Predicting anti-TNF treatment failure in patients with inflammatory bowel disease

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Submitted by Neil Chanchlani, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Medical Studies, September 2023.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Neil Chanchlani

Abstract

About one-third of patients with inflammatory bowel disease (IBD) are treated with anti-TNF therapy. Unfortunately, treatment failure is common, and only about one-third of patients continue to take anti-TNF therapy treatment one year after starting treatment. Early identification of patients at-risk of treatment failure may help direct anti-TNF treatment monitoring, early dose optimisation, and use of mitigating strategies to allow these drugs to be used in a safer, more cost-effective manner.

The aim of this thesis was to identify and explore multiple patient, disease, and drug related factors that have been implicated in anti-TNF treatment failure, and identify management strategies in the setting of treatment failure.

Chapter 1 provides an introduction to the diagnosis, investigation, and treatment of IBD. I provide an overview of treatment paradigms when managing IBD, including how to define anti-TNF treatment failure and what management options are available in clinical practice.

Chapter 2 provides an overview of the generic methodology used throughout the thesis, including my role within each study, laboratory work, data collection, cleaning, and management steps, and statistical analyses undertaken.

Chapter 3 describes an independent calibration of infliximab and adalimumab anti-drug antibody positivity thresholds, using a drug-tolerant assay. I newly defined thresholds from healthy controls to explore associations with anti-TNF drug level and treatment outcomes in a large therapeutic drug monitoring (TDM) service

cohort and patients recruited to the Personalised Anti-TNF Therapy in Crohn's disease (PANTS) study.

Chapter 4 explored the association of serum free (f) triiodothyronine-to-thyroxine (fT3/fT4) ratio and treatment failure to anti-TNF therapy in patients recruited to the PANTS study. I found that lower baseline serum fT3/fT4 was associated with treatment failure at week 14 of treatment, but not at the end of the first year.

Chapter 5 assessed whether pre-treatment 25-hydroxyvitamin D concentrations predicted treatment failure to infliximab and adalimumab in patients recruited to the PANTS study. Although Vitamin D deficiency was a common finding amongst patients with active Crohn's disease, unlike previous studies, I was unable to demonstrate that pre-treatment vitamin D concentrations were predictive of treatment failure throughout the first year of treatment.

Chapter 6 reports data from the two-year extension to the PANTS study, including the effectiveness of infliximab and adalimumab at two and three years. I found that loss of response to anti-TNF therapy during three years of treatment was common, and was most likely to occur during the first year of treatment secondary to low anti-TNF drug concentration. I also described how clinicians managed patients with loss of response and how effective those actions were.

Chapter 7 describes a UK-wide, multicentre retrospective cohort study reporting rates of anti-TNF antibody development and treatment failure of second-line anti-TNF therapies. I found, irrespective of drug sequence, development of anti-TNF

antibodies to the first anti-TNF was associated with development of anti-TNF antibodies to the second anti-TNF. In patients with low anti-TNF drug concentrations and presence of antibodies to their first anti-TNF therapy, introduction of an immunomodulator led to increased drug persistence to second anti-TNF therapy, compared to patients who had adequate anti-TNF drug concentrations in the absence of anti-TNF antibodies.

The work carried out in Chapter 8 was performed during the COVID-19 pandemic. In this experiment, I assessed whether anti-TNF therapy attenuates serological responses to SARS-CoV-2 infection. I found that anti-TNF treatment was associated with lower SARS-CoV-2 nucleocapsid seroprevalence and antibody reactivity when compared to vedolizumab-treated patients. Compared to patients with detectable anti-TNF drug concentrations, patients with undetectable drug concentrations were more likely to be seropositive for SARS-CoV-2 antibodies, potentially supporting a causal relationship.

An overview of the major findings of each chapter, their implications, and potential future research is discussed in Chapter 9.

Publications in this thesis

*denotes equal contribution

Chapter 3. Nice R*, **Chanchlani N***, Green H, Bewshea C, Ahmad T, Goodhand JR, McDonald TJ, Perry MH*, Kennedy NA*. Validating the positivity thresholds of anti-infliximab and anti-adalimumab drug-tolerant assays. Aliment Pharmacol Ther 2020 Nov 23.

Chapter 4. Lin S*, **Chanchlani N***, Carbery I*, Janjua M, Nice R, McDonald TJ, Bewshea C, Kennedy NA, Ahmad T, Selinger CP, Goodhand JR; PANTS consortium. Understanding anti-TNF treatment failure: Does serum triiodothyronine-to-thyroxine (T3/T4) ratio predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease? Aliment Pharmacol Ther. 2022 Sep;56(5):783-793.

Chapter 5. **Chanchlani N***, Lin S*, Smith R, Roberts C, Nice R, McDonald TJ, Hamilton B, Bishara M, Bewshea C, Kennedy NA, Goodhand JR, Ahmad T. Pretreatment Vitamin D Concentrations Do Not Predict Therapeutic Outcome to Anti-TNF Therapies in Biologic-Naïve Patients With Active Luminal Crohn's Disease. Crohns Colitis 360. 2023 May 15;5(3):otad026. doi: 10.1093/crocol/otad026.

Chapter 6. **Chanchlani N**, Lin S, Bewshea C, Hamilton B, Thomas A, Smith R, Roberts C, Bishara M, Nice R, Lees CW, Sebastian S, Irving PM, Lindsay JO, Russell RK, McDonald TJ, Goodhand JR, Ahmad T,

Kennedy NA; PANTS Consortium. Understanding the mechanisms and management of loss of response to anti-TNF therapy: three-year data from the PANTS study. Accepted and in press at *The Lancet Gastroenterology and Hepatology*.

Chapter 7. **Chanchlani N**, Lin S, Auth MK, Lee CL, Robbins H, Looi S, Murugesan SV, Riley T, Preston C, Stephenson S, Cardozo W, Sonwalkar SA, Allah-Ditta M, Mansfield L, Durai D, Baker M, London I, London E, Gupta S, Di Mambro A, Murphy A, Gaynor E, Jones KDJ, Claridge A, Sebastian S, Ramachandran S, Selinger CP, Borg-Bartolo SP, Knight P, Sprakes MB, Burton J, Kane P, Lupton S, Fletcher A, Gaya DR, Colbert R, Seenan JP, MacDonald J, Lynch L, McLachlan I, Shields S, Hansen R, Gervais L, Jere M, Akhtar M, Black K, Henderson P, Russell RK, Lees CW, Derikx LAAP, Lockett M, Betteridge F, De Silva A, Hussenbux A, Beckly J, Bendall O, Hart JW, Thomas A, Hamilton B, Gordon C, Chee D, McDonald TJ, Nice R, Parkinson M, Gardner-Thorpe H, Butterworth JR, Javed A, Al-Shakhshir S, Yadagiri R, Maher S, Pollok RCG, Ng T, Appiahene P, Donovan F, Lok J, Chandy R, Jagdish R, Baig D, Mahmood Z, Marsh L, Moss A, Abdulgader A, Kitchin A, Walker GJ, George B, Lim YH, Gulliver J, Bloom S, Theaker H, Carlson S, Cummings JRF, Livingstone R, Beale A, Carter JO, Bell A, Coulter A, Snook J, Stone H, Kennedy NA, Goodhand JR, Ahmad T; IMSAT study investigators. Implications for sequencing of biologic therapy and choice of second anti-TNF in patients with inflammatory bowel disease: Results from

the IMmunogenicity to Second Anti-TNF therapy (IMSAT) therapeutic drug monitoring study. Aliment Pharmacol Ther. 2022 Oct;56(8):1250-1263.

Chapter 8. **Chanchlani N***, Lin S*, Chee D, Hamilton B, Nice R, Zehra A, Bewshea C, Cipriano B, Derikx LAAP, Dunlop A, Greathead L, Griffiths RL, Ibraheim H, Kelleher P, Kok KB, Lees CW, MacDonald J, Sebastian S, Smith PJ, McDonald TJ, Irving PM, Powell N, Kennedy NA, Goodhand JR, Ahmad T. Adalimumab and infliximab impair SARS-CoV-2 antibody responses: results from a therapeutic drug monitoring study in 11422 biologic-treated patients. J Crohns Colitis. 2021 Sep 2:jjab153.

COVID impact statement

The COVID-19 pandemic outbreak started in March 2020, during the second year of my doctoral studies. As a result:

1) My primary funder, the patient charity *Crohn's & Colitis UK,* temporarily paused expenditure on research, including my salary/funding withdrew my funding due to significant income drop and unpredictable financial forecast. Therefore, I took an interruption of my doctoral studies for 5 months (April – September 2020) to re-enter clinical training and assist with high clinical workload/pressures at the Royal Devon University Healthcare NHS Foundation Trust (Exeter) during this time period. I restarted my doctoral studies in September 2020.
2) Two of my six pre-planned projects ceased, due to inability to recruit and consent patients face-to-face and obtain biological research samples, closure of university lab space to process patient samples, and changes in hospital-, university-, and national health research authority policy to prioritise COVID-related research only. This affected the following experiments: a) Validating the genetic association of HLA-DQA1*05 and immunogenicity in a non-IBD cohort and b) Identifying novel, and validating postulated, proteomic biomarkers to predict response to anti-TNF therapy.
3) I adapted my project plan and thesis to instead lead an experiment related to the Impact of Biologic and Immunomodulatory Therapy on SARS-CoV-2 Infection and Immunity in Patients with Inflammatory Bowel Disease study (Chapter 8 of my thesis). This publication informed international guidance and public policy related to COVID-19 vaccination (50 citations to date).

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List of commonly used abbreviations

IBD	Inflammatory bowel disease
UC	Ulcerative colitis
IBDU	Inflammatory bowel disease unclassified
STRIDE	Selecting Therapeutic Targets in Inflammatory Bowel Disease program
PCDAI	Paediatric Crohn's Disease Activity Index
PUCAI	Paediatric Ulcerative Colitis Activity Index
SES-CD	Simple Endoscopic Score Crohn's disease
TDM	Therapeutic drug monitoring
RCT	Randomised controlled trial
5-ASA	Aminosalicylates
TNF	Tumour necrosis factor
IL	Interleukin
ELISA	Enzyme-linked immunosorbent assay
PANTS	Personalised anti-TNF therapy in Crohn's disease study
AUC	Area under the receiver operating characteristic curve
ACE2	Angiotensin-converting enzyme-2
RR	Relative risk
HR	Hazard ratio
COVID-19	Coronavirus disease 2019
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SECURE-IBD	Surveillance Epidemiology of Coronavirus Under Research Exclusion study

Chapter 1: Introduction

Inflammatory bowel disease (IBD) encompasses a group of chronic, immune-mediated, relapsing-remitting conditions that affect the gastrointestinal tract. They consist of three conditions: Crohn's disease, where transmural inflammation affects any part of the gastrointestinal tract, ulcerative colitis (UC), where superficial inflammation usually affects the rectum and colon only, and inflammatory bowel disease unclassified (IBDU), where characteristics of both Crohn's disease and UC are present [1].

