving of a therapeutic level at trough were also constructed (Figures 3B and D). These show, for example, that the values of drug levels that

corresponded to an 80% predicted probability of achieving therapeutic trough drug level were 7.94 (95% CI: 6.53-16.49) μg/mL at visit 1 and 7.35 (95% CI: 6.21 – 12.09) μg/mL at visit 2.

DISCUSSION

The treating clinician's confidence in whether a single ADA drug level test is clinically applicable – both in terms of across and within cycles of treatment – is critical to applying therapeutic drug monitoring to patients on ADA with Crohn's disease. The current prospective observational study assessed such issues within and across consecutive cycles with several findings of clinical significance. First, the drug level at any point in a cycle reliably predicts the levels in the subsequent cycle. Secondly, drug levels were relatively stable in the first 9 days of a 2-week cycle, but a consistent decline in levels were noted in the second week towards the nadir of the trough level, which has been used as the 'gold standard' for decision-making via therapeutic drug monitoring. Thirdly, a threshold similar to that taken at trough when tested within the first 9 days of a cycle predicted a therapeutic trough level, with a very high sensitivity but specificity of 65-70%. Finally, non-temporal factors - syringe rather than pen as delivery device (albeit with very small numbers) and current smoking - were independently associated with trough drug levels. These enabled predictive models to be created, which, incorporating drug levels at either visit 1 or 2, accounted for 66% and 80% of the variation in trough levels respectively.

Therapeutic drug monitoring is established as a highly useful tool for clinicians managing patients with IBD. For instance, multiple studies have consistently demonstrated an inverse relationship between IFX drug levels and outcomes.[31] Sub-therapeutic IFX drug levels, measured after induction, are associated with future secondary loss of response.[15,17] Other groups have confirmed the utility of therapeutic drug monitoring,[18,32-34] even though therapeutic cut-off values associated with clinical remission vary due to factors such as differences in study design, the definition of remission used and the population being studied. Nevertheless, with ELISA-based platforms a threshold of between 2-3 μg/mL has been recurrently proposed,[18,31] although higher drug levels may be required to achieve mucosal healing.[35]

Regarding ADA, fewer data exist on the relationship between drug levels and outcomes; a meta-analysis of 5 studies reporting on 459 patients with Crohn's disease found improved rates of remission if trough levels were above a pre-defined cut-off of 4.85-5.9 μg/mL.[23] However, others have found no such

relationship. In a post-hoc analysis of the CLASSIC I and II registration trials amongst 275 patients with moderate-to-severe Crohn's disease, higher ADA drug levels were associated with clinical remission at week 4 in CLASSIC I and II and week 24 in CLASSIC II ($p < 0.005$), but no difference in drug levels was observed at week 56 ($p = 0.34$).[22] Further, threshold cut-off values that could discriminate between patients by remission status could not be identified due to high inter-patient variation in drug levels with significant overlap between those with and without remission.

Therapeutic drug monitoring is traditionally performed at trough, defined as just before the next scheduled dose, when anti-TNF concentrations have reached their nadir. This is easy with patients attending their scheduled IFX infusion, but more difficult with ADA when administered at home. Some have proposed that ADA therapeutic drug monitoring can be performed at any time point in a treatment cycle, due to the relatively flat peak-trough pharmacokinetics observed with subcutaneously administered monoclonal antibodies.[27,36,37] Unlike IFX,[38] few data are available describing the pharmacokinetics of ADA in patients with Crohn's disease. After a single 40 mg intravenous dose in healthy subjects, time to maximum plasma concentration was 5.5 ± 2.3 days.[24] In a post-hoc analysis of 341 ADA samples collected from 65 patients with Crohn's disease, large inter-individual differences in volume of distribution and clearance were observed, and elimination half-life in the absence of antibodies to ADA was 22 days.[39] In patients with rheumatoid arthritis, the time-to-peak plasma concentration was 9.1 days, clearance being increased in men and those with higher weight.[40]

The primary aim of the study was to assess ADA drug level variability within and across subsequent 14-day treatment cycles in patients with CD. No significant differences in intra-patient drug levels was observed between consecutive cycles suggesting the results of a single drug level may be interpreted with confidence and does not need to be repeated. Moreover, this study confirmed the flat peak-trough pharmacokinetics previously reported with ADA given that drug levels were relatively stable over the first 9 days. Also, though there were statistically significant declines from day 4-6 to trough (-1.06

μg/mL) and day 7-9 to trough (-0.89 μg/mL, each $p<0.001$), these small magnitudes were not necessarily clinically significant.

Nevertheless, further analysis was then performed in order to ascertain whether a model incorporating other factors could improve the ability of testing ADA drug levels at any point in time in the treatment cycle to make valid therapeutic decisions. Through linear regression modelling, two non-temporal covariates, smoking and the drug delivery device used, were identified. Combining these with the drug level at visit 1 or 2 accounted for 66 and 80% of the variance in trough drug levels, respectively. To our knowledge, this is the first study demonstrating that smoking might influence ADA pharmacokinetics. Although the deleterious effect interaction between smoking and Crohn's disease is well recognised,[41] studies have yet to demonstrate a difference in anti-TNF drug levels according to smoking status.[27,42-45]

A finding of potential interest was the apparent influence of the delivery device used in predicting ADA trough levels. No discrepancy in the bioavailability of ADA between the delivery device (pen or syringe) or the injection site (abdomen vs thigh) has been reported.[26] Intuitively, patient factors such as administration technique or inconsistent bioavailability due to variable absorption and subsequent lymphatic clearance prior to reaching the circulation might explain these findings, but this warrants further evaluation. Other patient characteristics such as BMI, weight and concomitant immunomodulation and disease factors such as active inflammation and serum albumin have been shown elsewhere to influence anti-TNF pharmacokinetics.[27,46-48] Interestingly, we found no such relationship in this cohort, which may be explained by the limited sample size in this study.

Anecdotally, it can be difficult for patients to attend blood tests on a specified day in order to obtain a trough ADA level. Thus, this study also considered whether an early cycle drug level at a specific cut-off value could accurately predict a therapeutic trough concentration, in this case 4.9 μg/mL, as reported elsewhere.[20,49] This was approached using two related statistical methods. The first was ROC analysis that identified with a respectable AUC (>0.85) threshold drug concentrations at visits 1 or 2 which were

almost the same as the therapeutic trough cut-off itself (Figs 3A and 3C). The second approach was to compute logistic regression curves in which the probability of achieving a therapeutic level at trough could be determined. If an 80% probability was desired, then a drug level of 7.94 after 4-6 days and 7.35 μg/mL after 7-9 days would need to be seen. The clinical application of this information that might initially appear somewhat paradoxical depends upon the precision and predictability required by the physician. Thus, using the ROC analysis, a drug level any day with in the first 9 days of the ADA treatment cycle can be considered qualitatively equivalent of the likely trough level in that cycle if a false-positive rate of 30-35% is considered acceptable clinically (as it is for many tests used). The precision of prediction together with its 95% confidence intervals can be evaluated using the logistic regression curves.

There are several limitations in this study. First, because of its small sample size, conclusions should be interpreted with caution and require validation in larger replication cohorts before they can be implemented in everyday practice. It is likely that the precision of regression equations and cut-off values observed would be improved with much larger samples. Secondly, ADA administration was unsupervised, hence patients may have not administered ADA strictly every 14 days, which may have influenced findings regarding variability of intra-patient drug level. Non-adherence to medical therapies is well recognised in IBD,[50] including patients treated with ADA.[51] However, this effect is likely to be small given no significant differences in intra-patient drug levels were observed between paired visits across subsequent cycles ($p = 0.6$). Thirdly, in the linear and logistic regression analyses the clustering of cycles (almost always two cycles) within patients was not explicitly modelled – a future study in a larger cohort could explore correlations between and within cycles. However, we did note, in exploratory mixed model analyses of the drug levels, that complex within-cycle correlation structures such as autoregressive and banded models, did not improve the goodness of fit of the model used to calculate the visit means in Table 2. Fourthly, the finding that syringe delivery device was an independent predictor of trough drug level should be interpreted with caution given only three patients administered ADA by this method. Fifthly, amongst smokers, we did not quantify the number of

cigarettes per day or duration of smoking. Some studies in patients with Crohn's disease have found no impact of smoking on disease outcomes and that, rather, the degree of smoking may be more important.[52] Hence, individual patient smoking patterns may influence the value identified as a covariate in the models we have proposed. Finally, we used faecal calprotectin as a surrogate of mucosal healing, defining a cut-off of > 150 μg/mL as being associated with active disease. A range of cut-off values have been proposed in the literature ranging from 50-400 μg/mL.[53] Acceptable correlation with validated endoscopic activity scores in Crohn's disease have been reported,[54] but whether this accuracy extends to isolated small bowel disease remains debated.[55,56]

In conclusion, this study has demonstrated that ADA drug levels vary little during the first nine days of a 14-day treatment cycle, but then decline thereafter to trough in patients with Crohn's disease. The results of a single drug level can be interpreted with confidence as intra-patient drug levels appear to remain consistent between subsequent cycles. Trough concentrations might be more accurately estimated from drug levels obtained during the first 9 days by considering the drug delivery device used and the negative effect of smoking status in each case. In the absence of such factors, the therapeutic trough level cut-off value can be applied to ADA levels taken during the first 9 days of the cycle, but with the caveat that there is a 1 in 3 false-positive rate in that assessment. Although larger studies are needed before these recommendations can be incorporated into everyday clinical practice, this study adds further valuable understanding of the utility of therapeutic drug monitoring for anti-TNF therapies in patients with Crohn's disease

Author Contributions

Guarantor of the article: MG Ward

MGW conceived the study, participated in data acquisition and analysis and drafted the manuscript. PAT, LB, and JH participated in data acquisition and revised the manuscript. GR performed laboratory analysis and revised the manuscript. JR performed data and statistical analysis and revised the

manuscript. DVL, PRG and MPW conceived the study, participated in data analysis and revised the manuscript. All authors approved the final version of the manuscript.

Acknowledgements

Declaration of personal interests: MGW has served as a speaker for Janssen, Abbvie, Ferring and Takeda and has received research funding from Abbvie. LB has served as a speaker for Abbvie. PAT, JH and GR have no disclosures. JR holds shares in Novartis AG. MPS has served as a speaker, a consultant and an advisory board member for Janssen, Abbvie, Ferring, Takeda and Hospira and has received research funding from Ferring and Orphan. PRG has served as consultant or advisory board member for AbbVie, Ferring, Janssen, Merck, Nestle Health Science, Danone, Allergan and Takeda. His institution has received speaking honoraria from AbbVie, Janssen, Ferring, Takeda, Fresenius Kabi, Mylan and Pfizer. He has received research grants for investigator-driven studies from AbbVie, Janssen, Falk Pharma, Danone and A2 Milk Company. His Department financially benefits from the sales of a digital application and booklets on the low FODMAP diet. He has published an educational/recipe book on diet.

Declaration of funding interests: The study was funded in part by AbbVie. AbbVie reviewed the manuscript prior to submission but were not involved in study design, interpretation or analysis of data and did not contribute to the content of the manuscript.

REFERENCES

1. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006 Feb;130(2):323-33

2. Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007 Sep;56(9):1232–9.

3. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002 May 4;359(9317):1541–9.

4. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med. 2004 Feb 26;350(9):876–85.

5. Reinisch W, Sandborn WJ, Hommes DW, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut. 2011 Jun;60(6):780–7.

6. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005 Dec 8;353(23):2462–76.

7. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther. 2011 May;33(9):987–95.

8. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003 Feb 13;348(7):601–8.

9. Karmiris K, Paintaud G, Noman M, et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009 Nov;137(5):1628–40.

10. Brandse JF, van den Brink GR, Wildenberg ME, et al. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. Gastroenterology. 2015 Aug;149(2):350–2.

11. Colombel J-F, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010 Apr 15;362(15):1383–95.

12. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol. 2006 Oct 1;4(10):1248–54.

13. Steenholdt C, Bendtzen K, Brynskov J, et al. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. Scand J Gastroenterol. 2011 Mar;46(3):310–8.

14. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59(1):49–54.

15. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut. 2014 Nov;63(11):1721–7.

16. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. Inflamm Bowel Dis. 2013 Nov;19(12):2568–76.

17. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. Journal of Crohn's and Colitis. 2013 Oct 1;7(9):736–43.

18. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015 Jun;148(7):1320–3.

19. Bodini G, Savarino V, Fazio V, et al. Sa2045 Relationship Between Drug Serum Concentration and Clinical Activity in Patients With Crohn Disease Who Achieved Remission With Adalimumab-a Prospective Study. Gastroenterology. 2012;142(5):S–388.

20. Roblin X, Marotte H, Rinaudo M, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2014 Jan;12(1):80–2.

21. Ward MG, Kariyawasam VC, Arkir Z, et al. Clinical utility of measuring adalimumab trough levels and antibodies to adalimumab in patients with inflammatory bowel diseases. J Gastroenterol Hepatol. 2013 Sep 5;28(Suppl.2):100.

22. Chiu Y-L, Rubin DT, Vermeire S, et al. Serum Adalimumab Concentration and Clinical Remission in Patients with Crohn's Disease. Inflamm Bowel Dis. 2013 May;19(6):1112–22.

23. Paul S, Moreau AC, Del Tedesco E, et al. Pharmacokinetics of Adalimumab in Inflammatory Bowel Diseases. Inflamm Bowel Dis. 2014 Jul;20(7):1288–95.

24. Ordás I, Mould DR, Feagan BG, et al. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin Pharmacol Ther. 2012 Apr;91(4):635–46.

25. Colombel J-F, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of biologics for inflammatory bowel disease. Inflamm Bowel Dis. 2012 Feb;18(2):349-58

26. Paulson S, Noertersheuser P, Garimella T, et al. Assessment of relative bioavailability, safety, and tolerability of single doses of adalimumab administered via an autoinjector pen and a prefilled syringe. J Am Acad Dermatol. 2007;56(2):AB9–AB9.

27. Lie MRKL, Peppelenbosch MP, West RL, et al. Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment. Aliment Pharmacol Ther. 2014 Nov;40(10):1202–8.

28. Van Assche G, Dignass A, Panés J, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. Journal of Crohn's and Colitis 2010. Feb;4(1):7–27.

29. Harvey RF, Bradshaw MJ. Measuring Crohn's disease activity. Lancet. 1980. May 24;1(8178):1134–5.

30. Satsangi J, Silverberg MS, Vermeire S, Colombel J-F. The Montreal classification of

inflammatory bowel disease: controversies, consensus, and implications. Gut 2006. Jun;55(6):749–53.

31. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. Journal of Crohn's and Colitis. 2016 May;10(5):619–25.

32. Steenholdt C, Brynskov J, Thomsen OØ, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. Gut. 2014 Jun;63(6):919-27.

33. D'haens G, Vermeire S, Lambrecht GL, et al. OP029. Drug-concentration versus symptom-driven dose adaptation of Infiximab in patients with active Crohn's disease: a prospective, randomised, multicentre trial (Tailorix) Journal of Crohn's and Colitis. 2016 Feb 13;10(Suppl 1):S24–4.

34. Yanai H, Lichtenstein L, Assa A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. Clin Gastroenterol Hepatol. 2015 Mar;13(3):522–2.

35. Ungar B, Levy I, Yavne Y, et al. Optimizing Anti-TNF-alpha Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol. 2016 Apr 1;14(4):550–57.

36. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. J Clin Pharmacol. 2015 Mar;55 Suppl 3:S39–50.