1.1 Epidemiology

In 2017, 6.8 million cases of IBD were reported globally [2], and by 2030, about 1% of people living in high-income countries are anticipated to have IBD [3,4]. The prevalence and incidence of IBD is increasing in both high- and low-middle income countries, but particularly across North America and Western Europe. In one systematic review of 147 population-based studies investigating the incidence of IBD from 1990 to 2015, the highest reported prevalence values for Crohn's disease were in Germany (322/100 000 persons), Canada (319/100 000 persons), and for UC were in Norway (505/100 000 persons) and USA (286/100 000 persons) [5]. Similar prevalence rates have been reported across the United Kingdom [3,4]. Countries in Africa, America, and South America are also seeing a rapid rise in annual incidence of IBD [5–7]. For example, in Bahrain (from 3 to 12/100 000 persons from 1984 to 2014) and Malaysia (from 0.36 to 1.46/100 000 persons from the 1980s to 2018) [7]. Factors associated with the increases in global incidence and prevalence include the development of standardised case definitions and reporting systems and increased availability of resources that aid diagnosis [6–8].

Our understanding of genetic, dietary, and lifestyle risk factors associated with the development of IBD has also changed substantially over the past two decades.

1.2. Aetiology

The aetiology of IBD remains unknown, but multiple complex interactions between genetic, environmental, and microbiome-related factors are hypothesised to contribute to developing the disease [9]. Together, these factors lead to aberrant host immune responses across the intestinal epithelium, including immunodysregulation with respect to host autophagy, T-cell response, and epithelial barrier function [1,10].

About 4 - 13% of patients with IBD have a family history, suggesting a genetic risk for development [11–13]. Trans-ancestral genome-wide association studies have identified over 240 loci related to IBD risk, with over 45 associations to a single causal variant [14–16]. Onward large-scale sequencing analyses from a cohort of 120 000 patients with Crohn's disease and population controls have identified 10 genes and rare variants [17], further cementing the role of host genetics in susceptibility to developing Crohn's disease.

Multiple environmental risk factors have been identified to be associated with the development of IBD, largely through case-control studies, and few have been rigorously evaluated in prospective cohorts. In one systematic review of 53 meta-analyses of 71 environmental risk factors, 9 were concluded to increase risk of IBD (smoking, urban living, appendectomy, tonsillectomy, antibiotic exposure, oral contraceptive use, consumption of soft drinks, vitamin D deficiency, and non-Helicobacter pylori-like enterohepatic Helicobacter species), and 7 concluded to decrease risk (physical activity, breastfeeding, bed sharing, tea consumption, high levels of folate, high levels of vitamin D, and *Helicobacter pylori* infection) [18].

Understanding the independent effects of environmental factors on development of IBD remains a challenge, and on ongoing area of research, particularly administration of antibiotics in early-life, diet, and impact of mental health and lifestyle [9,19].

Of all the risk factors associated with IBD, our understanding of the role of host microbiome and risk of IBD has advanced most substantially. The diversity and composition of the microbiota within the gut microbiome has been found to be often imbalanced in patients with IBD; 'dysbiosis' [20]. Studies evaluating impact of genetics, immunity, and diet on commensal gut bacteria, increasing metabolic dysbiosis (bacterial proteases, short-chain fatty acid production, tryptophan biosynthesis), taxonomic dysbiosis (*Bilophila wadsworthia, Akkermansia muciniphila*), and presence of pathobionts have shed new light on potential interaction networks of the intestinal epithelium [21–23]. In one prospective cohort study of 3483 health first-degree relatives of patients with Crohn's disease, five taxa in the human microbiome, *Ruminococcus torques, Blautia, Colidextribacter*, an uncultured genus-level group from *Oscillospiraceace,* and *Roseburia*, were identified to be associated with future onset of Crohn's disease [24].

1.3 Diagnosis

Diagnosis of IBD is based on a combination of clinical history, baseline tests including bloods and faecal calprotectin, endoscopy, and imaging.

Patients classically present with at least a six-week history of abdominal pain, bloody diarrhoea in UC, bloody or non-bloody diarrhoea in Crohn's disease, and weight loss. Other non-classic symptoms can also be present, and may be associated with longer time to diagnosis. Extra-intestinal manifestations occur in about one-quarter patients with IBD, and occur more commonly in patients with Crohn's disease than UC [25]. They include skin (erythema nodosum, pyogenic gangrenosum, oral ulceration), eye (uveitis, episcleritis), joint (arthritis, ankylosing spondylitis), and liver involvement (autoimmune liver disease or primary sclerosing cholangitis).

Although there is no single test to diagnose IBD, initial investigations suggestive of the condition include anaemia, which may be microcytic, thrombocytosis, and increased markers of inflammation (platelets, C-reactive protein, erythrocyte sedimentation rate, and plasma viscosity), and reduced albumin [26]. Faecal calprotectin, a neutrophil protein biomarker which measures intestinal inflammation, has now been incorporated in international guidelines to aid diagnosis of IBD, and a cut-off value of >250 ug/g is considered elevated in older children and adults [27,28].

In the setting of a clinically suggestive history, raised inflammatory markers, and elevated faecal calprotectin, healthcare professionals should refer patients for

diagnostic evaluation of IBD. Upper and lower gastrointestinal endoscopy, accompanied by histology consistent with IBD, remains the gold standard for diagnosing IBD. Adjunctive imaging, such as with computed tomography and small bowel magnetic resonance, may help determine presence of inflammation in areas inaccessible by endoscopy.

Once a diagnosis is confirmed, patients are stratified by disease phenotype, with the most common classification for both Crohn's disease and UC being the Montreal classification [29].

1.4 Assessment

Disease assessment (including in response to treatment) can be assessed crudely by interpreting the subjective reporting of patient symptoms and treating physician's clinical assessment. This information can be supplied to enumerate a physician global assessment or more formally, a validated disease activity score. Most scores incorporate a range of patient- and physician- reported information including severity of number of stools, abdominal pain, general well-being, and presence of extraintestinal manifestations. A pre-defined cut-off score will then categorise the patient as having responded to treatment, or having active disease, with further stratification of mild, moderate, or severe disease. The most common scores used in clinical practice are Harvey-Bradshaw Index [30] and Crohn's disease activity index (CDAI) [30] for Crohn's disease, and the Mayo score [31] and simple clinical colitis activity index [32] for UC. On their own, disease activity scores are poor predictors, with suboptimal sensitivity and specificity, for the presence of inflammation on endoscopic assessment [33–35].

More objectively, as at time of diagnosis, inflammatory burden can be assessed using a combination of patient-reported symptoms, clinical assessment, blood tests, and faecal calprotectin. Compared with patient or clinician assessment alone, incorporation of these additional variables to validated assessment tools improves the diagnostic accuracy of predicting intestinal inflammation substantially [36,37]. The use of endoscopy to assess treatment response remains the gold standard but is highly resource-intensive and impractical to perform routinely across most healthcare settings.

Continued areas of debate in clinical practice are the standardisation of subjective definitions across different healthcare settings from different clinicians, and implementing the same definitions from clinical research to the ‘real-world’ settings. Without overcoming these challenges, there continues to be substantial limitation in defining estimates of treatment response and failure accurately.

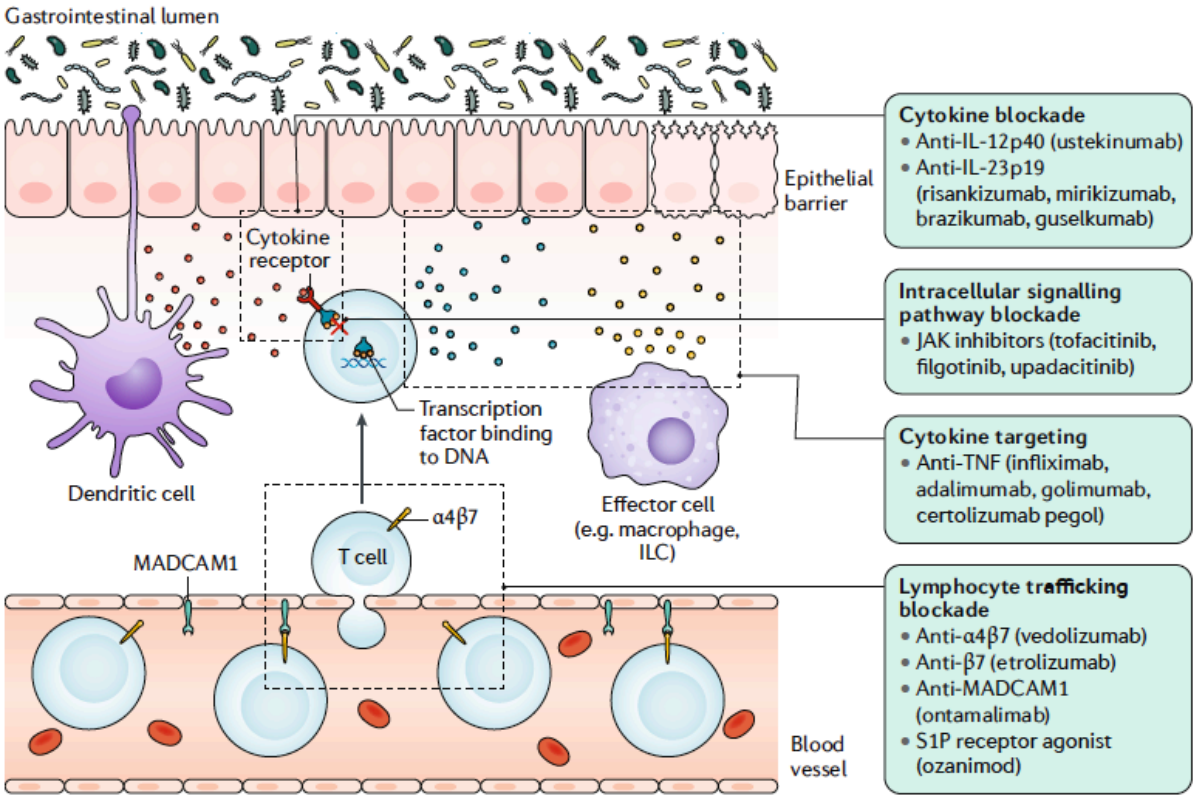
1.5 Treatment

Management of IBD consists of lifestyle modification, dietary manipulation, pharmacological therapy, and surgery. Adapting treatment to the patient's needs and preferences is imperative to treatment success and achieving disease remission.

Pharmacological therapies for IBD have traditionally been limited to dietary therapy, aminosalicylates, corticosteroids, immunomodulators, and anti-TNF therapies. The last decade, however, has seen a substantial increase in the number of potential cellular and molecular targets for IBD therapies (Figure 1), including newer biologics (veodolizumab, ustekinumab) as well as small molecule therapies (upadacitinib and tofacitinib) [38].

Historically, surgery has been reserved for patients whose IBD has been refractory to medical therapies or complicated, such as the development of haemorrhage, fibrosis, abscess, fistula, or infection [39,40]. However, recent evidence supports early surgical intervention in isolated, limited, and uncomplicated disease, particularly in patients who may have been unsuccessful with medical therapies [41,42]. As a result, there is an increased tendency to discuss surgical intervention for IBD early in the diagnostic and treatment course [43].

Figure 1: Drug targets in IBD [38]. Drug targets in IBD can be broadly separated into: cytokine blockade (for example, IL-12p40 with ustekinumab, IL-23p19 with risankizumab, mirikizumab, guselkumab or brazikumab) T cell intracellular signalling pathway blockade (for example, JAK inhibitors such as tofacitinib, folgotinib or upadacitinib) cytokine targeting (for example, anti- TNF agents such as infliximab, adalimumab, golimumab or certolizumab pegol) and lymphocyte trafficking blockade (anti- α4β7 agents such as vedolizumab, anti- β7 agents such as trolizumab, anti- mucosal addressin cell adhesion molecule 1 (MADCAM1) agents such as PF-00547659 and sphingosine 1-phosphate (S1P) receptor antagonists such as ozanimod). ILC innate lymphoid cell.



1.5.1 Dietary therapy

Diet is a modifiable risk factor for IBD onset and severity [44]. It is currently incorporated in treatment algorithms for Crohn's disease, either as a first-line, as for paediatric patients, or as adjunctive nutritional support alongside other medical therapies. The overall aim of dietary manipulation is to reduce intestinal inflammation and restore eubiosis. A range of dietary interventions can be initiated to do this, including exclusive enteral nutrition, partial enteral nutrition, and different elimination diets which contain a variety of included and excluded food groups depending on the diet. However, high-quality, long-term data assessing the efficacy of dietary therapies on reducing intestinal inflammation remains sparse. A 2019 Cochrane systematic review of 18 randomised controlled trials (RCTs) assessing 1878 patients with IBD found that the effects of dietary interventions for the induction and maintenance of remission of IBD remained uncertain and no firm conclusions could be drawn, in part due to high risk of bias and heterogeneity across studies [45]. A more recent systematic review and meta-analysis of 27 prospective controlled trials of solid food diets for the induction or maintenance of remission of IBD found that there may be a potential benefit with partial enteral nutrition when combined with selected dietary components for Crohn's disease, however certainty of evidence remains variable depending on the intervention studied [45].

1.5.2 Aminosalicylates

For UC, treatment with aminosalicylates (5-ASA) is often used first-line to induce or maintain remission of mild to moderate disease. Topical preparation at the site of inflammation, often administered rectally, is preferred, and has reduced systemic

absorption [46]. Multiple mechanisms of action have been proposed including activation of a peroxisome proliferator-activated receptor-gamma that transrepresses several genes responsible for inflammation in the colonic mucosa, and inhibition of inflammatory mediators including lipoxygenase and cyclooxygenase, interleukin(IL)-1, IL-2 and tumour necrosis factor-alpha (TNFa) [46]. In one systematic review and network meta-analysis of 40 RCTs for induction of remission in UC, topical preparation with mesalazine ranked first for clinical and endoscopic remission combined, followed by oral and topical mesalazine [47].

1.5.3 Corticosteroids

Corticosteroids are effective for inducing clinical remission in IBD, however they do not maintain remission and are associated with multiple adverse events. They can be administered orally, rectally, or systemically via intravenous administration. They have significant immunosuppressive effects and work by binding to the glucocorticoid receptor, where it regulates gene expression and reduces the production of pro-inflammatory cytokines (IL-1 beta, TNFa, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor, in addition to other ILs and interferon-gamma [48]. Systematic reviews of RCTs have demonstrated that corticosteroids are effective in reducing remission in both UC and Crohn's disease in children and adults [49–51]. Although steroids are effective, between 15 to 40% of IBD patients in the UK prescribed corticosteroids are thought to have excess steroid exposure [52]. Excess steroid exposure can lead to steroid dependence and increased adverse events, including venous thromboembolism, fragility fractures, and infections [53].

1.5.4 Immunomodulators

Immunomodulators, inclusive of thiopurines (mercaptopurine, azathioprine) and methotrexate, are used in the treatment of mild to moderate IBD. They work by blocking de novo pathways of purine synthesis to modify the response of the immune system in response to inflammation [54]. Thiopurines act by inhibiting lymphocyte proliferation via the incorporation of active drug metabolites into cellular nucleotides, supressing T cell function and natural killer cell activity, whereas methotrexate interrupts DNA synthesis and increases adenosine, inhibiting IL-1, and supressing T-cell function [55]. Multiple meta-analyses have demonstrated the effectiveness of thiopurines for the induction and maintenance of remission for Crohn's Disease [56–58]. For UC, patients who have failed or cannot tolerate 5-ASA, or those who require multiple courses of steroids, are more likely to benefit from thiopurines to induce and maintain remission [59–61]. The use of methotrexate as an induction agent for UC is less well established than for its use as an agent to maintain remission, and data, overall, are more low quality than for that of thiopurines. Use of methotrexate to induce and maintain remission in Crohn's Disease is well established and efficacious [62,63], whereas use in UC continues to be debated [64,65].

1.5.5 Biological therapies

Multiple biological therapies are used for the induction and maintenance of both moderate to severely active Crohn's disease and UC. These are large monoclonal antibodies that inhibit the proinflammatory cascade, and they inhibit activation and proliferation of T cells throughout the gastrointestinal tract [66]. Anti-TNF therapies, inclusive of infliximab and adalimumab, have garnered the most amount of

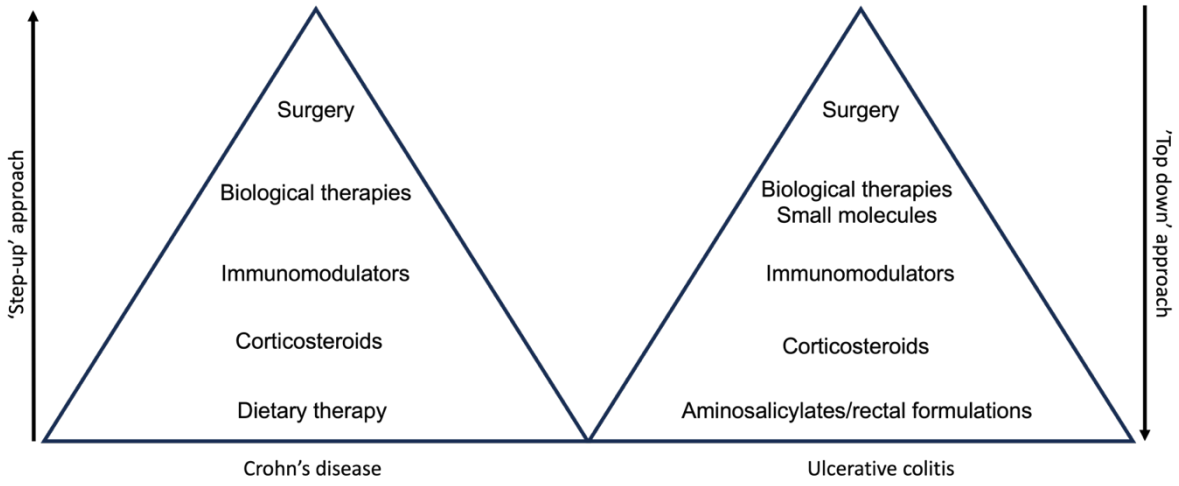
evidence, and our understanding of their efficacy and safety profile has advanced substantially over the last two decades. Anti-TNF therapies are efficacious and cost-effective for the induction and maintenance of remission for both Crohn's disease [67–69] and UC [70,71].

Other recently licensed biologics for both Crohn's disease and UC include vedolizumab, a gut-selective anti-integrin α4β7 monoclonal antibody [72,73], ustekinumab, an IL-12 and IL-23 inhibitor [74,75].

1.5.6 Paradigms

Recently, there has been a shift towards 'personalised' medicine for patients with IBD, with a focus on tailoring treatment strategy based on prediction of disease course, suspected prognosis, and risk of adverse events from medical therapies to an individual. Previously patients may be offered medical therapies in a prescriptive or formulaic fashion, often by starting with least-intensive option and 'stepping up' to more immunosuppressive medications [76]. Now, where appropriate, patients are being offered a 'top down' approach to treatment, whereby more-intensive and immunosuppressive therapies are offered as initial treatment in an effort to induce and maintain disease remission more effectively compared to the traditional 'step up' approach (Figure 2). Most recently included in these treatment algorithms is surgical resection, previously considered a last-resort, as initial treatment for patients with isolated Crohn's disease not-responsive to medical therapy [41,42].

Figure 2: Treatment paradigms for IBD [76].



1.5.7 Goals

There is no cure for IBD, but with appropriate treatment, patients can enter disease remission, whereby they are not symptomatic and the disease is inactive. Overall, the goal of IBD treatments is to reduce or remove inflammation, and prevent disease progression.