37. Hendy P, Hart A, Irving P. Anti-TNF drug and antidrug antibody level monitoring in IBD: a practical guide. Frontline Gastroenterology. 2016 Mar 9;7(2):122–8.

38. Fasanmade AA, Adedokun OJ, et al. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. Clin Ther. 2011 Jul;33(7):946–64.

39. Ternant D, Karmiris K, Vermeire S, et al. Pharmacokinetics of adalimumab in Crohn's disease. Eur J Clin Pharmacol. 2015 Jun 27;71(9):1155–7.

40. Ternant D, Ducourau E, Fuzibet P, et al. Pharmacokinetics and concentration-effect relationship of adalimumab in rheumatoid arthritis. British Journal of Clinical Pharmacology. 2015 Feb;79(2):286–97.

41. Ding NS, 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. 2015 Oct 30;43(1):30-51

42. Brandse JF, Vos LMC, Jansen J, et al. Serum concentration of anti-TNF antibodies, adverse effects and quality of life in patients with inflammatory bowel disease in remission on maintenance treatment. Journal of Crohn's and Colitis. 2015 Jun 26;9(11):973-81

43. Yarur AJ, Kubiliun MJ, Czul F, et al. Concentrations of 6-Thioguanine Nucleotide Correlate With Trough Levels of Infliximab in Patients With Inflammatory Bowel Disease on Combination Therapy. Clin Gastroenterol Hepatol. 2015 Jun 1;13(6):1118–1124.

44. Dotan I, Ron Y, Yanai H, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. Inflamm Bowel Dis. 2014 Dec;20(12):2247–59.

45. Mazor Y, Almog R, Kopylov U, et al. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. Aliment Pharmacol Ther. 2014 Sep;40(6):620–8.

46. Fasanmade AA, Adedokun OJ, Ford J, et al. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. Eur J Clin Pharmacol. 2009 Dec;65(12):1211-28.

47. Noertersheuser P, Eckert D, Sharma S, et al. P493 Factors affecting adalimumab pharmacokinetics in adult patients with moderate to severe CD. Journal of Crohn's and Colitis. 2013 Feb 9;7(S1):S207.

48. Mostafa NM, Eckert D, Pradhan RS, et al. Sa1195 Exposure-Efficacy Relationship (ER) for Adalimumab During Induction Phase of Treatment of Adult Patients With Moderate to Severe Ulcerative Colitis. Gastroenterology. 2013 Apr 23;144(5):S225–S226.

49. Bodini G, Giannini EG, Savarino EV, et al. Adalimumab Trough Levels and Response to Biological Treatment in Patients With Inflammatory Bowel Disease: A Useful Cutoff in Clinical Practice. Am J Gastroenterol. 2015 Mar 1;110(3):472–3.

50. Horne R, Parham R, Driscoll R, et al. Patients' attitudes to medicines and adherence to maintenance treatment in inflammatory bowel disease. Inflamm Bowel Dis. 2009 Jun;15(6):837–44.

51. Lopez A, Billioud V, Peyrin-Biroulet C, et al. Adherence to anti-TNF therapy in inflammatory bowel diseases: a systematic review. Inflamm Bowel Dis. 2013 Jun;19(7):1528–33.

52. Parsi MA. Does smoking decrease the response to infliximab in patients with Crohn's disease? Inflamm Bowel Dis. 2008 Oct;14 Suppl 2:S18–9.

53. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin Experiment Gastroenterol. 2016;9:21–9.

54. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal Calprotectin Correlates More Closely With the Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, Blood Leukocytes, and the CDAI. Am J Gastroenterol. 2009 Sep 15;105(1):162–9.

55. Gecse KB, Brandse JF, van Wilpe S, et al. Impact of disease location on fecal calprotectin levels in Crohn's disease. Scand J Gastroenterol. 2015 Jul;50(7):841–7.

56. Stawczyk-Eder K, Eder P, Lykowska-Szuber L, et al. Is faecal calprotectin equally useful in all Crohn's disease locations? A prospective, comparative study. Arch Med Sci. 2015 Apr 25;11(2):353–61.

Table 1. Patient characteristics (n = 19)

Characteristic		Value
Female		11 (57.9%)
Mean age (±SD]		39.2 (± 9.5) years
Median disease duration (IQR)		11 (6 - 18) years
Montreal classification	A1	3 (15.8%)
	A2	15 (78.9%)
	A3	1 (5.3%)
	B1	10 (52.6%)
	B2	6 (31.6%)
	B3	3 (15.8%)
	L1	8 (42.1%)
	L2	3 (15.8%)
	L3	8 (42.1%)
Current smoker		7 (36.8%)
Mean weight (± SD)		84.1 (± 15.1) kg
Mean body mass index (± SD)		28.7 (± 5.3)
Number of visits with active disease	Harvey Bradshaw Index ≥5	31 (29.5%)[a]
	C-reactive protein >3 mg/L	40 (36.4%)[b]
	Faecal calprotectin ≥150 μg/g	13 (35.1%)
Mean serum albumin (± SD)		37.8 (± 2.9) g/L[b]
Previous infliximab therapy		13 (68.4%)
Concurrent immunomodulator use	Any	14 (73.7%)
	Thiopurine	11 (57.9%)
	Methotrexate	2 (10.5%)
	Thioguanine	1 (5.3%)
Adalimumab therapy	Median duration (IQR)	27 (5 - 49) months
	Administered via a pen	16 (84.2%)
	Administered into abdomen	19 (100%)

[a] *Harvey=Bradshaw Index not calculated for 1 patient with a stoma.*

[b] *blood testing missing from 1 visit*

Table 2. Comparison of drug levels between visits (V) within and across cycles

Visit	Cycle 1 mean drug level (SEM)	Cycle 2 mean drug level (SEM)	p-value	Visit drug level (averaged across cycles) mean (SEM)	Pairwise p-value (between visits)
1 (day 4-6)	4.81 (0.47)	5.21 (0.58)	0.244	5.01 (0.37)	V1 vs V2: 0.491
2 (day 7-9)	4.86 (0.51)	4.82 (0.62)	0.905	4.84 (0.40)	V2 vs V3: < 0.001
3 (trough, day 13-14)	3.95 (0.48)	3.95 (0.53)	0.986	3.95 (0.35)	V1 vs V3 < 0.001

Table 3. Linear regression analysis of relationship between patient and disease factors and trough drug level

		Univariate analyses			Multivariate analyses (3-factor model with DL at Visit 1).			Multivariate analyses (3-factor model with DL at Visit 2).		
Variable	Factor level or units	Estimate of ß	SE	Wald test p-value	Estimate	SE	Wald test p-value	Estimate of ß	SE	Wald test p-value
Sex (Reference = Female)	Male	0.056	0.730	0.939	-0.321	0.432	[0.463]	-0.081	0.333	[0.810]
Cycle (Reference = 1st cycle)	2	-0.037	0.717	0.959	-0.254	0.419	[0.548]	0.023	0.321	[0.943]
Smoker (Reference = Non-smoker)	Yes	-1.486	0.695	0.040	**-1.038**	**0.468**	**0.034**	**-0.864**	**0.359**	**0.022**
DDD (Reference = Pen)	Syringe	1.990	0.912	0.036	**1.795**	**0.597**	**0.005**	**1.602**	**0.458**	**0.001**
Weight	kg	-0.034	0.024	0.156	0.0012	0.015	[0.923]	0.013	0.012	[0.273]
BMI	kg/m2	-0.105	0.097	0.298	-0.033	0.059	[0.581]	0.003	0.049	[0.951]
CIM (Reference = No)	Yes	1.008	0.818	0.226	0.227	0.520	[0.666]	0.093	0.399	[0.818]
DL Visit 1	µg/mL	0.734	0.104	<0.001	**0.625**	**0.100**	**<0.001**	0.032	0.147	[0.832]
DL Visit 2	µg/mL	0.770	0.077	<0.001	0.655	0.138	<0.001	**0.681**	**0.072**	**<0.001**
Albumin	g/L	0.171	0.123	0.173	-0.051	0.082	[0.540]	-0.009	0.058	[0.871]
HBI	remission	1.239	0.788	0.125	0.737	0.476	[0.132]	0.600	0.364	[0.110]
CRP (Reference ≤3)	Active as per >3 mg/L	-0.048	0.780	0.951	0.204	0.463	[0.662]	0.441	0.324	[0.182]
FC150 (Reference level = active)	Remission as per <150 µg/g	-0.772	0.752	0.311	-0.551	0.472	[0.252]	-0.411	0.357	[0.259]

DDD = drug delivery device (pen vs syringe), BMI = body mass index, CIM = concomitant immunomodulation, DL = drug level, HBI = Harvey-Bradshaw index, CRP = c-reactive protein, FC = faecal calprotectin, SE = standard error. P-values in square brackets refer to the t-test for adding the associated extra term to the model.

Table 4. Logistic regression model of patient and disease factors associated with therapeutic trough level

Therapeutic Drug Level Achieved at Trough		Univariate analyses			Multifactor analyses (2-factor model with DL at Visit 1 and Smoking Status).			Multifactor analyses (2-factor model with DL at Visit 2 and Smoking Status).		
Variable	Factor level or units	Estimate of ß	SE	Wald test p-value	Estimate	SE	Wald test p-value	Estimate of ß	SE	Wald test p-value
Sex (Reference = Female)	Male	-0.829	0.723	0.251	-1.777	0.978	[0.069]	-1.330	1.060	[0.208]
Cycle (Reference = 1st cycle)	2	0.087	0.677	0.898	-0.215	0.863	[0.803]	0.117	0.980	[0.905]
Smoker (Reference = No)	Yes	-1.879	0.870	0.031	**-2.030**	**1.140**	**0.074**	**-3.480**	**1.820**	**0.055**
DDD (Reference = Pen)	Syringe	-0.234	0.939	0.804	0.110	1.500	[0.943]	-0.380	3.960	[0.924]
Weight	kg	0.007	0.023	0.776	0.025	0.036	[0.500]	0.090	0.057	[0.114]
BMI	kg/m2	0.046	0.093	0.617	0.151	0.157	[0.333]	0.367	0.239	[0.125]
CIM (Reference = No)	Yes	0.258	0.804	0.749	0.480	1.010	[0.636]	0.930	1.170	[0.428]
DL Visit 1	µg/mL	0.700	0.273	0.010	**0.708**	**0.289**	**0.014**	-0.051	0.435	[0.907]
DL Visit 2	µg/mL	0.877	0.310	0.005	1.274	0.609	[0.036]	**1.232**	**0.482**	**0.011**
Albumin	g/L	0.106	0.121	0.379	-0.154	0.151	[0.308]	-0.306	0.263	[0.245]
HBI	Remission <5	0.814	0.789	0.303	0.608	0.994	[0.541]	1.300	1.280	[0.311]
CRP (Reference = ≤3)	Active as per >3 mg/L	0.693	0.721	0.336	1.244	0.976	[0.202]	3.010	1.670	[0.071]
FC150 (Reference = active)	Remission as per < 150 µg/g	-0.981	0.716	0.171	-1.206	0.980	[0.218]	-2.690	1.660	[0.105]

DDD = drug delivery device (pen vs syringe), BMI = body mass index, CIM = concomitant immunomodulation, DL = drug level, HBI = Harvey-Bradshaw index, CRP = c-reactive protein, FC = faecal calprotectin, SE = standard error. P-values in square brackets refer to the Wald test for adding the associated extra term to the model.

FIGURES

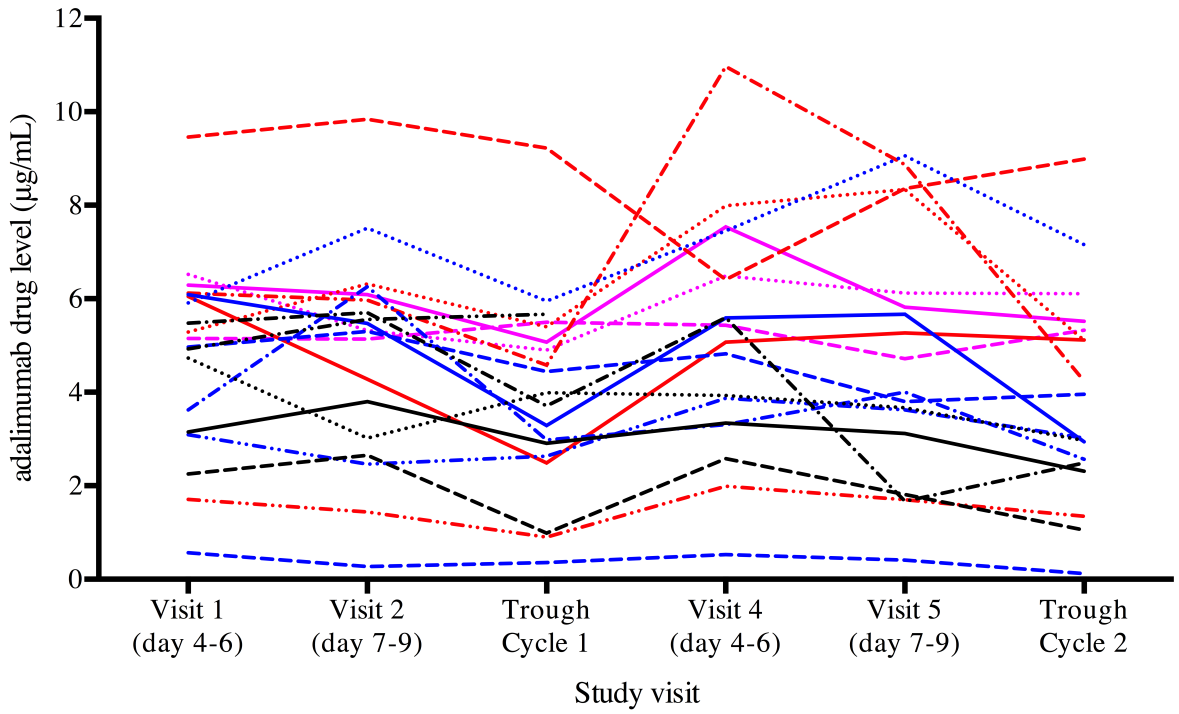


Figure 1. Adalimumab drug levels of all patients according to study visit

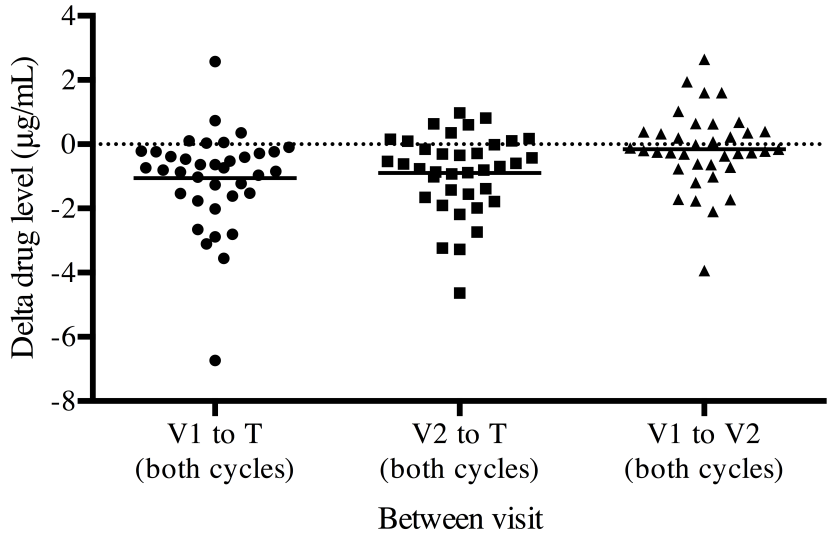
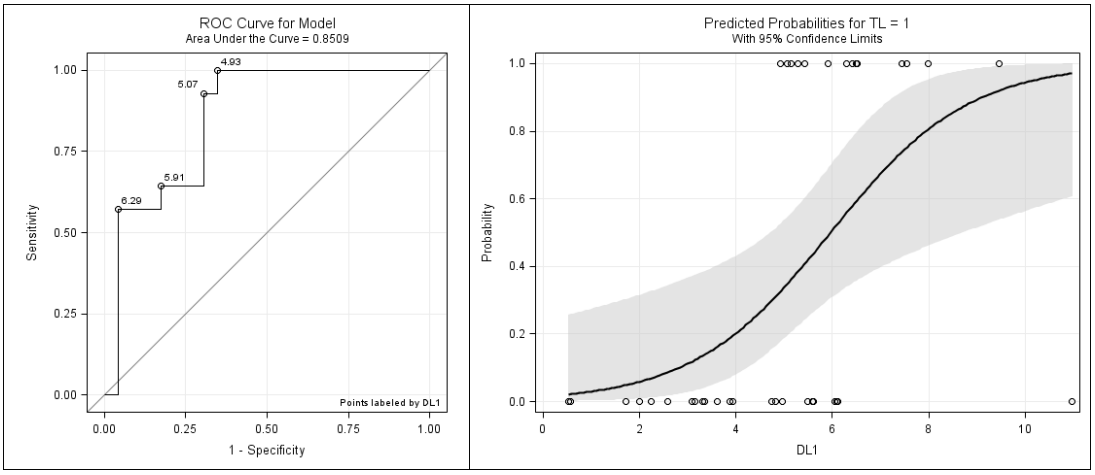