Remission has historically been defined as absence of patient-reported symptoms of IBD [77]. The expansion of the term to be subdivided to incorporate different features of assessment (clinical symptoms, biochemical results, and biopsy results) has been a welcome one to allow for a more objective assessment of disease state, where possible. In recent years, absence of patient-reported symptoms has been re-branded to 'clinical remission.' Normalisation of serological markers of inflammation and faecal calprotectin is now termed 'biochemical remission,' with normal macroscopic and microscopic appearances on endoscopy and biopsy being termed 'mucosal remission.' Not all three criteria need to be satisfied to be

considered, and depending on the clinical assessor, patients may be classified as in a state of non-remission if they are in clinical remission, but not biochemical or mucosal remission, for example.

Over the last decade, it has become increasingly accepted that a 'treat-to-target' approach should be applied and discussed with patients at the point of diagnosis. Treat-to-target aims to achieve disease remission by adjusting therapy according to achievement of predefined treatment response targets [78]. In 2015, the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) program was initiated by the International Organization for the Study of Inflammatory Bowel Diseases in order to examine potential treatment targets for IBD to be used for a treat-to-target clinical management strategy [79,80]. Patient acceptability of a treat-to-target approach is high, and in one UK cohort of patients in clinical remission, two-thirds agreed with a treat-to-target approach to remission of clinical symptoms and absence of mucosal inflammation [81].

For both UC and Crohn's disease, expert consensus agree clinical/patient-reported outcome remission combined with endoscopic assessment as the ideal treat-to-target outcomes [79,80]. For UC, clinical/patient-reported outcome remission was defined as resolution of rectal bleeding and diarrhoea/altered bowel habit, and for Crohn's disease, as resolution of abdominal pain and diarrhoea/altered bowel habit. Endoscopic assessment healing was considered a long-term target for both UC and Crohn's disease, whereas histologic or transmural remission were not for either disease, but were considered adjunctive targets (Table 1). In children, restoration of normal growth was considered a long-term treatment target.

Table 1: Selected Recommendations for Treating to Target in Crohn's disease and UC by the International Organization For the Study of IBD [80].

	Crohn's disease	**Ulcerative colitis**
Clinical (response)	Decrease of at least 50% in patient-reported outcome-2 (abdominal pain and stool frequency), and in children decrease in Paediatric Crohn's Disease Activity Score (PCDAI) of at least 12.5 points and in weighted PCDAI at least 17.5 points	Decrease of at least 50% in patient-reported outcome-2 (rectal bleeding and stool frequency), and in children decrease in Paediatric Ulcerative Colitis Activity Index (PUCAI) of at least 20 points
Clinical (remission)	Patient-reported outcome-2 abdominal pain $\leq$ 1 and stool frequency $\leq$ 3) or Harvey Bradshaw Index <5; in children by PCDAI (<10 points or <7.5 excluding the height item) or weighted PCDAI (<12.5 points)	Patient-reported outcome-2 (rectal bleeding = 0 and stool frequency = 0) or partial Mayo (<3 and no score >1), and in children PUCAI <10 points

Endoscopic/transmural assessment	Simple Endoscopic Score – Crohn's Disease (SES-CD) <3 points or absence of ulcerations (e.g. SES-CD ulceration subscores = 0)	Mayo endoscopic subscore = 0 points, or Ulcerative Colitis Endoscopic Index of Severity ≤ 1 points
Biomarkers	Normalisation of C-reactive protein (to values under the upper limit of normal) and faecal calprotectin (to 100–250 mg/g)	

The evidence underpinning the STRIDE recommendations, in particular the recommendations regarding mucosal healing, are predominantly based on indirect evidence from retrospective studies [82]. The authors acknowledge that implementation of a treat-to-target approach may not necessarily lead to an altered disease course, and the direct impact of treat-to-target on clinical short-term and long-term outcomes remains debated, although high-quality prospective trials (NCT01698307, NCT04259138) are currently evaluating these outcomes [82].

One systematic review identified a treat-to-target approach being associated with clinical remission [83], mucosal healing [84,85], and improved quality of life [86,87] compared to conventional clinical management. This was further supported by short- and long-term data from the CALM trial which evaluated endoscopic, clinical, and cost-effectiveness outcomes in patients with Crohn's disease receiving tight control versus clinical management alone [88,89]. In CALM, an open-label, multicentre RCT, 244 patients were randomly assigned to clinical management

(CDAI and prednisolone use) versus tight management (faecal calprotectin, C-reactive protein, CDAI, and prednisolone use). At week 48, patients in the tight control group were more likely to achieve mucosal healing with absence of deep ulcers compared to patients in the clinical management group (adjusted risk difference 16.1% [95%CI 3.9 - 28.3], p = 0.01). Three-year follow up data from the CALM study concluded that induction of deep remission early during the disease course was associated with decreased risk of progression, irrespective of tight control or conventional management therapy [90].

However, the cost-effectiveness data analyses from CALM concluded a tight control strategy was more effective than clinical management alone strategy, resulting in higher remission rates, fewer Crohn's-disease hospitalisations, and higher quality-adjusted life-years [82]. Savings were more pronounced when costs related to work productivity were incorporated.

Strategies supporting a treat-to-target approach include [82]:

a. management approach in which treatment decisions are based on close monitoring of outcome measures, such as faecal calprotectin and C-reactive protein
b. coordination of care by a multidisciplinary team, including the patient, and in instances where this cannot be done locally, remote monitoring and telemedicine may help
c. adherence to management regimens, and employing patient-friendly initiatives to improve concordance
d. therapeutic drug monitoring (TDM)

1.6 Anti-TNF therapies

The anti-TNF monoclonal antibodies are used first-line to treat patients with moderate to severe IBD refractory to conventional medical therapies [91–94]. Worldwide, there are four anti-TNF therapies licensed to treat IBD: infliximab, adalimumab, golimumab, and certolizumab pegol. Infliximab was first licensed by the US Food and Drug Administration in 1998 and adalimumab in 2002, therefore clinician experience and post-hoc data for these two anti-TNF therapies are the largest. Ongoing studies phase-3 studies are assessing the effectiveness of golimumab [95] and certolizumab pegol [96,97] in patients with IBD (NCT00488631).

1.6.1 Mechanism of action

TNF is a pleiotropic polypeptide pro-inflammatory cytokine and is upregulated in inflammatory conditions, infection, and tissue invasion [98]. In IBD, TNF mediates mucosal inflammation in the gastrointestinal tract by activation and proliferation of immune cells, induction of cytokine and chemokine production, stimulation of chemotaxis, angiogenesis, and extracellular matrix degradation [38]. These mechanisms lead to impairment in cell apoptosis as TNF acts by promoting epithelial cell death in the intestine and upregulating T cells that form immune complexes and lead to organ damage [99,100].

TNF is initially synthesised as a transmembrane protein from which the soluble form is released, and it subsequently binds to TNF receptor to mediate its effects [101]. The two most commonly prescribed anti-TNF monoclonal antibodies to treat IBD, infliximab and adalimumab, work by blocking the interaction between TNF and

TNF receptor. Infliximab, a chimeric human-mouse monoclonal IgG anti-TNF antibody, does so by binding inactive and active soluble TNF, and forming stable complexes with soluble TNF which allow dissociation of TNF and potential to form a reservoir for binding TNF [102,103]. Adalimumab, a fully human anti-TNF IgG monoclonal antibody against TNF, binds with high affinity to the soluble and transmembrane forms of TNF and neutralises the biologic activity of TNF [104].

1.6.2 Treatment outcomes to anti-TNF therapy

Multiple definitions of outcomes to IBD therapies have been proposed. Most use a combination of patient reported symptoms, clinical assessment, biochemical results, and if available, endoscopy results.

Treatment failure occurs when patients are newly initiated on an IBD therapy and upon assessment thereafter, they do not demonstrate clinical, biochemical, or mucosal response to treatment [105]. When classifying treatment failure for patients receiving anti-TNF therapy, timing of treatment failure forms the basis of the terminology used.

Primary non-response occurs when a patient demonstrates a lack of improvement in clinical signs of symptoms to an initial anti-TNF induction regime, typically assessed after 10 – 14 weeks of treatment [106,107]. The patient may require cessation of anti-TNF drug and escalation of therapy, including new prescription of or failure to taper corticosteroids, or escalation to resectional IBD surgery.

Secondary loss of response occurs, in those who initially respond to anti-TNF induction therapy, but subsequently develop symptomatic IBD activity during maintenance anti-TNF therapy that warrants an escalation of steroid, immunomodulatory, or anti-TNF therapy, resectional surgery, or cessation of anti-TNF drug [106,107].

Adverse events are unwanted side effects of medical therapies, and those arising from anti-TNF therapies range from mild symptoms of discomfort to life-threatening complications [108]. Significant events, such as infusion reactions, serious infection, or development of cancer, curtail treatment and lead to treatment discontinuation. Accurate rates and severity of TNF-related adverse events are limited by non-mandatory reporting, case severity bias, reliance on physician-reported registries, and limited prospective trial and observational data [109,110].

1.6.3 The PANTS study

Personalised anti-TNF therapy in Crohn's disease study (PANTS) is a UK-wide, multi-centre, prospective observational cohort reporting on treatment failure of the anti-TNF therapies, infliximab and adalimumab, in anti-TNF naïve patients with active luminal Crohn's disease (ClinicalTrials.gov number: NCT03088449) [111].

The primary objective of the study was to investigate the mechanisms that underlie treatment failure, and to develop a cost-effective, individualised anti-TNF treatment strategy for patients with Crohn's disease which maximizes benefit and minimises harm. Secondary outcomes included:

A) Identifying clinically meaningful serological and genetic markers that predict treatment failure,
B) Identifying clinical, biochemical and genetic predictors of durable clinical remission after anti-TNF withdrawal,
C) To report the initial UK experience of biosimilar infliximab including efficacy, safety, and pharmacokinetics using a prospective, open labelled study design.

Patients were recruited at the time of first anti-TNF exposure from 120 National Health Service trusts across the UK between March 7, 2013, and July 15, 2016. Patients were evaluated for three years or until drug withdrawal.

Patients were screened for inclusion in the cohort at the time of decision to treat with an anti-TNF drug and no more than 4 weeks before starting to receive the drug. The eligibility criteria were: age 6 years or older; diagnosis of Crohn's disease involving the colon, the small intestine, or both; and active luminal disease supported by a CRP of more than 3 mg/L 90 days before the first dose, faecal calprotectin of more than 50 µg/g between 90 days before and 28 days after first dose, or both. Exclusion criteria included previous exposure to, or contraindications for the use of, anti-TNF therapy. The protocol is available online at: https://www.ibdresearch.co.uk/wp-content/uploads/2018/09/PANTS-version-6-dated-20th-May-2016.pdf.