Figure 2. Absolute difference (delta) in adalimumab drug levels between visits. Long horizontal bars represent mean delta. Statistical differences in delta from zero were observed between V1 to T ($p < 0.0001$) and V2 to T ($p < 0.0001$, one sample t-test). No difference was seen between V1 to V2 ($p = 0.43$). V1 = visit 1 (day 4-6), V2 = visit 2 (day 7-9), T = trough (day 13 or 14)

A **B**



C **D**

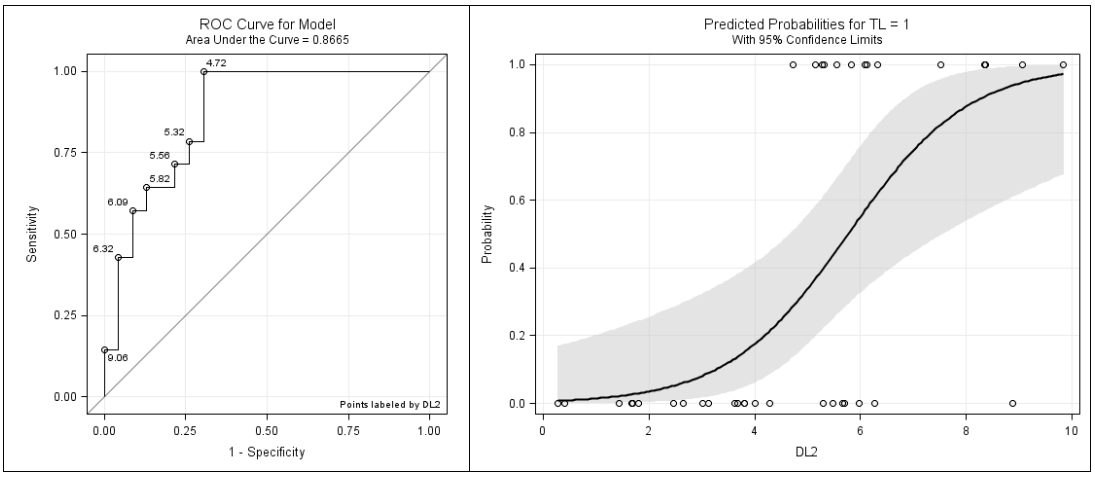


Figure 3. Relationship between drug levels at visit 1 (3A, 3B) and visit 2 (3C, 3D) and trough levels according to ROC analysis for achieving therapeutic trough level and logistic regression curves for probability of predicting a therapeutic trough level. Shaded areas on the logistic regression curves indicate 95% confidence limits for the probability of achievement of a therapeutic level at trough. DL1 = drug level at visit 1, DL2 = drug level at visit 2. TL=1 indicates achievement of a therapeutic trough level (>4.9 µg/ml)

SECTION 3. OPTIMISATION OF MICRONUTRIENTS

CHAPTER 9:

Prevalence of and risk factors for functional vitamin B12 deficiency in patients with Crohn's disease

Prevalence and risk factors for functional vitamin B_{12} deficiency in patients with Crohn's disease

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Disclosure of Funding: Nil

This is a non-final version of an article published in final form in Inflamm Bowel Dis 2015;21(12):2839-2847 (http://journals.lww.com/ibdjournal/pages/default.aspx)

ABSTRACT

Background: Crohn's disease (CD) is a risk factor for vitamin B_{12} deficiency due to frequent involvement of the terminal ileum. Conventional screening for B_{12} deficiency with serum B_{12} is relatively insensitive and measures total B_{12} concentration, of which a minority is present in a biologically active form. Holotranscobalamin (holoTC) combined with methylmalonic acid (MMA), is believed to be more accurate in identifying impaired B_{12} status. We evaluated the prevalence and risk factors for B_{12} deficiency using holoTC supported by MMA amongst patients with CD.

Methods: We performed a single centre service evaluation of 381 patients with CD that underwent B_{12} assessment (holoTC/MMA) and compared these with 141 patients with ulcerative colitis (UC). 89 patients with CD underwent paired serum B_{12} and holoTC. Amongst patients with CD, risk factors including terminal ileal resection length, ileal inflammation on endoscopy and disease characteristics on magnetic resonance imaging (MRI) were recorded.

Results: Prevalence of B_{12} deficiency amongst patients with CD was 33%, compared to 16% in UC, ($p<0.0001$). In 89 patients who underwent paired tests, conventional testing identified B_{12} deficiency in 5% of CD patients, which increased to 32% using holoTC/MMA. Independent risk factors for B_{12} deficiency were ileal resection length ≤20cm (OR 3.0; 95% CI 1.5 – 6.0, $p=0.002$) and >20cm (OR 6.7; 95% CI 3.0 – 14.7, $p<0.0001$) and ileal inflammation (OR 3.9; 95% CI 2.2 – 6.9, $p<0.0001$). On MRI, active terminal ileal inflammation ($p=0.02$), and an increased disease burden, (≥1 skip lesion, $p=0.01$ and pre-stenotic dilatation >3 cm, $p=0.01$) were associated with B_{12} deficiency.

Conclusions: Vitamin B_{12} deficiency is common in patients with CD. HoloTC supported by MMA identifies patients with B_{12} deficiency considered replete on conventional testing.

INTRODUCTION

Vitamin B_{12}, also known as cobalamin, is a water-soluble vitamin that is essential for effective erythropoiesis, functioning of the nervous system, DNA synthesis and carbohydrate, protein and fat metabolism.[1] Humans cannot synthesise vitamin B_{12}, and hence obtain it via the diet, where it is almost exclusively found in food of animal origin.[2] The daily requirement for B_{12} intake is 1-3 μg; body stores approximate 5 mg which explains why clinical manifestations of B_{12} deficiency often appear late.[3] Most absorption (98%) occurs within the distal terminal ileum in contrast to other water-soluble vitamins that are absorbed in the proximal small bowel. Accordingly patients with CD who frequently have ileal involvement or undergo ileal resection are at increased risk of B_{12} deficiency.

Deficiency is often asymptomatic in the early stages, however it can eventually present as megaloblastic anaemia or with neuropsychiatric manifestations, including subacute combined degeneration of the cord. Importantly, these potentially irreversible neurological complications have been reported in patients without macrocytosis or anaemia.[4] Further, B_{12} deficiency leads to hyperhomocysteinaemia which is an independent risk factor for ischaemic heart disease[5] and dementia.[6]

Serum B_{12} binds to two proteins in blood, transcobalamin I (haptocorrin) and transcobalamin II. Only the transcobalamin II-cobalamin complex (holotranscobalamin, holoTC) is utilized for receptor-mediated cellular uptake, and is considered metabolically active. The role of haptocorrin-bound B_{12} is unknown. Up to 15% of patients with low serum B_{12} are found to have low haptocorrin levels and this may contribute to the relatively low specificity of serum B_{12} levels in diagnosing B_{12} deficiency.[7] Biochemical sequelae of B_{12} deficiency are increased concentrations of plasma homocysteine and methylmalonic acid (MMA). Vitamin B_{12} is an essential cofactor in the conversion of homocysteine to methionine and methylmalonyl-CoA to succinyl-CoA. In B_{12} deficiency, the excess of methylmalonyl-CoA is hydrolysed to MMA. Measuring MMA is considered a good indicator of

functional B_{12} deficiency, but is hampered by cost and limited availability. Hyperhomocysteinaemia is less specific as it also occurs in folate, thiamine, vitamin B_6 and choline deficiency.[8]

Recognition of the limitations of measuring serum B_{12} in the assessment of B_{12} deficiency has led to the development of assays that measure holoTC. A growing body of evidence comparing holoTC with serum B_{12} has demonstrated that holoTC is a superior test in the assessment of B_{12} deficiency.[7,9] To date, no studies have reported the utility of holoTC in the assessment of B_{12} status in patients with IBD.

We therefore conducted a service evaluation to report the prevalence of B_{12} deficiency in patients with CD using holoTC and to identify risk factors associated with deficiency. In addition we compared holoTC with serum B_{12} testing to establish the B_{12} status of our cohort.

MATERIALS AND METHODS

As part of routine care,[10] patients attending the IBD service at Guy's and St. Thomas' NHS Foundation Trust, London routinely undergo annual B_{12} assessment. We included all patients with CD who had holoTC measured between January 2012 and March 2013, identified retrospectively by review of the electronic patient record. Patients with UC who underwent B_{12} measurement during this period were included as disease controls. Patients receiving vitamin B_{12} replacement or with a past history of vitamin B_{12} deficiency unrelated to IBD or those who had undergone previous gastrectomy were excluded. 33 patients with IBD-unclassified were excluded. The diagnosis of IBD was based on standard endoscopic, histopathological and radiological criteria.[11,12] Patients with CD were phenotyped according to the Montreal classification.[13] A subset of 89 consecutive patients with CD underwent paired testing of serum B_{12} and holoTC to compare differences in rates of B_{12} deficiency between tests.

Potential risk factors of interest for B_{12} deficiency were selected *a priori* and included age, gender, smoking status, disease phenotype, current treatment with immunomodulators (thiopurines, methotrexate or tioguanine) or anti TNF agents (infliximab or adalimumab), disease duration, ileal resection length (0 cm, 1-20 cm and >20 cm) and disease activity. For CD the Harvey Bradshaw Index (HBI)[14] was used with active disease being defined as a score ≥5.[15] Patients with UC were assessed according to the Simple Colitis Clinical Activity Index (SCCAI)[16] with a score of ≤3 being defined as clinical remission.[17] Clinical disease activity is routinely calculated at each patient visit to the clinic and documented in the electronic patient record. Biochemical evidence of active disease was defined as C-reactive protein (CRP) ≥5 mg/L. Ileal resection length was obtained from histopathology specimen reports or, where these were unavailable, from operative notes. Ileocolonoscopy or magnetic resonance imaging (MRI), performed within 6 months of vitamin B_{12} testing, was used to assess ileal inflammation. All MRIs underwent review by 2 radiologists with expertise in IBD. At endoscopy, active ileal inflammation was classified subjectively according to the presence or lack of macroscopic inflammation as reported by the endoscopist. Clinical data was collected independently by four authors and was then reviewed by the first and senior authors.

Laboratory Methods

Serum holoTC has been used as a first line screening test for B_{12} deficiency in our institution since January 2012[18] and was measured using the AxSYM assay (Abbott Diagnostics, Abbott Park, IL, USA)[19]. A value <25 pmol/L was defined as B_{12} deficiency and >50 pmol/L considered replete.[20] Values between 25 pmol/L and 50pmol/L were classified as intermediate [18] and underwent MMA analysis, subject to an estimated glomerular filtration rate of ≥60 mL/min/1.73m^2, using liquid chromatography-tandem mass spectrometry with electrospray ionisation as previously described.[21] MMA values >280nmol/L confirmed B_{12} deficiency in patients <65 years old, or >360nmol/L in patients >65 years.[20,22] Serum B_{12} was measured using the ARCHITECT assay (Abbott Diagnostics, IL, USA). Patients with values <107pmol/L were defined as B_{12} deficient as per local laboratory ranges. A separate analysis was performed using the National Health and Nutrition Evaluation Survey

(NHANES) serum B_{12} cut-off for diagnosing B_{12} deficiency of <147pmol/L.[23] Anaemia was defined as haemoglobin (Hb) <116g/L in females and <129g/L in males and macrocytosis as mean cell volume (MCV) >96fL as per local laboratory values.

MRI sub-analysis

168/381 (44%) CD patients underwent MRI within 6 months of B_{12} testing. All MRI studies were performed on a 1.5T MRI scanner (Siemens, Erlangen, Germany). Patients were given 1 litre of an oral 2.5% mannitol solution and imaged at 40 minutes according to previously published MR imaging parameters.[24] The images were reviewed for the presence (>3 mm mural thickening), length and activity of Crohn's disease within the small bowel. The jejunum was defined on MRI as small bowel extending from the duodenojejual flexure and seen within the left side of the abdomen, the terminal ileum was arbitrarily defined as small bowel within 20 cm of the ileocaecal valve and the ileum as the intervening small bowel between the jejunum and terminal ileum. The number of skip lesions was recorded as well as total length of small bowel involvement. Pre-stenotic dilatation was also noted, and defined as a small bowel diameter of >3 cm immediately proximal to a skip lesion. Strictures were defined as luminal narrowing with pre-stenotic dilatation. Active disease was defined as mural thickening >6 mm with mural enhancement greater than adjacent non-inflamed bowel.[25,26] Lesions demonstrating between 3-6mm mural thickness with less degree of mural enhancement were considered inactive. A sub-analysis was then performed exploring associations between B_{12} status and disease characteristics on MRI.

Statistical Analysis

Categorical variables are presented as number and percentage. Quantitative data are presented as mean with standard deviation or median with interquartile range (IQR) as appropriate. Comparisons between patient groups were carried out using Pearson x^2, independent sample *t*-test or Mann-Whitney U-test. Two sided p values <0.05 were considered significant. Multivariate analysis was performed using binary logistic regression where covariates of interest identified *a priori* were

entered into a forward step-wise model. Variables with p values of <0.1 were initially entered into the model and variables with p values of <0.05 were retained in the model. Results are reported as adjusted odds ratios (OR) and 95% confidence intervals (CI). For statistical analysis, holoTC values above the upper limit of quantification (>128 pmol/L) were assigned a value of 129 pmol/L. Analyses were carried out using SPSS v21.0 (SPSS Inc., Chicago, IL, USA).

Ethical consideration

According to the guidelines of the UK Health Research Authority[27] as the data collected were done so as part of routine clinical care and were evaluated retrospectively, ethical approval was not required.

RESULTS

Patient Characteristics (Table 1)

381 patients with CD, (male n=195, (51%)) and 141 with UC, (male n=55, (39%)) were included. Patients with CD had a longer disease duration (8 vs 6 years, p<0.01) and were more likely to be treated with immunomodulators and/or biologics than patients with UC, (63% vs 38%, p<0.0001 and 35% vs 9%, p<0.0001, respectively). There was no significant difference in mean Hb or MCV between CD and UC patients. No patients who underwent MMA analysis had renal impairment, (defined as eGFR < 60 mL/min/1.73m^2).

137/381 (36%) patients with CD underwent a total of 199 small bowel resections. 92 patients had one resection, 34 had two, 7 had three and 4 patients had 4 or more resections. Data on small bowel resection length was available from 150/199 (75%) operations. The median (IQR) cumulative length of resected small bowel amongst those who underwent surgery was 18 cm (11-30).

holoTC concentration

Median (IQR) holoTC was lower amongst patients with CD: 48pmol/L (33-70) compared to UC 67pmol/L (46-95), p<0.0001. Amongst patients with CD 46/381 (12%) had holoTC < 25 pmol/L.

153/381 (40%) had holoTC in the intermediate range and underwent MMA analysis. Of these 80/153 (52%) were deficient. Amongst patients with UC 23/141 (16%) were B_{12} deficient; 7/141 (5%) using holoTC alone which increased to 23/141 (16%) when combining holoTC with MMA. The prevalence of B_{12} deficiency using holoTC and MMA analysis was significantly greater in CD than in UC patients (33% vs 16%, p<0.0001).

holoTC vs serum B_{12} for assessment of B_{12} deficiency

89 CD patients had B_{12} status assessed with paired serum B_{12} and holoTC. Using local laboratory ranges, serum B_{12} identified B_{12} deficiency in 4/89 (5%) compared to 13/89 (15%) using holoTC alone. The latter group increased to 28/89 (32%) when intermediate range holoTC results were analysed by MMA (Figure 1). In addition, 1/4 (25%) patients assessed as deficient on serum B_{12} testing had an intermediate holoTC value (44 pmol/L), however they were found to be replete on MMA testing. The remaining three patients with B_{12} deficiency on serum testing were also deficient on holoTC testing alone. Applying the NHANES cut-off for diagnosing B_{12} deficiency, (<147 pmol/L), serum B_{12} identified B_{12} deficiency in 22/89 (25%). 11/22 (50%) of patients deficient on serum B_{12} were replete using holoTC and MMA. Further, 22/67 (33%) classified as replete using serum B_{12} were found to have B_{12} deficiency using holoTC and MMA.

Univariate and Multivariate Analysis of Disease Characteristics and B_{12} Status

On univariate analysis (Table 2) ileal disease location (L1/L3 vs L2) significantly predicted B_{12} deficiency, (OR 2.8; 95% CI: 1.6 – 4.8, p<0.0001), as did a complicated phenotype (stricturing OR 2.0; 95% CI: 1.2 – 3.3, p=0.005 and penetrating OR 2.4; 95% CI: 1.4 – 4.3, p=0.002 compared to non-penetrating, non-stricturing disease).

Patients with a history of ileal resection were more likely to be B_{12} deficient compared to those without surgery, (OR 3.2; 95% CI: 2.0 – 4.9, p<0.0001). Increasing ileal resection length was associated with B_{12} deficiency with OR 1.04; 95% CI: 1.02-1.05, p<0.0001 for each 1 cm resected. B_{12} deficiency was found in 24%, 48% and 65% of patients with resections of 0 cm, ≤ 20 cm, (OR

2.9; 95% CI: 1.6 – 5.2, p<0.0001), and > 20 cm, (OR 5.8; 95% CI: 3 – 11.3, p<0.0001) respectively, (Figure 2).

Patients with active disease, assessed through clinical indices, CRP or on endoscopy/imaging, were also significantly more likely to have B_{12} deficiency (Figure 3), (OR 2.5; 95% CI 1.5 – 4.1, p<0.0001, OR 1.6; 95% CI 1.0 – 2.5, p=0.03, OR 4.5; 95% CI 2.7 – 7.7, p<0.0001 for HBI≥5, CRP>5 ng/mL and active inflammation at endoscopy/imaging, respectively).

292/381 (77%) of patients had complete data and were entered into the multivariate analysis (Table 3). Increasing ileal resection length (OR 3.0; 95% CI 1.5 – 6.0, p=0.002 and OR 6.7; 95% CI 3.0 – 15.0, p<0.0001 for ≤20cm and >20cm resected, respectively) was an independent predictor of B_{12} deficiency. Ileal inflammation (endoscopy/ imaging) also remained significant after multivariate analysis, (OR 3.9; 95% CI 2.2 – 6.9, p<0.0001). There was a trend to clinically active disease (HBI≥5) being an independent predictor of B_{12} deficiency, (OR 1.9; 95% CI 0.98 -3.7, p=0.6). None of the other variables, including disease location, nor behaviour were independently associated with B_{12} status.

Relationship between MRI findings and B_{12} status

168/381 (44%) patients underwent an MRI within 6 months of B_{12} assessment, and were included in a sub-analysis in order to explore further the relationship between small bowel disease involvement, activity and B_{12} status. 63/168 (38%) were B_{12} deficient.

On univariate analysis, active terminal inflammation, was significantly associated with B_{12} deficiency, (OR 2.3; 95% CI 1.2 – 4.7, p=0.02). Pre-stenotic dilatation (OR 2.9; 95% CI 1.3 – 6.8, p=0.01) and segmental small bowel disease (1 or more skip lesions), (OR 2.3; 95% CI 1.2 – 4.4, p=0.01) were also associated with B_{12} deficiency. A trend towards ileal strictures and B_{12} deficiency was observed, (OR 2.3; 95% CI 0.9 – 5.8, p=0.09).

The 94/168 (56%) patients that had inflammation involving the terminal ileum or ileum were analysed with respect to length of small bowel involvement. One outlier with 165 cm of ileal and 35 cm of jejunal involvement who was B_{12} replete was excluded. Within this group 43/93 (46%) patients were B_{12} deficient. The length of inflamed ileum was significantly greater in those with B_{12} deficiency, mean (SD) = 14.1 cm (14.9) than in patients who were B_{12} replete mean (SD)= 8.6cm (9.5), p=0.04. Of note, uninflamed but involved ileum was not associated with B_{12} deficiency.

DISCUSSION

This study, the first using holoTC and MMA, and the largest to date assessing B_{12} status amongst patients with CD, demonstrates that B_{12} deficiency is common and that holoTC coupled with MMA identifies B_{12} deficiency in patients otherwise considered replete on traditional serum testing (serum B_{12} alone). Increasing ileal resection length and ileal inflammation were independent predictors of B_{12} deficiency. In addition, using MRI to assess the relationship between the burden of small bowel disease and B_{12} status we demonstrated that terminal ileal disease with active inflammation, skip lesions and pre-stenotic dilatation were associated with B_{12} deficiency.

A recent review found that 5.6-38.0% of patients with CD were B_{12} deficient.[28] The largest study, a single centre retrospective review of 201 adult patients with CD[29], found that 18.4% of patients were deficient using serum B_{12} measurement; multivariate analysis identified that prior ileal (OR 7.22; 95% CI 1.97 – 26.51) or ileocolonic (OR 5.81; 95% CI 2.09 – 16.12) resection and the need for ongoing medical therapy (OR 2.59; 95% CI 1.03 – 6.47) were independent risk factors for B_{12} deficiency.

Our findings suggest that low B_{12} status may be present in a third of patients with CD, a generally higher prevalence than that reported in most studies.[29-31] This is likely to be due to the differences in methodologies used and the cut-offs applied for holoTC, serum B_{12} and MMA tests. Therefore when interpreting results related to vitamin B_{12} status these factors should be taken into consideration.

Indeed, there is a growing body of evidence that holoTC offers improved sensitivity and specificity when compared to serum B_{12}.[7,32-34] In this regard our results demonstrate that in paired samples, serum B_{12} identified B_{12} deficiency in 5% of CD patients compared to 32% using holoTC and MMA. In addition, in one patient found to be deficient on serum B_{12} testing, holoTC and MMA values were within reference ranges. Applying the higher NHANES cut-off of 147 pmol/L the prevalence of B_{12} deficiency increased to 25%. However the specificity was poor and a significant proportion of patients with evidence of functional B_{12} deficiency remained unidentified. Although MMA is the preferred test to confirm functional B_{12} deficiency[35,36] it has yet to be widely adopted. It should also be noted that MMA is influenced by other factors independently of B_{12} deficiency such as renal impairment and bacterial overgrowth, the latter being of particular relevance in CD with previous surgery or small bowel disease. [37] It should be emphasised that although holoTC offers theoretical advantages over serum B_{12} as a first-line screening test there are limitations. Coupling MMA to holoTC improves specificity, however there remains no true gold standard to diagnose functional B_{12} deficiency. Little is known about other conditions or factors that may influence holoTC. Whether a single deficient holoTC value progresses or spontaneously regresses has not been elucidated. There are also unanswered questions around whether holoTC measures B_{12} metabolism, absorption or both[38]. Further studies in mixed populations are required to determine the relevance of subclinical B_{12} deficiency detected with holoTC.

The possible reasons for high rates of B_{12} deficiency amongst patients with CD are protean. First, disease or resection of the terminal ileum increases the risk of B_{12} deficiency. The terminal ileum alone is involved in 45% of patients with a further 18% having an ileocolonic distribution,[39] whilst the risk of surgery at 1, 5 and 10 years in patients with CD approximates 16%, 33% and 47%.[40] In addition, small bowel bacterial overgrowth can impair B_{12} absorption. The results of studies assessing the impact of terminal ileal resection length on subsequent B_{12} deficiency in patients with CD have been conflicting. Resections >60 cm invariably result in B_{12} deficiency[41,42] whereas 48-53% are rendered B_{12} deficient with resections between 20-60 cm.[42,43] Amongst those with resections <20 cm,

one study reported that no patients developed B_{12} deficiency [43] whereas another found that 38% of patients with a resection of <10 cm were B_{12} deficient.[42] However, these studies did not consider other factors that may influence B_{12} absorption. In our study, we confirmed on multivariate analysis that increasing ileal resection length was predictive of B_{12} deficiency. Our finding that 31/48 (65%) of patients with a resection of >20 cm had B_{12} deficiency is in broad agreement with previous studies, the slightly higher prevalence possibly being due to the greater sensitivity of holoTC/MMA testing. Ileal disease involvement and a history of stricturing or penetrating phenotype were also associated with B_{12} deficiency on univariate analysis but were not found to be independent predictors on multivariate analysis and are probably markers for surgical intervention.

This study is the first to explore the relationship between MRI disease characteristics and B_{12} status amongst patients with CD. An interesting observation was that active terminal ileal inflammation, rather than chronic or inactive disease was significantly predictive of B_{12} deficiency. We found that patients with B_{12} deficiency had, on average, more extensive active small bowel inflammation (mean 14.1 vs 8.6 cm, p=0.04). Similarly an increasing number of skip lesions and the presence of pre-stenotic dilatation were also associated with B_{12} deficiency and a trend towards small bowel strictures was observed. Small bowel strictures and dilatation may predispose to B_{12} deficiency through several mechanisms, including bacterial overgrowth. These findings underscore the importance of an intact terminal ileum in meeting adequate dietary B_{12} absorption and imply, as one might expect, that with increasing disease burden, B_{12} deficiency becomes more prevalent.

Our finding of B_{12} deficiency in 16% of patients with UC was unexpected. Amongst patients with UC, the prevalence of B_{12} deficiency has been shown to approximate that of the general population[44] except in patients who have undergone restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) in whom the prevalence of B_{12} deficiency is as high as 25%.[45] Whereas all patients with CD at our institution undergo annual B_{12} assessment, patients with UC are checked at the physician's discretion. This may have led to selection bias. As patients with UC have inflammation confined to

the colon, rates of B_{12} deficiency generally parallel those with age-matched healthy controls.[30,31] Backwash ileitis may impair dietary B_{12} absorption[46] and IPAA may also lead to impaired B_{12} absorption due to faecal stasis and small bowel bacterial overgrowth.[47] Amongst our cohort of patients with UC 11/141 (8%) had undergone colectomy with IPAA, of which 4/11 (36%) were B_{12} deficient. Interestingly, using identical analytical platforms and reference range cut offs, the prevalence of patients with low holoTC and/or elevated MMA in our mixed general patient population at our institution was 11%[18]

There are several limitations with this study. First, the cohort represents patients managed in a tertiary referral centre who may have a more aggressive disease phenotype with a higher prevalence of surgery compared to the wider IBD population. However disease location, behaviour and surgical rates are comparable to large population based studies.[39,40,48-50] Our finding of B_{12} deficiency in 33% of patients with CD did not consider patients who were already identified as being B_{12} deficient who were receiving supplementation, nor did it consider non-prescribed B_{12} intake with multi-vitamin therapy. Thus, it may underestimate the true prevalence. We did not routinely screen for other causes of B_{12} deficiency however the impact is likely to be small. Pernicious anaemia, responsible for 20-50% of all causes of vitamin B_{12} deficiency[51] for example, has a prevalence of only 0.1% in the general population and a median age at diagnosis of 60 years.[52,53] The validity of findings on ileocolonoscopy or MRI up to six months from B_{12} sampling has limitations, however, the median time between B_{12} measurement and either endoscopic or MRI assessment was short (1 month). In this regard total body stores of vitamin B_{12} are several orders of magnitude greater than daily requirements and therefore the development of B_{12} deficiency will develop slowly when absorption is impaired.[54] The retrospective design of the study limits the strength of the conclusions. Prospective measurement of all three tests (serum B_{12}, holoTC and MMA) with receiver operator curve (ROC) analysis should be performed in this population in the future. Further, a comparison between serum B_{12}/MMA and holoTC/MMA amongst those with intermediate B_{12} would give further insights into the relative

performance of each test. Amongst our sub-group of paired serum B_{12} and holoTC we performed MMA only on those with intermediate holoTC values.

In conclusion, this study demonstrates that assessing B_{12} status in patients with CD using holoTC and MMA identifies impaired B_{12} status in patients otherwise considered replete with traditional serum testing. HoloTC and MMA also excludes B_{12} deficiency in patients otherwise considered deficient using serum testing. Although these results suggest that holoTC offers higher sensitivity compared to serum B_{12} as first line screening for B_{12} deficiency amongst patients with IBD, it comes at the expense of performing MMA (in this cohort approximately 40%). Further, both holoTC and MMA are not reimbursable tests in many countries throughout the world. B_{12} deficiency is common in patients with CD, particularly those with a previous history of ileal resection and current ileal inflammation. Further studies are required to determine the effect of B_{12} supplementation in such patients and also to identify the optimal dose and route of delivery of B_{12} replacement therapy.

REFERENCES

1. Sanz-Cuesta T, González-Escobar P, Riesgo-Fuertes R, et al. Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). *BMC Public Health*. 2012;12:394.

2. Herrmann W, Schorr H, Obeid R, et al. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am. J. Clin. Nutr*. 2003;78:131–136.

3. Kulnigg S, Gasche C. Systematic review: managing anaemia in Crohn's disease. *Aliment Pharmacol Ther*. 2006;24:1507–1523.

4. Carmel R. Subtle and atypical cobalamin deficiency states. *Am J Hematol*. 1990;34:108–114.

5. Nygård O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med*. 1997;337:230–236.

6. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002;346:476–483.

7. Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. *Clin. Chem*. 2007;53:963–970.

8. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin. Chem*. 2004;50:3–32.

9. Hvas AM, Nexo E. Holotranscobalamin--a first choice assay for diagnosing early vitamin B deficiency? *J. Intern. Med.* 2005;257:289–298.

10. Carter MJ, Lobo AJ, Travis SPL, et al. Guidelines for the management of inflammatory bowel disease in adults. *Gut.* 2004;53 Suppl 5:V1–16.

11. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis*: 2012;6:991–1030.

12. Van Assche G, Dignass A, Panés J, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis*. 2010;4:7–27.

13. Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut.* 2006;55:749–753.

14. Harvey RF, Bradshaw MJ. Measuring Crohn's disease activity. *Lancet.* 1980;1:1134–1135.

15. Bernstein CN, Fried M, Krabshuis JH, et al. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. *Inflamm Bowel Dis.* 2010;16:112–124.

16. Walmsley RS, Ayres RC, Pounder RE, et al. A simple clinical colitis activity index. *Gut.* 1998;43:29–32.

17. Leighton JA, Kane SV, Korzenik JR, et al. M1235 Different Simple Clinical Colitis Activity Index (SCCAI) Cut-off Points Used to Define Remission of Ulcerative Colitis (UC) in Large Mesalamine Study. *Gastroenterology.* 2010;138:S–360–360.

18. Sobczyńska-Malefora A, Gorska R, Pelisser M, et al. An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. *Clin. Biochem.* 2014;47:82–86.

19. Brady J, Wilson L, McGregor L, et al. Active B12: a rapid, automated assay for holotranscobalamin on the Abbott AxSYM analyzer. *Clin. Chem.* 2008;54:567–573.

20. Lloyd-Wright Z, Hvas A-M, Møller J, et al. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. *Clin. Chem.* 2003;49:2076–2078.

21. Schmedes A, Brandslund I. Analysis of methylmalonic acid in plasma by liquid chromatography-tandem mass spectrometry. *Clin. Chem.* 2006;52:754–757.

22. Vogiatzoglou A, Oulhaj A, Smith AD, et al. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. *Clin. Chem.* 2009;55:2198–2206.

23. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. *Arch. Intern. Med.* 1999;159:1289–1298.

24. Griffin N, Grant LA, Anderson S, et al. Small bowel MR enterography: problem solving in Crohn's disease. *Insights Imaging.* 2012;3:251–263.

25. Koh DM, Miao Y, Chinn RJ, et al. MR imaging evaluation of the activity of Crohn's disease. *AJR Am J Roentgenol.* 2001;177:1325–1332.

26. Miao YM, Koh DM, Amin Z, et al. Ultrasound and magnetic resonance imaging assessmentof active bowel segments in Crohn's disease. *Clin Radiol*. 2002;57:913–918.

27. http://www.hra.nhs.uk/documents/2013/09/defining-research.pdf Accessed - Nov 2014. 2013:1–4.

28. Battat R, Kopylov U, Szilagyi A, et al. Vitamin B12 deficiency in inflammatory bowel disease: prevalence, risk factors, evaluation, and management. *Inflamm Bowel Dis*. 2014;20:1120–1128.

29. Headstrom PD, Rulyak SJ, Lee SD. Prevalence of and risk factors for vitamin B(12) deficiency in patients with Crohn's disease. *Inflamm Bowel Dis*. 2008;14:217–223.

30. Vagianos K, Bernstein CN. Homocysteinemia and B vitamin status among adult patients with inflammatory bowel disease: a one-year prospective follow-up study. *Inflamm Bowel Dis*. 2012;18:718–724.

31. Yakut M, Üstün Y, Kabaçam G, et al. Serum vitamin B12 and folate status in patients with inflammatory bowel diseases. *Eur. J. Intern. Med*. 2010;21:320–323.

32. Valente E, Scott JM, Ueland PM, et al. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B_{12} status in the elderly. *Clin. Chem*. 2011;57:856–863.

33. Heil SG, de Jonge R, de Rotte MCFJ, et al. Screening for metabolic vitamin B12 deficiency by holotranscobalamin in patients suspected of vitamin B12 deficiency: a multicentre study. *Ann. Clin. Biochem*. 2012;49:184–189.

34. Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clin. Chem*. 2006;52:278–285.

35. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *American Journal of Clinical Nutrition*. 2011;94:348S–358S.

36. Allen RH, Stabler SP, Savage DG, et al. Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. *Am J Hematol*. 1990;34:90–98.

37. Dibaise JK, Young RJ, Vanderhoof JA. Enteric microbial flora, bacterial overgrowth, and short-bowel syndrome. *Clin Gastroenterol Hepatol*. 2006;4:11–20.

38. Carmel R. Holotranscobalamin: not ready for prime time. *Clin. Chem*. 2012;58:643–5– author reply 645–6.

39. Thia KT, Sandborn WJ, Harmsen WS, et al. Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort. *Gastroenterology*. 2010;139:1147–1155.

40. Frolkis AD, Dykeman J, Negrón ME, et al. Risk of surgery for inflammatory bowel diseases has decreased over time: a systematic review and meta-analysis of population-based studies. *Gastroenterology*. 2013;145:996–1006.

41. Thompson WG, Wrathell E. The relation between ileal resection and vitamin B12 absorption. *Can J Surg*. 1977;20:461–464.

42. Behrend C, Jeppesen PB, Mortensen PB. Vitamin B12 absorption after ileorectal anastomosis for Crohn's disease: effect of ileal resection and time span after surgery. *Eur J Gastroenterol Hepatol*. 1995;7:397–400.

43. Duerksen DR, Fallows G, Bernstein CN. Vitamin B12 malabsorption in patients with limited ileal resection. *Nutrition*. 2006;22:1210–1213.

44. Fernandez-Banares F, Abad-Lacruz A, Xiol X, et al. Vitamin status in patients with inflammatory bowel disease. *Am J Gastroenterol*. 1989;84:744–748.

45. Coull DB, Tait RC, Anderson JH, et al. Vitamin B12 deficiency following restorative proctocolectomy. *Colorectal Dis*. 2007;9:562–566.

46. M'Koma AE, Wise PE, Schwartz DA, et al. Prevalence and outcome of anemia after restorative proctocolectomy: a clinical literature review. *Diseases of the Colon & Rectum*. 2009;52:726–739.

47. Hylander E, Rannem T, Hegnhøj J, et al. Absorption studies after ileal J-pouch anastomosis for ulcerative colitis. A prospective study. *Scand. J. Gastroenterol*. 1991;26:65–72.

48. Bernstein CN, Loftus EV, Ng SC, et al. Hospitalisations and surgery in Crohn's disease. *Gut*. 2012;61:622–629.

49. Cosnes J, Cattan S, Blain A, et al. Long-term evolution of disease behavior of Crohn's disease. *Inflamm Bowel Dis*. 2002;8:244–250.

50. Lovasz BD, Golovics PA, Vegh Z, et al. New trends in inflammatory bowel disease epidemiology and disease course in Eastern Europe. *Dig Liver Dis*. 2013;45:269–276.

51. Loukili NH, Noel E, Blaison G, et al. [Update of pernicious anemia. A retrospective study of 49 cases]. *Rev Med Interne*. 2004;25:556–561.

52. Andres E, Serraj K. Optimal management of pernicious anemia. *J Blood Med*. 2012;3:97–103.

53. Toh BH, Van Driel IR, Gleeson PA. Pernicious anemia. *N Engl J Med*. 1997;337:1441–1448.

54. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. *Neurology*. 1995;45:1435–1440.

Table 1. Patient Demographics

Characteristic	**Crohn's Disease n = 381**	**Ulcerative Colitis n = 141**	***p* value**
Male/female N (%)	195(51%)/186 (49%)	55(39%)/86(61%)	0.02
Age, years median, (IQR)	35 (29-47)	36 (30-47)	0.50

Current smoker, N	59 (16%)	18 (13%)	0.52
Disease duration, years median (IQR)	8 (3-16)	6 (2-12)	0.01
Immunomodulator, N	239 (63%)	53 (38%)	<0.0001
Azathioprine/mercaptopurine	212 (56%)	48 (34%)	
Methotrexate	11 (3%)	2 (1%)	
Tioguanine	16 (4%)	3 (2%)	
Biologic, N	133 (35%)	6 (4%)	<0.0001
Abdominal surgery, N	150 (39%)	13 (9%)	<0.0001
Total small bowel resection length, (cm) median (IQR)	18 (11-30)	Not applicable	
Hb g/L,mean (SD)	131.3 (±15.8)	130.1 (±16.1)	0.62
MCV fL (median, IQR)	92 (88-96)	91 (87-95)	0.51
Elevated CRP (≥5ng/mL), N	129 (34%)	35 (25%)	0.06
Active disease (HBI/SCCAI), N	78 (21%)	40 (28%)	0.07
Ileal inflammation (endoscopy/MRI), N^	94 (30%)	Not applicable	

^ ileal inflammation available in 317/381 (83%) patients

Table 2. Univariate Analysis between Covariates and B_{12} status.

Variable	**B_{12} deficient n = 126/381 (33%)**	**B_{12} replete n = 255/381 (67%)**	**Unadjusted Odds Ratio**	**95% CI**	***p* value**
Gender					
Female	54 (43%)	132 (52%)	1.0		
Male	72 (57%)	123 (48%)	1.4	0.9 – 2.1	0.1
Age (years)					
<18	2 (2%)	4 (2%)	1.0		
18 – 25	15 (12%)	35 (13%)	0.9	0.1 – 5.2	0.9
26 – 35	46 (37%)	90 (35%)	1.0	0.2 – 5.8	1.0
36 – 50	39 (31%)	80 (32%)	1.0	0.2 – 5.6	1.0
>50	24 (18%)	46 (18%)	1.0	0.2 – 6.1	1.0
Smoker					
No	102 (81%)	220 (86%)	1.0		
Yes	24 (19%)	35 (14%)	1.5	0.8 – 2.6	0.2
Age at diagnosis*					
<16 years	15 (12%)	31 (12%)	1.0		
16-40 years	94 (75%)	185 (74%)	1.1	0.5 – 2.0	0.9
>40 years	16 (13%)	35 (14%)	0.9	0.4 – 2.2	0.9
Behaviour					

Non-stricturing/non penetrating					
Stricturing	42 (33%)	133 (52%)	1.0		
Penetrating	50 (40%)	78 (31%)	2.1	1.2 – 3.3	0.005
	34 (27%)	44 (17%)	2.4	1.4 – 4.3	0.002
Ileal disease					
L2	19 (15%)	84 (33%)	1.0		
L1/L3	107 (85%)	171 (67%)	2.8	1.6 - 4.8	<0.0001
Duration disease					
0-2 years	28 (22%)	63 (25%)	1.0		
>2-10 years	44 (35%)	92 (36%)	1.1	0.6 – 1.9	0.8
> 10 years	54 (43%)	100 (39%)	1.2	0.7 – 2.1	0.5
Treatment with immunomodulator					
No	48 (38%)	94 (37%)	1.0		
Yes	78 (62%)	161 (63%)	0.9	0.6 – 1.5	0.8
Treatment with biologic					
No	80 (64%)	168 (66%)	1.0		
Yes	46 (34%)	87 (34%)	1.1	0.7 – 1.7	0.6
Ileal resection					
No	58 (46%)	186 (73%)	1.0		
Yes	68 (54%)	69 (27%)	3.2	2.0 – 4.9	<0.0001
Anaemic^					
No	91 (73%)	199 (79%)	1.0		
Yes	33 (27%)	54 (21%)	1.3	0.8 – 2.2	0.3
Macrocytosis^					
No	103 (83%)	192 (76%)	1.0		
Yes	21 (17%)	61 (24%)	1.6	0.4 – 1.1	0.1

***available in n=376 ^ available in n=377**

Table 3. Multivariate analysis of covariates independently associated with B_{12} status

Variable	**Adjusted Odds Ratio**	**95% CI**	***p* value**
Ileal resection			
None			
0-20cm	3.0	1.5 – 6.0	0.002
>20cm	6.7	3.0 – 15.0	<0.0001
Ileal inflammation	3.9	2.2 – 6.9	<0.0001
Active disease (HBI)	1.9	0.98 – 3.7	0.06

Table 4. Univariate analysis of MRI findings and B_{12} status

Variable	B_{12} deficient n=63 (38%)	B_{12} replete n=105 (62%)	Unadjusted Odds Ratio	95% CI	*p* value
Terminal ileal disease (distal 20cm)					
No					
Inactive disease	25 (40%)	58 (55%)	1.0		
Active inflammation	9 (14%)	18 (17%)	1.1	0.5 – 2.9	0.8
	29 (46%)	29 (28%)	2.3	1.2 – 4.7	0.02
Ileal disease					
No	54 (86%)	96 (91%)	1.0		
Inactive disease	3 (5%)	2 (2%)	2.6	0.4 – 16.5	0.3
Active inflammation	6 (9%)	7 (7%)	1.5	0.5 – 4.8	0.5
Jejunal disease					
No	60 (95%)	102 (97%)	1.0		
Inactive disease	2 (3%)	0 (0%)			
Active inflammation	1 (2%)	3 (3%)	0.6	0.06 – 5.6	0.6
SB skip lesions					
None	20 (32%)	54 (51%)	1.0		
≥1	43 (68%)	51 (49%)	2.3	1.2 – 4.4	0.01
Pre-stenotic dilatation					
No	47 (75%)	94 (90%)	1.0		
Yes	16 (25%)	11 (10%)	2.9	1.3 – 6.8	0.01
Stricture					
No	52 (83%)	96 (91%)	1.0		
Yes	11 (17%)	9 (9%)	2.3	0.9 – 5.8	0.09

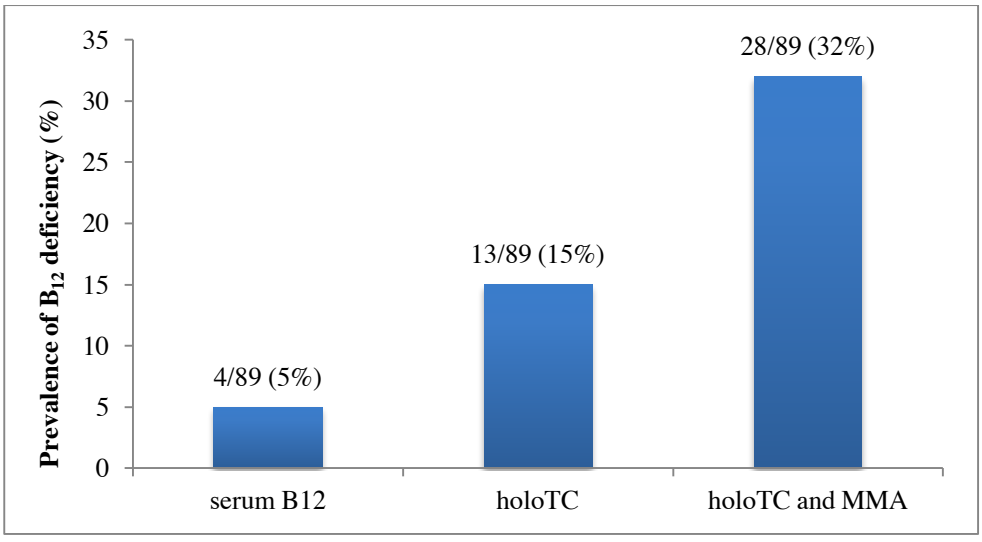

Figure 1. Prevalence of B_{12} deficiency using different tests (MMA;methylmalonic acid)