The choice of anti-TNF was at the discretion of the treating physician and prescribed according to the licensed dosing schedule. Study visits were scheduled at first dose (week 0), postinduction (week 14), at weeks 30 and 54 after first dose, and six-monthly thereafter until end of study or drug withdrawal. Additional visits were planned for infliximab-treated patients at each infusion, and for both groups at the time of treatment failure or treatment discontinuation.

At baseline, sites recorded demographic data, smoking status, age at diagnosis, disease duration, Montreal classification of disease location and behaviour, previous medical and drug history, and previous Crohn's disease-related surgeries. At every visit, disease activity score, weight, therapy, and adverse events were recorded. Blood and stool samples were processed through the central laboratory at the Royal Devon and Exeter National Health Service Foundation Trust.

Findings from the first year of study were published in 2019 [111]. In this largest prospective study of anti-TNF therapy in inflammatory bowel disease, consistent with the registration studies, about a quarter of patients had primary non-response to anti-TNF therapy, a third of initial responders lost response, and only a third were in remission at week 54. Other relevant findings from the study are discussed in various sections below.

1.6.4 Therapeutic drug monitoring (TDM)

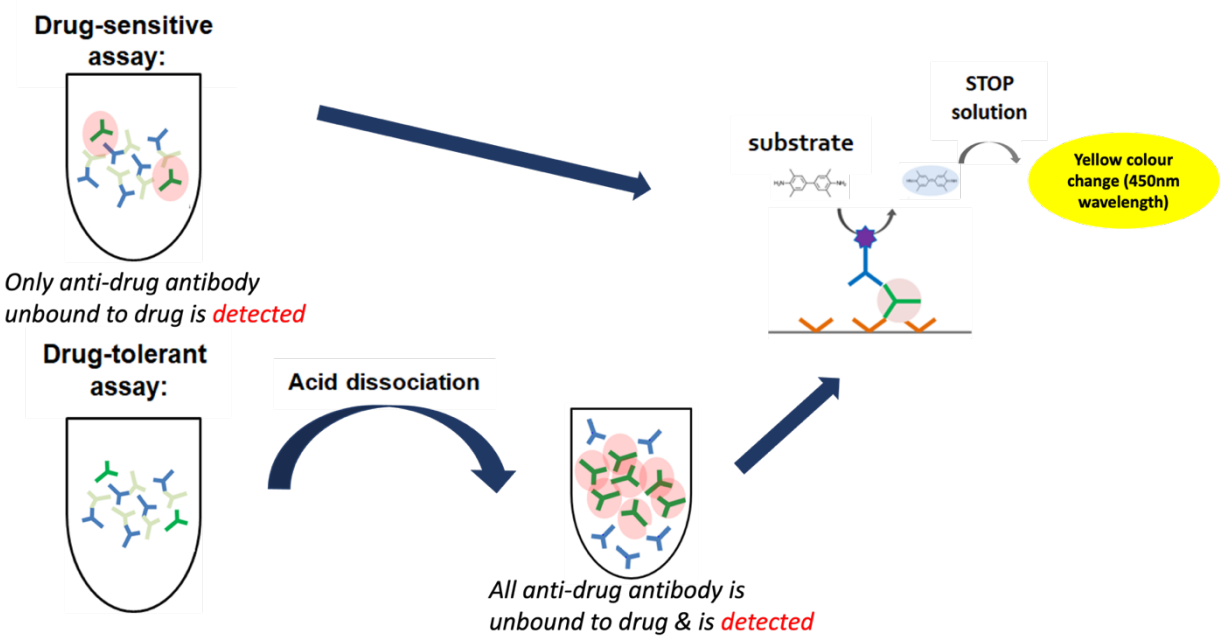
TDM of infliximab and adalimumab is gradually being adopted into routine clinical practice in the United Kingdom [28], United States of America, and other high-income countries [112]. The aim of TDM, measuring an individual's trough drug

level and anti-drug antibody levels in whole blood (obtained via venepuncture), is to assess compliance, drug metabolism, and immunogenicity with a view to guide adjustments or change in management in order to improve clinical outcomes.

The assumptions underlying TDM are that drug absorption, distribution, and metabolism varies between patients, and are influenced by demographic-, disease-, and treatment-related factors. Therefore, the plasma level of a drug is more closely related to the drug's therapeutic effect or toxicity than is the prescribed dosage.

There are a range of anti-TNF antibody and drug level assays that can be used to perform TDM [113,114]. Many institutions measure antibodies using a ‘drug-sensitive’ or ‘free’ antibody assay, which can only measure anti-drug antibodies once all available drug is bound and antibodies are in excess of the drug. This differs from ‘drug-tolerant’ or ‘total’ assays, which include a pre-analytical acid antibody-drug disassociation step that allows antibodies to be measured in the presence of drug (Figure 3). These assays permit quantitative measurement of free therapeutic drug in serum, by following a standard enzyme-linked immunosorbent assay (ELISA) format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody [115,116]. One potential benefit of using drug tolerant over drug-sensitive assays it that clinicians may be able to measure anti-drug antibodies earlier in the treatment course, allowing a window of opportunity to intervene and mitigate the risk of treatment failure associated with immunogenicity [117].

Figure 3: Schematic outlining the differences between drug-sensitive and drug-tolerant antibody assays



1.6.4.1 When to perform TDM

TDM can be performed proactively, whereby routine measurement of drug level and anti-drug antibody regardless of clinical outcome is taken, or reactively, where measurement of drug level and anti-drug antibody is taken in the setting of loss of response [118]. Compared to empirical dosing alone, TDM used reactively at the time of loss of response to an anti-TNF treatment, improves durability of response and safety and leads to significant cost-savings [119,120].

The evidence base supporting proactive over reactive TDM is less clear and the role for proactive TDM remains controversial. One systematic review of 9 RCTs suggests limited benefit of proactive over reactive TDM in patients with IBD [121]. However, with inclusion of observational data, there may be decreased risks of treatment failure and hospitalisation with use of proactive TDM [122].

Historic studies are difficult to interpret and the generalisability of their results are limited by important facets of their study design. For example, in the TAXIT study, optimisation of drug levels successfully recaptured response prior to study entry, but limited the heterogeneity of drug-levels making it more difficult to detect a positive effect of proactive TDM [123]. The early studies also used lower target drug levels (0.5 – 7 mg/L) than the optimal cut-offs observed in future studies (>7 mg/L) [123–125]. These studies are difficult to blind, have been performed in the maintenance phase of therapy only, and there is no consensus on when and how to increase the anti-TNF dose [126,127]. In the recent NORDUM trials, proactive TDM performed during induction therapy, unlike during maintenance therapy, did not demonstrate any significant benefit over conventional therapy [128,129]. However, investigators relied on subjective outcomes of loss of response and included a mixed disease cohort.

More recent trials have demonstrated improved clinical outcomes in patients with higher drug concentrations following dose intensification. In PAILOT, proactive TDM resulted in a greater likelihood of steroid-free clinical remission compared with the reactive TDM [130]. In patients with UC, but not Crohn's disease, the SERENE studies demonstrated higher rates of clinical remission in those treated with higher adalimumab maintenance dosing compared to lower dosing [131,132]. In PRECISION, compared to standard dosing, a Bayesian pharmacokinetic dashboard guiding aiming to maintain a minimum infliximab concentration of 3 μg/ml during maintenance therapy led to improved clinical remission rates and reduced the incidence of loss of response episodes at 1 year [133]. Currently, proactive testing is not yet recommended in clinical practice.

1.6.4.2 Choosing a target drug level

Optimal therapeutic range of anti-TNF drug is poorly defined and not well understood. Most data are derived from small, retrospective observational studies, however few well-powered prospective cohorts and RCTs have been established, including the PANTS study [111,134].

There are multiple factors that influence target trough levels. These include differences in a) assay technique, such as ELISA or homogenous mobility shift assay, b) treatment goal, such as biochemical or histological remission, c) timing of test, such as during induction or maintenance therapy, and d) reason for TDM, such as proactive or reactive testing.

In a recently published systematic review of 43 studies, the authors determined the lower limit of therapeutic range for anti-TNF therapy [135] Recommendations were based on a small number of studies and varied according to clinical setting and desired target. For infliximab, this ranged from 3 – 3.7 μg/mL (area under the curve [AUC] 0.71 – 0.75) in the maintenance phase with the treatment target of clinical remission to 10.1 μg/mL (AUC 0.82) for fistula healing in active perianal inflammatory disease. For adalimumab, this ranged from 5.0 – 11.8 μg/mL (AUC 0.66 – 0.83) in the maintenance phase with a treatment target of biomarker remission to 4.9 – 7.1 (AUC 0.7 – 0.77) in the maintenance phase with a treatment target of mucosal healing. The PANTS study concluded the lower limit of infliximab at the end of induction to be at least 7 μg/mL in order to predict clinical outcome at one-year, and for adalimumab, the lower limit was 12 μg/mL (AUC 0.75) [111].

1.6.4.3 Combination therapy with an immunomodulator

Combination therapy, co-prescription of an anti-TNF agent and an immunomodulator, such as a thiopurine or methotrexate, is effective in preventing the development of immunogenicity and maintaining optimal anti-TNF drug level. Although the mechanism is incompletely understood, it is hypothesised that the influence of immunomodulator on the pharmacokinetics of anti-TNF may occur as a result of a shared mechanism of apoptosis for the two drugs [136–138]. However, the enhanced effect of immunosuppression from two agents rather than one is also likely contributory.

Supporting initial registration trials, real-world data demonstrate anti-TNF therapy in combination with an immunomodulator, thiopurine or methotrexate, is effective in reducing treatment failure. In the double-blind SONIC trial, at week 26 and 50, treatment-naïve patients with Crohn's disease treated with combination therapy with infliximab and azathioprine were more likely to achieve clinical, endoscopic, and pharmacologic outcomes than monotherapy with infliximab alone [139,140]. The benefit of combination may be due to the effect of azathioprine's influence on the pharmacokinetics of infliximab in achieving optimal drug level [141].