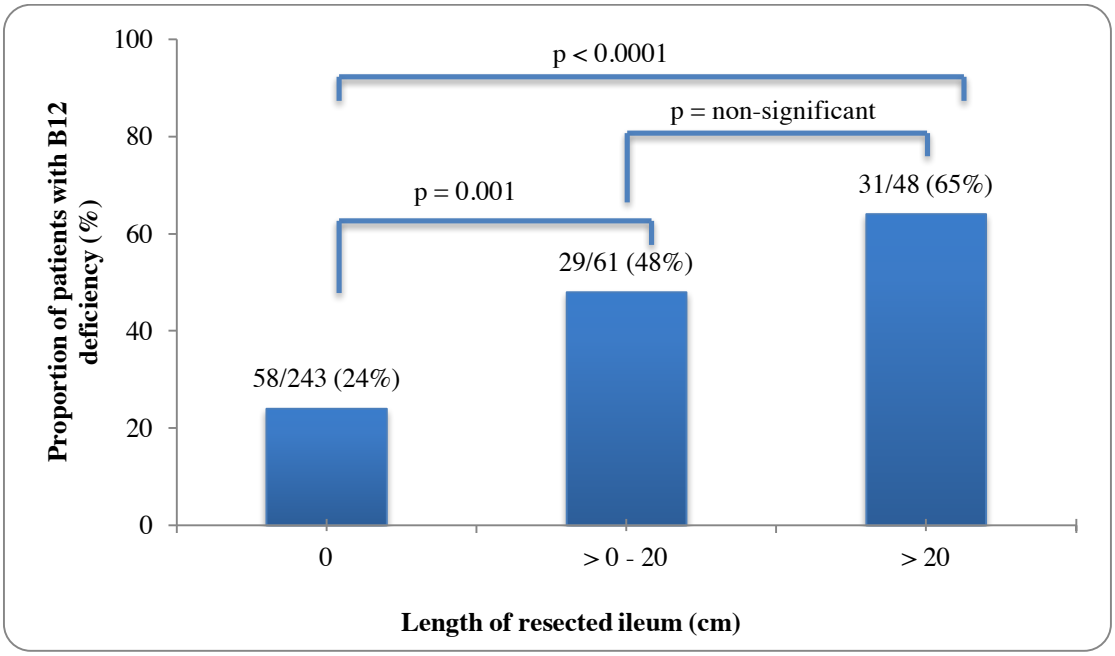

Figure 2. Association between B_{12} status and length of ileum resected

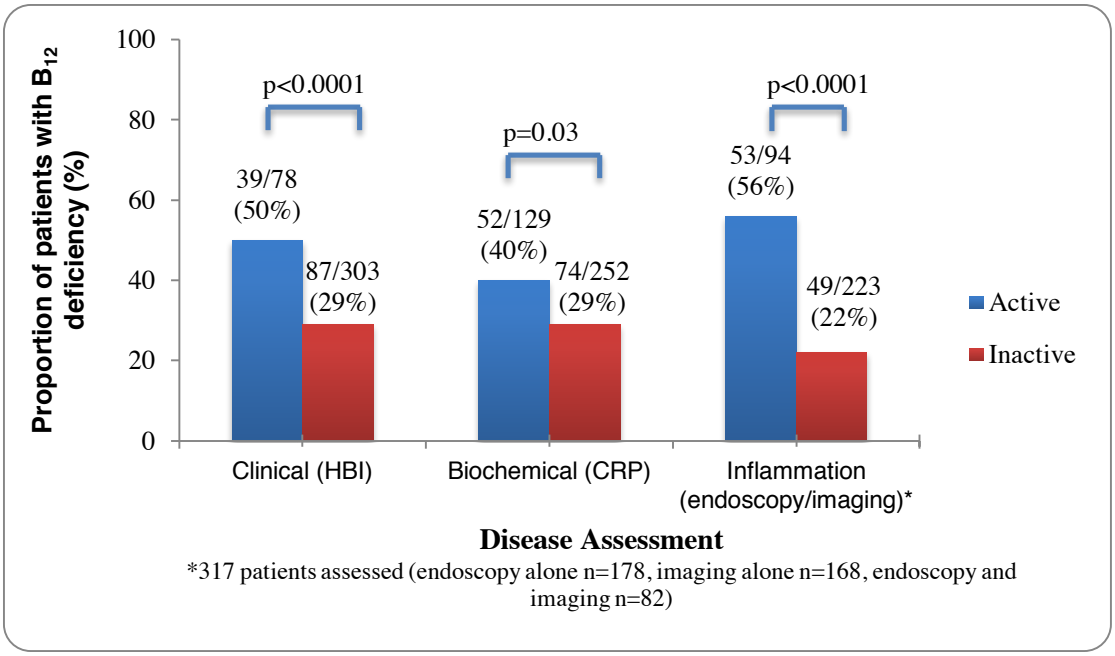

Figure 3. Proportion of B_{12} deficient patients according to active vs inactive disease. (HBI; Harvey Bradshaw Index, CRP; C-reactive protein)

SECTION 5.

CHAPTER 10:

Integrative discussion

INTERGRATIVE DISCUSSION

As the management of IBD continues to evolve, so do the expectations of our patients. Previous therapies, such as corticosteroids and immunomodulators, have little-to-no impact on the natural history of the disease[118] and come at the expense of side effects and toxicity which limit their use.[22,52,119] In recent decades strategies have shifted from a reactive symptom-based approach to one of individualised risk stratification with tailored management targeting healing of the mucosa.[28] The ultimate goal is to restore patient's quality of life and prevent disease progression, through the use of personalised therapy, drawing on medical and surgical therapy where appropriate. Less frequently considered factors, such as nutritional deficiencies[41], fatigue[120] and the psychological burden of a chronic disease must be addressed. Taken together, therapeutic optimisation of patients with IBD is a critical issue and, accordingly, forms the basis for the work contained within this thesis.

Thiopurines, and to a lesser extent, MTX, have been a mainstay of drug therapy in the treatment of IBD for over 30 years. The rates of intolerance and toxicity, both in the short and long term, are well recognised. Further, due to their slow onset of action, they are not appropriate induction agents.[121] However, in a proportion of patients, they are effective for maintaining remission.[49,122] With the introduction of highly efficacious and relatively safe anti-TNF therapy some 15 years ago, interest in the use of thiopurines in some parts of the world waned. Despite their remarkable impact, anti-TNF primary non-response and, more commonly, secondary loss of response are frequently encountered,[38] which is clinically relevant as few alternative therapeutic options exist. We have come to understand that secondary loss of response is intrinsically linked to low concentrations of drug, which in turn is influenced by patient and disease factors that increase clearance and by immunogenicity. In this regard, the results from SONIC provided a valuable signal; thiopurines in combination with IFX improved outcomes compared to either agent alone, and, although not designed to investigate the pharmacokinetic mechanisms underpinning this benefit, higher

drug levels and reduced anti-drug antibody formation were observed in the combination arm. This, and work by others,[56,62] has led to the strategy of combination therapy over anti-TNF monotherapy in an effort to improve drug levels and reduce immunogenicity, with the goal of improving the durability of response to anti-TNF. In spite of this, data demonstrating a benefit of combination therapy when using ADA are lacking. Further, whether the intensity of concomitant thiopurine use, as measured by the active metabolites, TGNs, is of relevance has not been addressed in this setting. This led our group to perform the study which comprises Chapter Three of this thesis. In a well described cohort of 123 consecutive patients with CD who initiated ADA, clinical response at week 12 was observed in 83% of those treated with combination therapy compared to 61% of those on ADA monotherapy ($p = 0.02$). Of particular interest was the finding that the intensity of thiopurine dosing was of importance; 87% of those with a TGN >235 pmol/$8x10^8$RBC were responders, compared to 70% with sub-therapeutic TGNs and 61% in patients treated with monotherapy ($p = 0.011$). On multivariate analysis, therapeutically dosed-thiopurines were found to be an independent predictor of response to induction (OR 4.32, 95% CI: 1.41-13.29, $p = 0.01$). This benefit extended to maintenance therapy, where remission semesters were observed more frequently in patients treated with combination therapy compared to monotherapy, (81 vs 60%, $p < 0.0001$) and again therapeutic TGNs were independently associated with semesters of remission (OR 3.71; 95% CI: 1.87-7.34, $p < 0.0001$). Although not the focus of the study, safety was comparable between patients treated with combination therapy compared to ADA monotherapy. Limitations of the work include the lack of randomisation to different treatment arms, the absence of therapeutic drug monitoring of ADA, the assessment of induction outcomes according to physician global assessment and the high proportion of patients with therapeutic TGNs. Nevertheless, the findings from this study build on those by others[123] supporting the use of combination therapy with ADA in CD. Higher response rates at induction and during maintenance in those with therapeutic TGNs compared to sub-therapeutic TGNs were observed, which in turn were better than those treated with ADA monotherapy. Considering rates of thiopurine non-adherence, and the inability to achieve a therapeutic TGN in the proportion of patients with a skewed 'hypermethylation' profile,[124] the lack of metabolite-adjusted thiopurine dosing in studies investigating the role of combination therapy may, in part,

explain the lack of benefit that has been reported. In view of this, future prospectively designed studies which stratify patients receiving thiopurines to different TGN concentrations, and which, in turn, examine this impact of anti-TNF drug levels and antibodies, are eagerly anticipated.

In this regard the findings from Chapter Seven offer some insight into the differing pharmacokinetic-pharmacodynamic relationship between IFX and ADA in CD, and the influence of patient and disease factors, including the intensity of thiopurine dosing as measured by TGNs, on drug levels. In a well characterised cohort of 191 patients we found no difference in drug levels between patients treated with combination therapy compared to anti-TNF monotherapy ($p = 0.86$). Despite this being in contrast to the findings of SONIC and those reported by some,[61,64,125] others have failed to demonstrate such an association. In a retrospective study of 217 IBD patients, ADA drug levels were no different in those treated with monotherapy compared to combination therapy (11.5 vs 13.1 μg/mL, p = not significant).[63] Ungar et al found higher IFX levels in combination treated patients but not with ADA.[98] Considering the current study, the lack of association cannot be explained by the intensity of concurrent immunomodulation, given drug levels were similar across a range of thiopurine doses, according to TGNs. How do we, therefore, reconcile our findings, particularly in light of the benefit of therapeutically dosed thiopurines with ADA in the study comprising Chapter Three? First, it is plausible that these diametric conclusions are a result of the limitations which are inherent to all retrospective and cross-sectional studies. In the former study, response may have been influenced by high rates of corticosteroid use, and the proportion of patients with sub-therapeutic TGNs was relatively low. The definition of remission during maintenance was defined as not requiring treatment intensification or failing therapy, hence some patients treated with combination therapy and therapeutic TGNs may have had ongoing mild-to-moderate clinical disease but 'no-where to go'. We did not perform drug levels or assess for anti-drug antibodies, so the premise that the benefit of adequately dosed thiopurines in combination with ADA was due to an improvement in ADA pharmacokinetics was unproven. Considering the latter study, patients may have been escalated to combination therapy on

account of previous low anti-TNF drug levels which may explain the lack of difference that was observed. Conversely, given the impact of anti-drug antibodies on increasing drug clearance, and the observation that immunogenicity generally occurs within 12 months of starting anti-TNF therapy,[62] perhaps the lack of difference in drug levels between combination therapy and monotherapy in Chapter Seven is explained by the median timing in which sampling was performed, namely 22 months.

Other findings from the studies reported in Chapter Seven and Eight deserve further commentary. In agreement with others,[61,81,82,96] we demonstrated that IFX drug levels could discriminate between patients with active disease compared to those in remission, but no such relationship existed for ADA. For IFX, our proposed cut-off of > 1.5 μg/mL which best predicted clinical remission is in broad agreement with the literature.[93] Of clinical relevance, however, was our observation in Chapter Six, that significant differences in inter-kit performance of commonly used ELISAs limit the relative comparability of thresholds which have been reported in the literature. This translated into a significant misclassification rate of patients with therapeutic or sub-therapeutic trough levels when other assays were compared to the reference assay. We observed that higher IFX thresholds were needed to neutralise systemic inflammation and predict mucosal healing, which have been corroborated in recent publications.[97,98] Moving forward, this has clinical implications for future prospective studies that proactively dose anti-TNF therapy according to a treat-to-target paradigm.[118] The lack of association between ADA drug levels and end-points suggests there is a disconnect between what is happening in the serum and the point of action, the mucosa, in contrast to IFX. This has been addressed in the study by Yarur et al comparing serum and mucosal tissue levels of IFX and ADA; a signification correlation was observed with IFX ($r = 0.51$, $p = 0.017$) but not with ADA ($r = 0.23$, $p = 0.17$).[126] In our study, these results may be explained by the relatively low proportion of patients who had ADA sampled at trough, (21%), before drug levels theoretically reach a nadir. In support of this argument, the findings in Chapter Eight suggest that ADA drug levels do indeed decline significantly to trough, by an average of 1.06 and 0.89 μg/mL from day 4-6 and day 7-9, respectively.

Further important insights into the pharmacokinetics of IFX and ADA, and modulating factors thereof, were made. Considering ADA, within-patient variation in drug levels sampled at any point in a fortnightly treatment cycle do not differ significantly to the next. This is important, as it gives the clinician some confidence that decisions can be made on the basis of a single ADA drug level and do not need to be repeated. If drug levels are found to be in the therapeutic range during the first 9 days of therapy, then the trough level can be assumed to be therapeutic with the caveat of a 1 in 3 false-positive rate. The logistic regression curves contained within Chapter Eight can be applied to calculate the trough levels from samples obtained between day 4-6 or day 7-9. Cut-offs vary according to the precision desired by the clinician; for example, a level > 7.35 μg/mL correlated to an 80% probability of achieving a therapeutic trough level. Adding the drug level obtained at day 4-6 or day 7-9 in a current smoker administering ADA by syringe accounted for 66 or 80% (depending on the day, respectively) in the variation of drug levels. To date, there are no high quality studies which have performed intensive ADA sampling and which, in turn, address the relationship of patient and disease factors. Considering the results from Chapter Seven, weekly ADA dosing was associated with higher drug levels and low serum albumin and higher weight were independent predictors of lower levels. For IFX, markers of active inflammation (namely elevated faecal calprotectin and C-reactive protein) negatively influenced drug levels, and higher doses of IFX (10 mg/kg/q8 rather than 5 mg/kg/q6) predicted higher IFX levels. These findings propose several hypotheses that warrant further evaluation. First, the relationship between ADA dosing and weight adds to the argument that in some, individualised weight based therapy may be of benefit. Second, the positive relationship between inflammatory burden, mucosal inflammation and IFX drug clearance has been reported by others.[125,127] This implies that in patients with severe disease, such as in acute severe colitis, larger doses of anti-TNF may be required to maintain a therapeutic drug level, which in turn will likely lead to improved clinical outcomes. Prospective randomised controlled trials addressing this area are eagerly anticipated. Third, the ideal dose intensification strategy in secondary loss of response with IFX remains unclear. In a retrospective study of

168 patients with CD, higher response rates with doubling the dose to 10 mg/kg/q8 compared to halving the interval to 5 mg/kg/q4 were observed (77 vs 66%) although this was not statistically significant (p = 0.14).[128] Although not directly comparable, (given patients in our study were treated with 5mg/kg/q6, rather than q4), the favourable benefit seen with 10 mg/kg/q8 infers mechanistically that higher peak concentrations may be of relevance. It should be noted that these patient and disease factors which significantly influenced anti-TNF pharmacokinetics accounted for a relatively low variance in drug levels (23-31%), highlighting the complexity (and significant inter-individual variation) of monoclonal therapy operating within a biological system. Nevertheless, they add to the work done both others[101,102,107,129,130] and lay the foundation for further studies which, ideally, will one day lead us closer to the goal of delivering personalised therapy. Our findings add valuable understanding to the utility of therapeutic drug monitoring of anti-TNF in CD. Should large scale studies replicate and build on our findings, patients drug levels may soon be calculated at any time point in a treatment cycle with reasonable accuracy through the use of simple automated dashboard systems.[131] Taken together, the work contained within these studies adds significantly to our understanding of ways to optimise the use of thiopurines and anti-TNF therapy.

A significant proportion of patients develop side effects leading to thiopurine and MTX withdrawal.[48,50,132] Prior to the widespread availability of anti-TNF maintenance therapy this often rendered patients to corticosteroid dependence, ongoing active disease and, in many, resulted in surgery. Therefore, around the turn of the century, the need for alternative effective therapies was paramount, but few options existed. As discussed previously, even in the anti-TNF era, concomitant immunomodulation is still of relevance. These issues led to the use of thioguanine (TG), a non-conventional thiopurine, which, by way of its direct conversion to TGN, circumvents many of the intermediate metabolites in the classical thiopurine pathway.[72] This confers a putative benefit of avoiding many of the typical side effects seen with conventional thiopurines, including pancreatitis. Accordingly, pilot studies in both CD[73,133] and UC[134] were conducted which reported response and remission rates similar to those seen with conventional thiopurines. Soon after, high rates of nodular regenerative hyperplasia (NRH) of the liver, a common

cause of non-cirrhotic portal hypertension, were observed in patients treated with 40-80 mg TG, leading to it being abandoned outside of a select number of centres.[135,136] TG-induced NRH is felt to be dose-dependent and proponents of the drug have reported little-to-no NRH using low-dose TG (generally < 20mg daily).[72,137,138] TG has been in regular use at our institution (Guy's and St. Thomas' Hospital, London, UK) for 13 years. Chapter Four of this thesis reports our long term safety and efficacy data in 54 IBD patients treated with TG with 126 patient-years of follow-up. In a difficult-to-treat cohort of patients intolerant of, or refractory to conventional immunomodulators, TG dosed 20-40 mg daily was generally well tolerated, with 30% ceasing due to side-effects or toxicity. Pancreatitis did not recur in any of the patients (n = 19) who developed this complication with conventional thiopurines. No causes of NRH were observed in a protocol-directed surveillance program involving biochemistry, liver biopsy and/or magnetic resonance imaging, where appropriate. Short term (6 and 12 month) clinical response rates were similar to those seen with conventional immunomodulators. Finally, TGNs did not correlate with efficacy or toxicity, and were, as expected, higher than those observed with other thiopurines (median 740 pmol/$8x10^8$RBC).

The safety data from this study using low-dose TG are reassuring, and adds to the literature suggesting that the associated risk of NRH is possibly no higher than that observed with conventional thiopurines, or indeed IBD itself.[77,139,140] Further, the avoidance of commonly encountered side-effects seen with azathioprine and mercaptopurine, combined with the practicality of a single daily dose, make TG a potentially attractive therapeutic option. Given the current landscape, with an expanding therapeutic armamentarium which includes anti-TNF and adhesion blocking agents, the case for TG monotherapy is somewhat flawed. However, given the importance of the role of concomitant immunomodulation in limiting loss of response to anti-TNF, particularly during the first 12-24 months of therapy[62], research into low-dose TG in this context is warranted.

A section pertaining to optimisation of micronutrients, specifically vitamin B12, was included in this thesis to highlight that in this modern age of complex medical management decisions involving, but not limited to, immunomodulators and anti-TNF therapy, simple measures of clinical relevance are often overlooked. Micronutrient deficiency is common in IBD,[41,112,141] particularly in patients with CD. It is associated with a range of potentially serious manifestations, and is readily correctable when identified. Patients with CD, by way of ileal disease involvement, or indeed after surgical resection, are at particular risk of B12 deficiency due to its exclusive absorption at this site. In this regard the prevalence of B12 deficiency in CD has been reported to occur in 5.6-38.0%.[114] Sequelae of B12 deficiency includes megaloblastic anaemia and neuropsychiatric manifestations, but serious complications, such as sub-acute combined degeneration of the cord, are recognised. The assessment of B12 deficiency has traditional been through serum measurement, however this lacks specificity. Methlymalonic acid (MMA) is considered the gold standard however is expensive and not widely available. This has led to the development of the HoloTC test which measures the B12-transcoblamin II complex, which is considered metabolically active and has been found to be a superior method of assessing functional B12 deficiency.[116,142,143] Accordingly, the study comprising Chapter Nine of this thesis aimed to assess the prevalence of B12 deficiency using HoloTC, supported by MMA, and explored factors associated with B12 deficiency in patients with CD. Further, a sub-analysis was performed comparing the utility of HoloTC to traditional serum B12 testing. A particular strength of this retrospective study conducted in 381 patients with CD was the painstaking retrieval of estimates of ileal resection length amongst 199 small bowel resections, calculated after review of operative reports or histopathology specimens, where available. For the first time we also explored the relationship between the burden of terminal ileal disease, according to a variety of characteristics observed on magnetic resonance imaging (MRI). We found that 33% of patients were B12 deficient, emphasising the importance of assessing for this micronutrient deficiency in patients with CD. Findings of clinical relevance were that ileal resection ≤ 20 cm (OR: 3.0; 95% CI: 1.5-6.0, $p = 0.002$) and > 20 cm (OR 6.7; 95% CI: 3.0-14.7, $p < 0.0001$) and ileal inflammation (OR 3.9; 95% CI: 2.2-6.9, $p < 0.0001$) were independent predictors of B12 deficiency on multivariate analysis. Amongst the 44% of patients

who underwent an MRI, active terminal ileal inflammation ($p = 0.02$), increased disease burden (≥ 1 skip lesion, $p = 0.01$) and pre-stenotic dilatation > 3cm ($p = 0.01$) were associated with B12 deficiency on univariate analysis. These findings underscore, as one might expect, that with increasing disease burden, B12 deficiency becomes more prevalent. Finally, holoTC, supported by MMA, offered advantages over serum B12 testing in the assessment of B12 deficiency. HoloTC and MMA identified B12 deficiency in patients otherwise considered replete using serum testing. Our results add to the literature demonstrating that B12 deficiency is common in CD. The findings suggest that the use of holoTC, supported by MMA when appropriate, should be considered the gold standard moving forward. The identification of new risk factors (on MRI) and greater understanding into the impact of length of surgically resected terminal ileum are of clinical relevance. Taken together, this study offers valuable insights which can be used in the optimisation of vitamin B12 deficiency.

REFERENCES FROM CHAPTER 1 and 10

1. Solberg IC, Vatn MH, Hoie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol 2007;5(12):1430–8.

2. Peyrin-Biroulet L, Harmsen WS, Tremaine WJ, et al. Surgery in a population-based cohort of Crohn's disease from Olmsted County, Minnesota (1970-2004). Am J Gastroenterol 2012;107(11):1693–701.

3. Nguyen GC, Nugent Z, Shaw S, et al. Outcomes of patients with Crohn's disease improved from 1988 to 2008 and were associated with increased specialist care. Gastroenterology 2011;141(1):90–7.

4. Tozer PJ, Burling D, Gupta A, et al. Review article: medical, surgical and radiological management of perianal Crohn's fistulas. Aliment Pharmacol Ther 2011;33(1):5–22.

5. Reinisch W, Reinink AR, Higgins PDR. Factors associated with poor outcomes in adults with newly diagnosed ulcerative colitis. Clin Gastroenterol Hepatol 2015;13(4):635–42.

6. Samuel S, Ingle SB, Dhillon S, et al. Cumulative incidence and risk factors for hospitalization and surgery in a population-based cohort of ulcerative colitis. Inflamm Bowel Dis 2013;19(9):1858–66.

7. Bernstein CN, Loftus EV, Ng SC, et al. Epidemiology and Natural History Task Force of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD). Hospitalisations and surgery in Crohn's disease. Gut 2012;61(4):622–9.

8. Huppertz-Hauss G, Høivik ML, Langholz E, et al. Health-related quality of life in inflammatory bowel disease in a European-wide population-based cohort 10 years after diagnosis. Inflamm Bowel Dis 2015;21(2):337–44.

9. Bernklev T, Jahnsen J, Lygren I, et al. Health-related quality of life in patients with inflammatory bowel disease measured with the short form-36: psychometric assessments and a comparison with general population norms. Inflamm Bowel Dis 2005;11(10):909–18.

10. Blondel-Kucharski F, Chircop C, Marquis P, et al. Health-related quality of life in Crohn's disease: a prospective longitudinal study in 231 patients. Am J Gastroenterol 2001;96(10):2915–20.

11. Reinisch W, Sandborn WJ, Bala M, et al. Response and remission are associated with improved quality of life, employment and disability status, hours worked, and productivity of patients with ulcerative colitis. Inflamm Bowel Dis 2007;13(9):1135–40.

12. Langholz E, Munkholm P, Davidsen M, et al. Colorectal cancer risk and mortality in patients with ulcerative colitis. Gastroenterology 1992;103(5):1444–51.

13. Winther KV, Jess T, Langholz E, et al. Long-term risk of cancer in ulcerative colitis: A population-based cohort study from Copenhagen County. Clinical Gastroenterology and

Hepatology 2004;2(12):1088–95.

14. Card T, Hubbard R, Logan RFA. Mortality in inflammatory bowel disease: a population-based cohort study. Gastroenterology 2003;125(6):1583–90.

15. Loftus EV. Mortality in inflammatory bowel disease: peril and promise. Gastroenterology 2003;125(6):1881–3.

16. Palli D, Trallori G, Saieva C, et al. General and cancer specific mortality of a population based cohort of patients with inflammatory bowel disease: the Florence Study. Gut 1998;42(2):175–9.

17. Prideaux L, Kamm MA, De Cruz PP, et al. Inflammatory Bowel Disease in Asia - A Systematic Review. J Gastroenterol Hepatol 2012;27(8):1266-80.

18. Lovasz BD, Golovics PA, Vegh Z, et al. New trends in inflammatory bowel disease epidemiology and disease course in Eastern Europe. Dig Liver Dis 2013;45(4):269–76.

19. Niewiadomski O, Studd C, Hair C, et al. Prospective population-based cohort of inflammatory bowel disease in the biologics era: Disease course and predictors of severity. J Gastroenterol Hepatol 2015;30(9):1346–53.

20. Gearry RB, Richardson A, Frampton CMA, et al. High incidence of Crohn's disease in Canterbury, New Zealand: results of an epidemiologic study. Inflamm Bowel Dis 2006;12(10):936–43.

21. Burger D, Travis S. Conventional medical management of inflammatory bowel disease. Gastroenterology 2011;140(6):1827–1837.

22. Irving PM, Gearry RB, Sparrow MP et al. Review article: appropriate use of corticosteroids in Crohn's disease. Aliment Pharmacol Ther 2007;26(3):313–29.

23. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353(23):2462–76.

24. Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. New Engl J Med 1999;340(18):1398–405.

25. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002;359(9317):1541–9.

26. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology 2006;130(2):323–33

27. Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut 2007;56(9):1232–9.

28. Bouguen G, Levesque BG, Feagan BG, et al. Treat to target: a proposed new paradigm for the management of Crohn's disease. Clin Gastroenterol Hepatol 2015;13(6):1042–2.

29. Falvey JD, Hoskin T, Meijer B, et al. Disease activity assessment in IBD: clinical indices and biomarkers fail to predict endoscopic remission. Inflamm Bowel Dis 2015;21(4):824–31.

30. Ordás I, Feagan BG, Sandborn WJ. Early use of immunosuppressives or TNF antagonists for the treatment of Crohn's disease: time for a change. Gut 2011;60(12):1754–63.

31. Louis E, Dotan I, Ghosh S, et al. Optimising the Inflammatory Bowel Disease Unit to Improve Quality of Care: Expert Recommendations. J Crohns Colitis 2015;9(8):685-91.

32. Hernández-Sampelayo P, Seoane M, Oltra L, et al. Contribution of nurses to the quality of care in management of inflammatory bowel disease: a synthesis of the evidence. J Crohns Colitis 2010;4(6):611–22.

33. Aguas Peris M, Del Hoyo J, Bebia P, et al. Telemedicine in inflammatory bowel disease: opportunities and approaches. Inflamm Bowel Dis 2015;21(2):392–9.

34. Hunter J, Claridge A, James S, et al. Improving outpatient services: the Southampton IBD virtual clinic. Postgraduate Medical Journal 2012;88(1042):487–91.

35. Weinshilboum R. Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. 2001. p. 601–5.

36. Moreau AC, Paul S, Del Tedesco E, et al. Association between 6-thioguanine nucleotides levels and clinical remission in inflammatory disease: a meta-analysis. Inflamm Bowel Dis 2014;20(3):464–71.

37. Haines ML, Ajlouni Y, Irving PM, et al. Clinical usefulness of therapeutic drug monitoring of thiopurines in patients with inadequately controlled inflammatory bowel disease. Inflamm Bowel Dis 2011;17(6):1301–7.

38. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33(9):987–95.

39. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003;348(7):601–8.

40. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. J Clin Pharmacol 2015;55 Suppl 3:S39–50.

41. Hwang C, Ross V, Mahadevan U. Micronutrient deficiencies in inflammatory bowel disease: From A to zinc. Inflamm Bowel Dis 2012;18(10):1961–81.

42. Rosenblum Chow BF, Condon GP, et al. Oral versus parenteral administration of Co60-labeled vitamin B12 to rats. J Biol Chem 1952;198(2):915–28.

43. Present DH, Korelitz BI, Wisch N, et al. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. N Eng J Med 1980;302(18):981–7.

44. Roblin X, Rinaudo M, Del Tedesco E, et al. Development of an Algorithm Incorporating Pharmacokinetics of Adalimumab in Inflammatory Bowel Diseases. Am J Gastroenterol 2014;109(8):1250–6.

45. Afif W, Loftus EV, Faubion WA, et al. Clinical Utility of Measuring Infliximab and Human Anti-Chimeric Antibody Concentrations in Patients With Inflammatory Bowel Disease. Am J Gastroenterol 2010;105(5):1133–9.

46. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148(7):1320–3.

47. D'haens G, Vermeire S, Lambrecht GL, et al. OP029. Drug-concentration versus symptom-driven dose adaptation of In iximab in patients with active Crohn's disease: a prospective, randomised, multicentre trial (Tailorix). J Crohns Colitis 2016;10(Suppl 1):S24–4.

48. Chande N, Tsoulis DJ, Macdonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. Cochrane Database Syst Rev 2013;(4):CD000545.

49. Timmer A, McDonald JWD, Tsoulis DJ, et al. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev 2012;(9):CD000478.

50. Heap GA, Weedon MN, Bewshea CM, et al. HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants. Nat Genet 2014;46(10):1131–4.

51. Kotlyar DS, Lewis JD, Beaugerie L, et al. Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: a meta-analysis. Clin Gastroenterol Hepatol 2015;13(5):847–58.

52. Peyrin-Biroulet L, Khosrotehrani K, Carrat F, et al. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. Gastroenterology 2011;141(5):1621–28.

53. Cosnes J, Bourrier A, Laharie D, et al. Early administration of azathioprine vs conventional management of Crohn's Disease: a randomized controlled trial. Gastroenterology 2013;145(4):758–65.

54. Panés J, López-Sanromán A, Bermejo F, et al. Early azathioprine therapy is no more effective than placebo for newly diagnosed Crohn's disease. Gastroenterology 2013;145(4):766–74.

55. Louis E, Irving P, Beaugerie L. Use of azathioprine in IBD: modern aspects of an old drug. Gut 2014;63(11):1695-99.

56. Panaccione R, Ghosh S, Middleton S, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. Gastroenterology 2014;146(2):392–3.

57. Dulai PS, Siegel CA, Colombel J-F, et al. Systematic review: Monotherapy with antitumour

necrosis factor α agents versus combination therapy with an immunosuppressive for IBD. Gut 2014;63(12):1843–53.