This differed from the open-label DIAMOND trial, where, at week 26, treatment-naïve patients with Crohn's disease treated with combination therapy with adalimumab and azathioprine did not differ in treatment response from monotherapy with adalimumab alone [142]. Mucosal healing, however, was significantly higher in the group treated with combination therapy compared to the monotherapy group, and a post-hoc analysis demonstrated that higher adalimumab

trough levels at week 26 were associated with disease remission at week 52, suggesting that combination therapy may be beneficial for patients who have suboptimal drug levels [143]. The SONIC study may have been affected by low rates of immunogenicity, short duration of follow-up throughout the study period, or both.

In the PANTS study, immunomodulator use was associated with lower immunogenicity to both infliximab and adalimumab and higher drug concentrations for infliximab-treated patients compared with no immunomodulator use [111]. Data from 11,244 patients prescribed anti-TNF across four Canadian provinces confirmed these findings [144]. Looking at a composite outcome of IBD-related treatment failure, hospitalisation, surgery, new/recurrent corticosteroid switch, or anti-TNF switch, patients who received combination therapy were less likely to fail treatment for both Crohn's disease and UC and infliximab and adalimumab.

Data on the efficacy of methotrexate combination therapy in adult patients with IBD remains scarce, and largely limited to short-term follow-up. In a recently published multicenter, double-blind RCT of 297 paediatric patients with Crohn's disease newly initiating infliximab or adalimumab, combination therapy with adalimumab, but not infliximab, and methotrexate was associated with two-fold reduction in treatment failure compared to those treated with adalimumab monotherapy at two-year follow up [145]. For both anti-TNF therapies, there was no difference in risk of developing anti-drug antibodies.

One substantial concern of combination therapy is whether the risk of adverse events, particularly lymphoma, is increased amongst patients who receive combination therapy vs monotherapy. In the largest cohort to date of almost 190,000 patients, with a follow-up of 6.7 years, absolute risk for developing lymphoma was low [146]. However, the use of thiopurine or anti-TNF monotherapy was associated with a small increased risk of lymphoma, compared with exposure to neither medication, and the risk was higher with combination therapy than with each of these treatments used alone [147]. These findings have been replicated in subsequent systematic reviews and meta-analyses [148,149].

Reversal of immunogenicity, that is seroconversion of high antibodies and suboptimal drug level to low antibodies and optimal drug level, may be achieved with addition of an immunomodulator to anti-TNF therapy. The finding was demonstrated in a case report [150], however, the evidence base has since expanded to small retrospective cohorts. In one case series of 23 patients who developed antibodies to adalimumab monotherapy with clinical evidence of loss of response, addition of an immunomodulatory as salvage therapy resulted in reversal of antibody development, increase in drug level, and clinical improvement [151]. In a larger cohort, 17/159 patients who developed antibodies to infliximab and adalimumab were given immunomodulators. 13/17 had a resultant increase in median anti-TNF drug level, decrease in antibody to undetectable level within 11 months, and regained clinical response [152]. Replication from larger, prospective studies is awaited.

In PANTS, methotrexate, compared to thiopurines, had a similar effect on immunogenicity, and in contrast to findings from SONIC, immunomodulator use in infliximab-treated patients was associated with higher week 54 remission rates compared with no immunomodulator use, independent of week 54 drug concentration or immunogenicity status[111]. In COMMIT, patients with Crohn's disease receiving treatment with prednisolone, were treated with combination therapy with infliximab and methotrexate or monotherapy with infliximab [153]. There was no difference in patients treated with combination therapy over monotherapy. This differs from the latest Canadian data which suggest that azathioprine is preferential over methotrexate, particularly for UC, whereas in Crohn's disease, the recommendation is less clear [144].

1.6.5 Anti-TNF treatment failure

About one in three patients with IBD, 40% Crohn's disease and 15% UC, require treatment with anti-TNF therapy [154]. Successful treatment with an anti-TNF leads to mucosal healing [139], reduced hospitalizations and surgeries [155], and improvements in quality of life [156,157].

Although anti-TNF therapies have greatly advanced the treatment of IBD, they do not always provide durable remission. Anti-TNF treatment failure and drug discontinuation is common. Consistent with registration studies, in the PANTS study, about one-quarter of patients with a diagnosis of Crohn's disease who newly initiated anti-TNF therapy did not respond to treatment within the first 12 weeks, and one-third of initial responders lost response by the end of the first year of treatment [111].

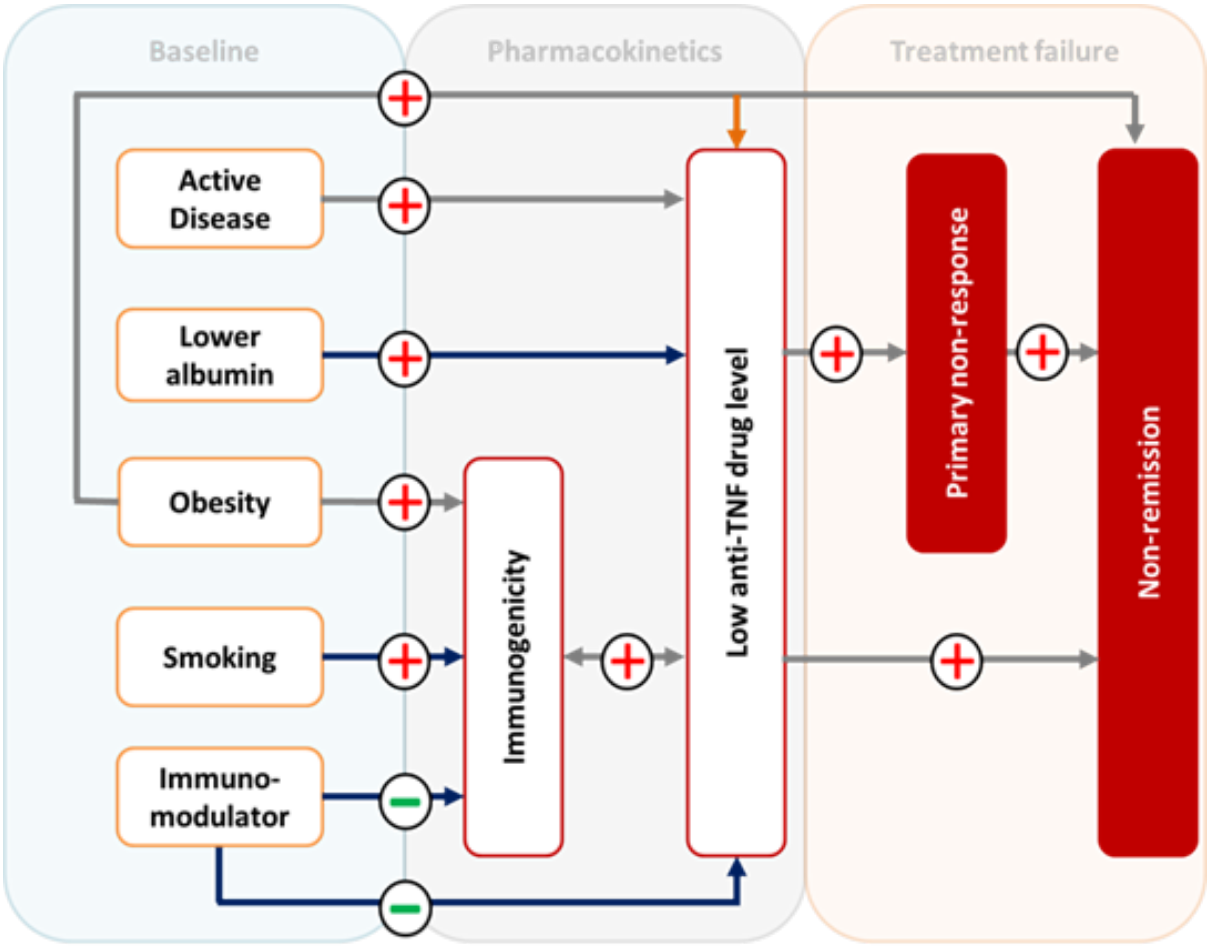
Multiple patient, disease, treatment, and multi-omic related factors been postulated to lead to anti-TNF treatment failure in patients with IBD (Table 2). For patients with Crohn's disease, the factors most strongly associated with anti-TNF treatment failure include increased disease activity, low anti-TNF drug concentrations, and development of anti-drug antibodies. In PANTS, a three-way relationship between these three factors was identified, whereby increased disease activity was associated with low drug levels and increased risk of immunogenicity, low drug levels were associated with increased disease activity and a major risk factor for subsequent development of immunogenicity, and immunogenicity, through drug clearance, was associated with low drug levels and increased disease activity (Figure 4).

Table 2: Significance of commonly used current biomarkers and novel putative biomarkers in the evaluation of anti-TNF therapeutic efficacy [158].

	Decreased or normalised in Crohn's disease			Decreased or normalised in ulcerative colitis		
	Response	Remission	Mucosal healing	Response	Remission	Mucosal healing
Commonly used current biomarkers						
Faecal calprotectin	+	+	+	+	+	+
C-reactive protein	+	+	+	+	+	+
Serum levels of anti-TNF drugs	+	+	+	+	+	+
Anti-drug antibodies	+	+	+	+	+	+
Novel putative biomarkers						
Mucosal transcripts:						
TNF-a	+	+	+	+	+	+
IL-17a	+	+	+	+	+	+
Oncostatin M	+	+		+	+	
	+			+		
	+			+		

IL-7R TREM1						
miRNAs	+			+		
Faecal and mucosal microbiota profile	+			+		
Proteomics	+					
Genomic	+			+		

Figure 4: Schematic outlining the relationship between baseline and pharmacokinetic factors on anti-TNF treatment failure, from the PANTS study



1.6.6 Drug-related anti-TNF treatment failure

There are three main drug-related mechanisms of anti-TNF treatment failure: pharmacokinetic, pharmacodynamic, and immunogenicity-related treatment failure (Table 3).

Table 3: Drug-related treatment failure, stratified by anti-TNF drug and antibody concentrations [106,107].

	Anti-TNF concentration	Anti-TNF antibody concentration	Mechanism of action
Pharmacokinetic	Suboptimal	Undetectable	Increase anti-TNF drug clearance
Pharmacodynamic	Optimal	Undetectable	Non-TNF pathways drive inflammation
Immunogenicity	Suboptimal	Detectable	Antibody development which compete at anti-TNF binding site and increase drug clearance

Pharmacokinetic: patients with IBD have a high inflammatory burden, with higher circulating serum and mucosal TNF levels and lower mucosal anti-TNF levels, compared to patients without IBD [159,160]. There are three main hypothesised mechanisms of pharmacokinetic clearance of therapeutic anti-TNF biologics [161]: (i) TNF absorbs and binds anti-TNF monoclonal antibodies and neutralises ant-TNF biologic, (ii) mononuclear phagocytes in the reticuloendothelial system clear immune complexes of TNF and anti-TNF through Fc receptor mediated endocytosis and proteolytic degradation [162], and (iii) immunoglobulin gut loss,

whereby anti-TNF biologics leak through inflamed mucosa into the stool, further compounds loss of biologic [163].

Pharmacodynamic: inflammation in the gut lumen occurs as a result of multiple cellular pathways activated, including cytokine release of IL-12 and 23 and anti-TNF, intracellular signalling pathways of janus kinase/signal transducer and activator of transcription signalling, and leucocyte adherence and migration across the intestinal endothelium [38,164]. Pharmacodynamic failure occurs when anti-TNF drug levels are adequate without anti-drug antibody formation. It is thought to occur when non-TNF pathways drive gut inflammation.

Immunogenicity: immunogenic-failure is due to the formation of anti-drug antibodies which compete at the anti-TNF binding site. Detectable antibodies may act by neutralising the effect of the anti-TNF drug or increase drug clearance [165]. Immunogenicity to anti-TNF therapy is a major cause of loss of response, primary non-response and secondary loss of response, and treatment discontinuation.

In the PANTS cohort, the first-ever genome-wide association study of immunogenicity to any biologic drug was performed, and the Human Leukocyte Antigen allele, HLA-DQA1*05, carried by approximately 40% of White Europeans, conferred an almost two-fold risk of immunogenicity (hazard ratio [HR] 1.90 [95%CI 1.60 to 2.25], $p<0.0001$) to anti-TNF therapy [166]. This association was seen for both risk of antibody development and drug persistence, and was consistent for patients treated with adalimumab or infliximab and for patients treated with anti-TNF therapy alone or in combination with an immunomodulator.

In a post-hoc analysis, separately testing the adalimumab and infliximab sub-cohorts for association to HLA-DQA1*05 four-digit resolution alleles, revealed low linkage disequilibrium between DQA1*05:01 and DQA1*05:05 [167]. The PANTS cohort, however, was underpowered to support this conclusion and fine-mapping and functional studies are required to ascertain the causal HLA-alleles across extended haplotypes [168].

This finding has since been replicated. First, in a retrospective cohort study of infliximab-treated patients only, carriage of the variant was associated with a high risk of immunogenicity (HR 7.39 [95%CI 2.97 to 17.191], p<0.0001) in addition to loss of response and treatment discontinuation [169]. Second, in a European multicohort prospective study of patients with multiple sclerosis, rheumatoid arthritis, and patients with IBD treated with 8 different biopharmaceuticals, carriage of the variant was associated with an increased rate of immunogenicity (adjusted HR = 3.9 [95%CI 1.923 to 5.976], p < 0.0001 for the homozygotes) [170]. More recently, a systematic review and meta-analyses of 3756 patients, with median follow-up of 12 months, confirmed that variants in HLA-DQA1∗05 were associated with an increased risk in immunogenicity and secondary loss of response in patients with immune-mediated inflammatory diseases treated with anti-TNF therapies [171].

Risk of immunogenicity may be mitigated, in variant-carriers in whom immunmodulators are tolerated, by combination therapy with adalimumab or infliximab therapy, or avoidance of anti-TNF in patients in whom immunomodulators are not tolerated. In non-variant carriers in whom

immunomodulators are tolerated, infliximab or adalimumab combination therapy is appropriate, or adalimumab monotherapy if immunomodulators are not tolerated. The role of proactive TDM to offset the risk of immunogenicity in carriers of the variant remains unknown but an active area of research [172].

Pharmacogenomic screening of HLA-DQA1*05 in patients with IBD being considered for treatment with infliximab, using the result to guide the application of mono- or combination therapy with an immumomodulator, is currently being trialled in Canada (NCT04109300).

1.7 COVID-19 and patients with IBD

The below section presented is selected from a peer-reviewed review article I co-first authored, which has been published in *Gut* [173] The selected contents have been reformatted to fit the style of the thesis.

As IBD is an immune-mediated inflammatory disease, there are potential intersections between the pathogenesis of COVID-19 and IBD at the molecular level. Epithelial expression of angiotensin-converting enzyme-2 (ACE2) and transmembrane serine protease 2 appear to be essential for viral entry of SARS-CoV-2 into host enterocytes, which result in unopposed renin-angiotensin pathway leading to acute lung injury [174]. In patients with IBD, who have inflammation of the gut and are often treated with immunosuppressive medications, the epithelial expression of ACE2 will remain unchanged or even downregulated [175–177], which may impact on the disease spectrum of COVID-19 and its clinical management.

Patients with IBD are at greater risk of developing serious infections and pneumonia [178–180], particularly those treated with biological drugs which are known to be associated with an increased risk of opportunistic infections [181]. At the beginning of the pandemic, concerns were raised as to whether patients with IBD may develop worse health outcomes. It also remained uncertain whether patients treated with immunosuppressive drugs have reduced vaccine response, as has been demonstrated previously for other vaccine-preventable infections [182–185]. Until 2022, patients with immune-mediated inflammatory diseases, including IBD, were excluded from the SARS-CoV-2 vaccine clinical development

programmes. Since the rollout of novel vaccine platforms internationally, many of which have not previously been studied in patients with IBD, many questions regarding the safety and effectiveness of SARS-CoV-2 vaccination in these patients have emerged.

With respect to patients with IBD treated with anti-TNF therapies, use of anti-TNF has not been associated with an increased risk of COVID-19 [186]. Furthermore, anti-TNF-treated patients did not demonstrate an increased risk for intensive care unit admission, mechanical ventilation, or death compared to non-anti-TNF treated patients [187]. Similar findings have been replicated in three population-based studies in the United States, France and Denmark [188–190].

Some studies suggest that the risk of developing severe COVID-19 may be lower in biological-treated patients potentially due to the effect of these drugs in suppressing cytokine inflammatory pathways that underlie COVID-19-associated inflammatory complications [191–193]. In one meta-analysis, pooled relative risks of hospitalisation (relative risk [RR] 0.34 [95%CI 0.19 to 0.61]), intensive care unit admission (RR 0.49 [95%CI 0.33 to 0.72]), and mortality (RR 0.22 [95%CI 0.13 to 0.38]) were lower in biological-treated patients, most of whom were treated with an anti-TNF, compared to patients treated with other non-biologicals for IBD [191]. A meta-analysis also found that patients treated with anti-TNF therapy had decreased risk of hospitalisation and intensive care unit admission compared to corticosteroids or 5-ASA [192].

However, patients treated with anti-TNF therapy, in combination with an immunomodulator, showed an increased risk of COVID-19 adverse outcomes. Although a French nationwide study found that in-patient mortality rates were similar between patients treated with anti-TNF monotherapy compared with anti-TNF combination therapy [190], data from the Surveillance Epidemiology of Coronavirus Under Research Exclusion (SECURE-IBD) study reported that patients treated with anti-TNF combination therapy had a higher risk of severe COVID-19 than those on anti-TNF monotherapy (8.8% vs 2.2%, RR 4.01 [95% CI 1.65 to 9.78]) [194]. In a pooled analysis from three international registries consisting of patients on different immune-mediated inflammatory diseases, anti-TNF monotherapy appeared to have the best safety profile than other commonly prescribed treatment regimens, including anti-TNF combination therapy [195].

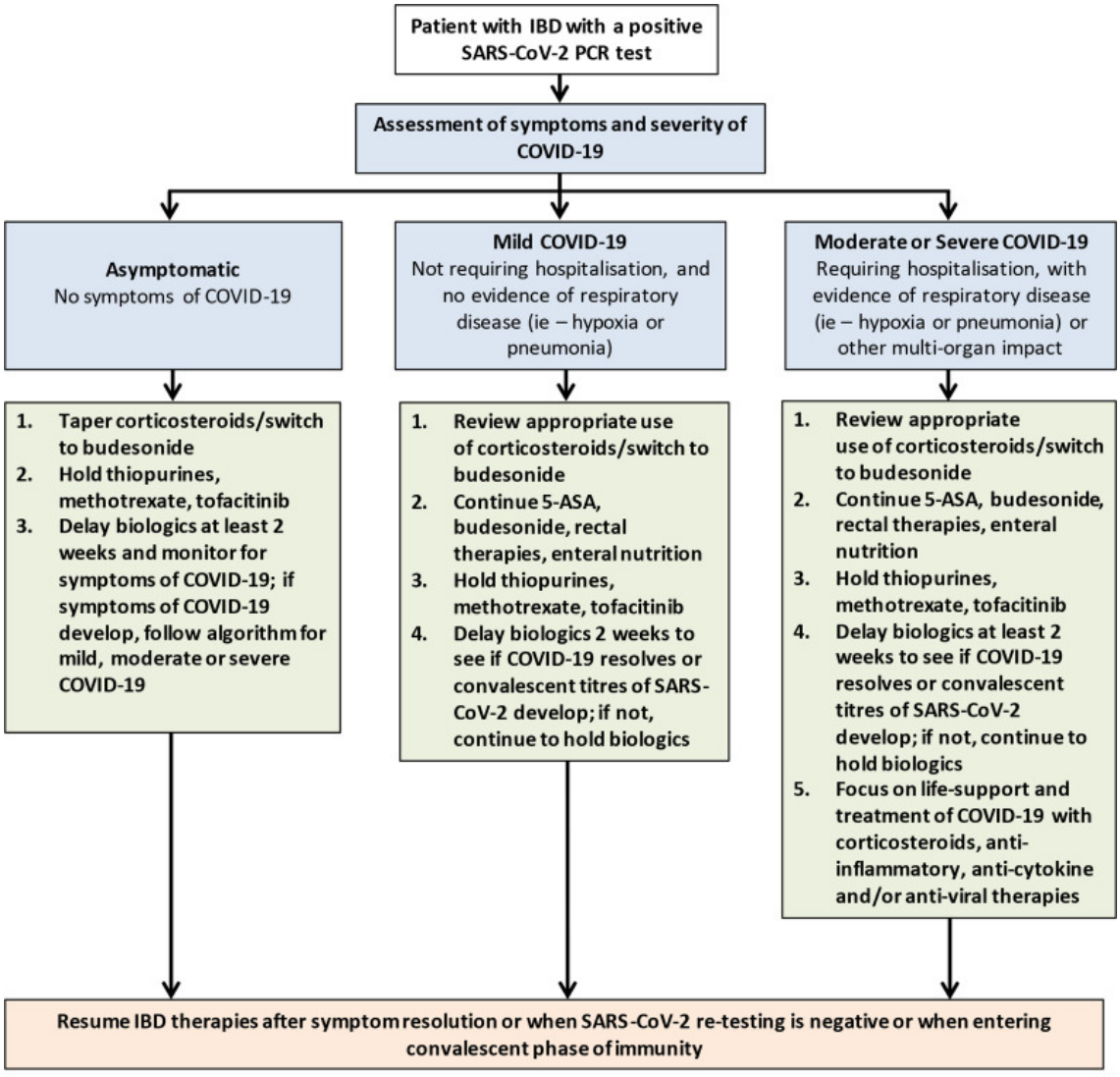
Data of the impact of vedolizumab, an anti-integrin, on COVID-19 outcomes has been conflicting. One report suggested that vedolizumab treatment was associated with an increased risk of developing COVID-19 (RR 1.70 [95%CI 1.16 to 2.48] compared to patients treated with 5-ASA alone [196]. Initial data from SECURE-IBD also suggested an increased risk of hospitalisations in vedolizumab- compared to anti-TNF treated patients (RR 1.39 [95%CI 1.001 to 1.90]), but not risk of severe COVID-19 [196,197].