58. Feagan BG, McDonald JWD, Panaccione R, et al. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. Gastroenterology 2014;146(3):681–1.

59. Jones JL, Kaplan GG, Peyrin-Biroulet L, et al. Effects of Concomitant Immunomodulator Therapy on Efficacy and Safety of Anti-Tumor Necrosis Factor Therapy for Crohn's Disease: A Meta-analysis of Placebo-controlled Trials. Clin Gastroenterol Hepatol 2015;13(13):2233–40.

60. Vermeire S, Noman M, Van Assche G, et al. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. Gut 2007;56(9):1226–31.

61. Colombel J-F, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362(15):1383–95.

62. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut 2014;63(8):1258–64.

63. van Schaik T, Maljaars JPW, Roopram RK, et al. Influence of combination therapy with immune modulators on anti-TNF trough levels and antibodies in patients with IBD. Inflamm Bowel Dis 2014;20(12):2292–8.

64. Yarur AJ, Kubiliun MJ, Czul F, et al. Concentrations of 6-Thioguanine Nucleotide Correlate With Trough Levels of Infliximab in Patients With Inflammatory Bowel Disease on Combination Therapy. Clin Gastroenterol Hepatol 2015;13(6):1118–1124

65. Cahill J, Zadvornova Y, Naik AS, et al. Tu1282 Azothioprine or 6-Mercaptopurnine Dose Does Not Effect Serum Infliximab Level or Rate of Antibody to Infliximab Formation. Gastroenterology 2015;148(4):S–847–847.

66. Matsumoto T, Motoya K, Watanabe T, et al. Comparison of adalimumab monotherapy and a combination with azathioprine for patients with Crohn's disease: a prospective, multicentre, open-labelled clinical trial (DIAMOND study). J Crohns Colitis 2016;10(1):S8.

67. Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum 2006;54(1):26–37.

68. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004;350(9):876–85.

69. Bouguen G, Sninsky C, Tang KL, et al. Change in erythrocyte mean corpuscular volume during combination therapy with azathioprine and infliximab is associated with mucosal healing: a post hoc analysis from SONIC. Inflamm Bowel Dis 2015;21(3):606–14.

70. Harvey RF, Bradshaw MJ. Measuring Crohn's disease activity. Lancet 1980;1(8178):1134–5.

71. Cassileth PA, Kahn SB, Silber R, et al. Induction therapy and intensive consolidation with daunorubicin, cytosine arabinoside, and 6-thioguanine in adult acute nonlymphoblastic leukemia. Cancer Clin Trials 1981;4(2):125–8.

72. Derijks LJJ, Gilissen LPL, Engels LGJB, et al. Pharmacokinetics of 6-thioguanine in patients with inflammatory bowel disease. Ther Drug Monit 2006;28(1):45–50.

73. Dubinsky MC, Hassard PV, Seidman EG, et al. An open-label pilot study using thioguanine as a therapeutic alternative in Crohn's disease patients resistant to 6-mercaptopurine therapy. Inflamm Bowel Dis 2001;7(3):181–9.

74. Herrlinger KR, Deibert P, Schwab M, et al. Remission maintenance by tioguanine in chronic active Crohn's disease. Aliment Pharmacol Ther 2003;17(12):1459–64.

75. Dubinsky MC, Vasiliauskas EA, Singh H, et al. 6-thioguanine can cause serious liver injury in inflammatory bowel disease patients. Gastroenterology 2003;125(2):298–303.

76. Seiderer J, Zech CJ, Reinisch W, et al. A multicenter assessment of liver toxicity by MRI and biopsy in IBD patients on 6-thioguanine. J Hepatol 2005;43(2):303–9.

77. Musumba CO. Review article: the association between nodular regenerative hyperplasia, inflammatory bowel disease and thiopurine therapy. Aliment Pharmacol Ther 2013;38(9):1025–37.

78. van Asseldonk DP, Seinen ML, de Boer NKH, et al.Hepatotoxicity associated with 6-methyl mercaptopurine formation during azathioprine and 6-mercaptopurine therapy does not occur on the short-term during 6-thioguanine therapy in IBD treatment. J Crohns Colitis 2012;6(1):95–101.

79. Ansari A, Elliott T, Fong F, et al. Further experience with the use of 6-thioguanine in patients with Crohn's disease. Inflamm Bowel Dis 2008;14(10):1399–405.

80. Laharie D, Bourreille A, Branche J, et al. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. Lancet 2012;380(9857):1909–15.

81. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol 2006;4(10):1248–54.

82. Steenholdt C, Bendtzen K, Brynskov J, et al. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. Scand J Gastroenterol 2011;46(3):310–8.

83. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut 2010;59(1):49–54.

84. Paul S, Moreau AC, Del Tedesco E, et al. Pharmacokinetics of Adalimumab in

Inflammatory Bowel Diseases. Inflamm Bowel Dis 2014;20(7):1288–95.

85. Casteele NV, Gils A, Singh S, et al. Antibody Response to Infliximab and its Impact on Pharmacokinetics can be Transient. 2013;108(6):962-71.

86. Kopylov U, Mazor Y, Yavzori M, et al. Clinical utility of antihuman lambda chain-based enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. Inflamm Bowel Dis 2012;18(9):1628–33.

87. Van Stappen T, Billiet T, Vande Casteele N, et al. An Optimized Anti-infliximab Bridging Enzyme-linked Immunosorbent Assay for Harmonization of Anti-infliximab Antibody Titers in Patients with Inflammatory Bowel Diseases. Inflamm Bowel Dis 2015;21(9):2172–7.

88. Steenholdt C, Ainsworth MA, Tovey M, et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. Ther Drug Monit 2013;35(4):530–8.

89. Imaeda H, Takahashi K, Fujimoto T, et al. Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn’s disease. J Gastroenterol 2013;

90. Guiotto C, Daperno M, Frigerio F, et al. Clinical relevance and inter-test reliability of anti-infliximab antibodies and infliximab trough levels in patients with inflammatory bowel disease. Digestive and Liver Disease 2016;48(2):138–45.

91. Vande Casteele N, Buurman DJ, Sturkenboom MGG, et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. Aliment Pharmacol Ther 2012;36(8):765–71.

92. Schmitz EMH, van de Kerkhof D, Hamann D, et al. Therapeutic drug monitoring of infliximab: performance evaluation of three commercial ELISA kits. Clin Chem Lab Med 2016;54(7):1211–9.

93. Moore C, Corbett G, Moss AC. Systematic Review and Meta-Analysis: Serum Infliximab Levels During Maintenance Therapy and Outcomes in Inflammatory Bowel Disease. Journal of Crohn's and Colitis 2016;10(5):619–25.

94. Roblin X, Marotte H, Rinaudo M, et al. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol 2014;12(1):80–2.

95. Mazor Y, Almog R, Kopylov U, et al. Adalimumab drug and antibody levels as predictors of clinical and laboratory response in patients with Crohn's disease. Aliment Pharmacol Ther 2014;40(6):620–8.

96. Chiu Y-L, Rubin DT, Vermeire S, et al. Serum Adalimumab Concentration and Clinical Remission in Patients with Crohn’s Disease. Inflamm Bowel Dis 2013;19(6):1112–22.

97. Zittan E, Kabakchiev B, Milgrom R, et al. Higher Adalimumab Drug Levels are Associated with Mucosal Healing in Patients with Crohn's Disease. Journal of Crohn's and Colitis

2016;10(5):510–5.

98. Ungar B, Levy I, Yavne Y, et al. Optimizing Anti-TNF-α Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol 2016;14(4):550–2.

99. Nanda KS, Cheifetz AS, Moss AC. Impact of Antibodies to Infliximab on Clinical Outcomes and Serum Infliximab Levels in Patients With Inflammatory Bowel Disease (IBD): A Meta-Analysis. Am J Gastroenterol 2012;108(1):40–7.

100. Lee LYW, Sanderson JD, Irving PM. Anti-infliximab antibodies in inflammatory bowel disease: prevalence, infusion reactions, immunosuppression and response, a meta-analysis. Eur J Gastroenterol Hepatol 2012;24(9):1078–85.

101. Fasanmade AA, Adedokun OJ, Ford J, et al. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. Eur J Clin Pharmacol 2009;65(12):1211–28.

102. Dotan I, Ron Y, Yanai H, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. Inflamm Bowel Dis 2014;20(12):2247–59.

103. Vande Casteele N, Feagan BG, Gils A, et al. Therapeutic Drug Monitoring in Inflammatory Bowel Disease: Current State and Future Perspectives. Curr Gastroenterol Rep 2014;16(4):378.

104. Ordás I, Mould DR, Feagan BG, et al. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin Pharmacol Ther 2012;91(4):635–46.

105. Vaughn BP, Sandborn WJ, Cheifetz AS. Biologic Concentration Testing in Inflammatory Bowel Disease. Inflamm Bowel Dis 2015;21(6):1435–1442.

106. Lie MRKL, Peppelenbosch MP, West RL, et al. Adalimumab in Crohn's disease patients: pharmacokinetics in the first 6 months of treatment. Aliment Pharmacol Ther 2014;40(10):1202–8.

107. Dotan I, Yanai H, Ron Y, et al. Sa1250 Population Pharmacokinetic Evaluation of Adlimumab Reveals Patient Factors That Increase Adalimumab Clearance and Shorten Half-Life in Inflammatory Bowel Disease Patients. Gastroenterology 2014;146(5):S–243–243.

108. Paulson S, Noertersheuser P, Garimella T, et al. Assessment of relative bioavailability, safety, and tolerability of single doses of adalimumab administered via an autoinjector pen and a prefilled syringe. 2007;56(2):AB9–AB9.

109. Ding NS, 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 2016;43(1):30–51.

110. Gee MI, Grace MG, Wensel RH, et al. Protein-energy malnutrition in gastroenterology outpatients: increased risk in Crohn's disease. J Am Diet Assoc 1985;85(11):1466–74.

111. Goh J, O'Morain CA. Review article: nutrition and adult inflammatory bowel disease. Aliment Pharmacol Ther 2003;17(3):307–20.

112. Krok KL, Lichtenstein GR. Nutrition in Crohn disease. Curr Opin Gastroenterol 2003;19(2):148–53.

113. Sanz-Cuesta T, González-Escobar P, Riesgo-Fuertes R, et al. Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). BMC Public Health 2012;12:394.

114. Battat R, Kopylov U, Szilagyi A, et al. Vitamin B12 deficiency in inflammatory bowel disease: prevalence, risk factors, evaluation, and management. Inflamm Bowel Dis 2014;20(6):1120–8.

115. Headstrom PD, Rulyak SJ, Lee SD. Prevalence of and risk factors for vitamin B(12) deficiency in patients with Crohn's disease. Inflamm Bowel Dis 2008;14(2):217–23.

116. Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clin Chem 2007;53(5):963–70.

117. Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006;55(6):749–53.

118. Peyrin Biroulet L, Sandborn W, Sands BE, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol 2015;110(9):1324–38.

119. Osterman MT, Haynes K, Delzell E, et al. Effectiveness and Safety of Immunomodulators With Anti-Tumor Necrosis Factor Therapy in Crohn's Disease. Clin Gastroenterol Hepatol 2015;13(7):1293-1301.

120. Kreijne JE, Lie MRKL, Vogelaar L, et al. Practical Guideline for Fatigue Management in Inflammatory Bowel Disease. Journal of Crohn's and Colitis 2015;10(1):105–11.

121. Khan KJ, Dubinsky MC, Ford AC, et al. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 2011;106(4):630–42.

122. Prefontaine E, Sutherland LR, Macdonald JK, et al. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 2009;(1):CD000067.

123. Reenaers C, Louis E, Belaiche J, et al. Does co-treatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? Aliment Pharmacol Ther 2012;36(11-12):1040–8.

124. Blaker PA, Arenas-Hernandez M, Smith MA, et al. Biochemical Pharmacology. Biochem Pharmacol 2013;86(4):539–47.

125. Papamichael K, Van Stappen T, Vande Casteele N, et al. Infliximab Concentration Thresholds During Induction Therapy Are Associated With Short-term Mucosal Healing in Patients With Ulcerative Colitis. Clin Gastroenterol Hepatol 2016;14(4):543–9.

126. Yarur AJ, Jain A, Sussman DA, et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. Gut 2016;65(2):249–55.

127. Brandse JF, van den Brink GR, Wildenberg ME, et al. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. Gastroenterology 2015;149(2):350–2.

128. Katz L, Gisbert JP, Manoogian B, et al. Doubling the infliximab dose versus halving the infusion intervals in Crohn's disease patients with loss of response. Inflamm Bowel Dis 2012;18(11):2026–33.

129. Fasanmade AA, Adedokun OJ, Blank M, et al. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. Clin Ther 2011;33(7):946–64.

130. Ternant D, Aubourg A, Magdelaine-Beuzelin C, et al. Infliximab pharmacokinetics in inflammatory bowel disease patients. Ther Drug Monit 2008;30(4):523–9.

131. Mould DR, Dubinsky MC. Dashboard systems: Pharmacokinetic/pharmacodynamic mediated dose optimization for monoclonal antibodies. J Clin Pharmacol 2015;55 Suppl 3:S51–9.

132. Moreno-Otero R, García-Buey L, Trapero-Marugán M. Safety of methotrexate for inflammatory bowel disease. Dig Liver Dis 2012;44(8):706–7.

133. Herrlinger KR, Kreisel W, Schwab M, et al. 6-thioguanine--efficacy and safety in chronic active Crohn's disease. Aliment Pharmacol Ther 2003;17(4):503–8.

134. Derijks LJJ, de Jong DJ, Gilissen LPL, et al. 6-Thioguanine seems promising in azathioprine- or 6-mercaptopurine-intolerant inflammatory bowel disease patients: a short-term safety assessment. Eur J Gastroenterol Hepatol 2003;15(1):63–7.

135. de Boer NKH, Reinisch W, Teml A, et al. 6-Thioguanine treatment in inflammatory bowel disease: a critical appraisal by a European 6-TG working party. Digestion 2006;73(1):25–31.

136. de Boer NKH, van Asseldonk DP, van Bodegraven A. ECCO consensus: evidence-based use of 6-thioguanine therapy in Crohn's disease? J Crohns Colitis 2010;4(4):484–5.

137. de Boer NKH, Zondervan PE, Gilissen LPL, et al. Absence of nodular regenerative hyperplasia after low-dose 6-thioguanine maintenance therapy in inflammatory bowel disease patients. Digestive and Liver Disease 2008;40(2):108–13.

138. Almer SHC, Hjortswang H, Hindorf U. 6-Thioguanine therapy in Crohn's disease--observational data in Swedish patients. Dig Liver Dis 2009;41(3):194–200.

139. Vernier-Massouille G, Cosnes J, Lemann M, et al. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. Gut 2007;56(10):1404–9.

140. de Boer NKH, Tuynman H, Bloemena E, et al. Histopathology of liver biopsies from a thiopurine-naïve inflammatory bowel disease cohort: prevalence of nodular regenerative hyperplasia. Scand J Gastroenterol 2008;43(5):604–8.

141. Fernandez-Banares F, Abad-Lacruz A, Xiol X, et al. Vitamin status in patients with inflammatory bowel disease. Am J Gastroenterol 1989;84(7):744–8.

142. Hvas AM, Nexo E. Holotranscobalamin--a first choice assay for diagnosing early vitamin B deficiency? J Intern Med 2005;257(3):289–98.

143. Sobczyńska-Malefora A, Gorska R, Pelisser M, et al.An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. Clin Biochem 2014;47(1-2):82–6.