In more recent data from SECURE-IBD, vedolizumab-treatment was found to be associated with a decreased risk of hospitalisation or death (RR 0.66 [95%CI 0.56 to 0.78]), and no association with risk of severe COVID-19, compared to patients who were not on vedolizumab [198]. It is plausible that vedolizumab-treated patients

may be at increased risk of COVID-19 compared to patients treated with other IBD medications, in part because the anti-integrin not only binds to effector memory cells in the gut, but also the upper respiratory tract [199,200]. It is more likely that initial data were underpowered to detect true differences between patients treated with different IBD medications. As data on patients treated with less commonly prescribed medications, such as vedolizumab, enriched over the course of the pandemic, the increased risk of adverse outcomes to COVID-19 disappeared.

Management of a patient with IBD who tests positive for SARS-CoV-2, with or without symptoms, remains controversial. Consensus from experts recommend modification of IBD therapy in patients who have confirmed COVID-19 (Figure 5) [201–203]. General principles of the guidelines include consideration to taper oral corticosteroids or switch to budesonide, with thiopurines, methotrexate, and tofacitinib, and delay biological therapy for two weeks until recovery. However, most of these recommendations are based on consensus only, and should be considered on an individual basis utilising the most recent data where possible. Whilst steroids have consistently come across as a risk factor for severe COVID-19, the proven benefit of steroids in managing hospitalised patients with COVID-19 suggests that steroids should not always be withdrawn in cases of patients with IBD hospitalised with COVID-19 [204].

Figure 5: Treatment considerations for patients with IBD who develop COVID-19 infection. Adapted from the latest European Crohn's and Colitis Organisation and American Gastroenterological Association guidelines [202,203]. *IBD=inflammatory bowel disease, PCR=polymerase chain reaction, 5-ASA=5-aminosalicylic acid*.



Clinical notes:

1) The International Organization for the Study of Inflammatory Bowel Diseases recommended that patients without symptoms but positive for SARS-CoV-2 withhold IBD therapies for a minimum of 10 days. In patients with a positive test for SARS-CoV-2 and symptoms of COVID-19, IBD therapy should also be withheld, and restarted when at least 3 days (72 hours) have passed since recovery, there is improvement in respiratory symptoms, and at least 10 days have passed since symptoms first appeared.

2) The severity of COVID-19 should be weighed up against IBD disease activity, and careful risk–benefit assessment regarding treatments for COVID-19 and escalating treatments for IBD should be considered on an individual basis

3) Guidance from early on in the pandemic recommended tapering of systemic steroids in patients with IBD and confirmed SARS-CoV-2 infection, however, decisions regarding risk-benefit ratio should be made in light of active IBD symptoms, and also take into consideration the role of steroids in the management of COVID-19 infection

4) Recommendations are largely based on expert consensus due to limited published data

The International Organization for the Study of Inflammatory Bowel Diseases recommend that patients without symptoms but positive for SARS-CoV-2 withhold IBD therapies for a minimum of 10 days [201]. In patients with a positive test for SARS-CoV-2 and symptoms of COVID-19, IBD therapy should also be withheld, and restarted when at least 3 days (72 hours) have passed since recovery, there is improvement in respiratory symptoms, and at least 10 days have passed since symptoms first appeared.

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Chapter 2: Methods

2.1 Study design

This thesis is comprised of data from multiple, longitudinal observational cohorts that were both retrospectively and prospectively established, and analysed subsequently. Because cohort studies measure exposure to risk factors of interest prior to the occurrence of an outcome, they are particularly useful in assessing studying rare, often multiple, outcomes, and defining the natural history of disease and order in which disease events occur [1].

Across all cohorts of this thesis, selection of participants was based on pre-specified inclusion and exclusion criteria. Compared to prospective cohorts, historical cohorts, such as those established in Chapters 3, 7, and 8 of this thesis were faster to perform. However, the data on exposure and potential known and unknown confounders were more inaccurate. Data collected for prospective cohorts, such as those established in Chapters 4, 5, 6, were collected in a more standardised way, before the onset of the outcomes of interest, and collected in a way that maximised collection of follow-up data.

Long-term, these cohorts, particularly the prospective ones, were expensive to run. They all relied on records of exposure being available, reliable, and accurate, and there was loss to follow-up over the time periods that needed to be accounted for statistically in order to avoid selection bias. Furthermore, as cohorts were analysed over a number of years across multiple sites in the UK, careful attention needed to be paid to exposure and outcome status over the conduct of the studies.

2.2 Laboratory work

2.2.1 Anti-TNF drug level and antibody measurements

Alongside a senior biomedical scientist from the Royal Devon and Exeter NHS Foundation Trust department of biochemistry, Rachel Nice, we made an application to the Exeter Ten Thousand cohort (https://exetercrfnihr.org/about/exeter-10000/) to obtain sera from a random sample of 498 healthy volunteers. In line with the study aims and objectives, our application to test for antibodies to infliximab and adalimumab was approved. Under the direct supervision from Rachel Nice, I used the Immundiagnostik IDKmonitor infliximab and adalimumab drug level ELISA assays and total infliximab and adalimumab anti-drug antibody ELISA assays to determine the positivity threshold for these assays [2–6]. I followed the manufacturer's test instructions, which are summarised below.

Test principles for the drug level assay are [5,6]:

- In a first incubation step, the free adalimumab from the standards/controls/diluted samples was bound to the specific monoclonal anti-adalimumab antibody coated on the plate.
- To remove all unbound substances, a washing step was carried out.
- In a further incubation step, peroxidase-labelled antibody was added.
- Tetramethylbenzidine was used as a substrate for peroxidase.
- Finally, an acidic stop solution was added to terminate the reaction. The colour changes from blue to yellow.

- The intensity of the yellow colour was directly proportional to the concentration of free adalimumab in the sample.
- A dose response curve of the absorbance unit (optical density) vs. concentration was generated, using the values obtained from standard.
- The concentrations of free adalimumab in the samples were determined directly from this curve.

Test principles for the total anti-drug antibody assay are [7,8]:

- During sample preparation, the anti-drug antibodies were separated from the therapeutic antibody in order to acquire free anti-drug antibodies.
- By adding the conjugate (peroxidase labelled therapeutic antibody) and the tracer (biotinylated therapeutic antibody), the unmarked therapeutic antibodies were replaced and the marked antibodies formed a complex with the anti-drug antibodies.
- This complex bound via biotin to the streptavidin coated microtiter plate. It was detected via the peroxidase conjugate with the peroxidase converting the substrate tetramethylbenzidine to a blue product.
- The enzymatic reaction was stopped by adding an acidic solution. The samples converted from blue to yellow.
- The colour change was measured in a photometer at 450 nm. The interpretation was made using the cut-off control.

2.2.2 Serum TSH, triiodothyronine, and thyroxine measurements

Over the course of five weeks, I was responsible for the storage, management and processing of 1171 serum samples from the PANTS study, held at the University of

Exeter central laboratory, for TSH, triiodothyronine, and thyroxine measurements. In order to run the Roche Elecsys TSH immunoassay, the department of biochemistry laboratory required each serum sample to be delivered to them in a unique tempus tube, in aliquots of 50 µL (the volume of sample required to undertake the assay), and thawed. As study lead and coordinator, I was responsible for preparing each sample, and all data and results management within the study, including liaising internally with the lab.

2.2.3 Serum 25-hydoxyvitamin D measurement

Over the course of seven weeks, I was responsible for the storage, management and processing of 1374 serum samples from the PANTS study, held at the University of Exeter central laboratory, for 25-hydoxyvitamin D measurement. In order to run the Roche Elecsys 25-hydoxyvitamin electrochemiluminescence immunoassay, the department of biochemistry laboratory required each serum sample to be delivered to them in a unique tempus tube, in aliquots of 15 µL (the volume of sample required to undertake the assay), and thawed. As study lead and coordinator, I was responsible for preparing each sample, and all data and results management within the study, including liaising internally with the lab.

2.2.4 SARS-CoV-2 nucleocapsid antibody measurement

Over the course of three months, I was responsible for storage, management, and processing of 14,106 surplus serum samples (kept by laboratories for routine therapeutic drug monitoring from six UK laboratories [Barts Health NHS Trust, NHS Greater Glasgow and Clyde, Guy's and St Thomas' NHS Foundation Trust, North West London Pathology, Royal Devon and Exeter NHS Foundation Trust, and

Royal Wolverhampton NHS Trust]) for SARS-CoV-2 nucleocapsid antibody testing. Samples were not stored in a standardised manner by laboratories, and were received frozen in a variety of volumes and containers, and with heterogenous clinical details provided.

In order to run the Roche Elecsys Anti-SARS-CoV-2 nucleocapsid immunoassay, the department of biochemistry laboratory required each serum sample to be delivered to them in a unique tempus tube, in aliquots of 150 µL (the volume of sample required to undertake the assay), and thawed. As study lead and coordinator, I was responsible for preparing each sample, and all data and results management within the study, including liaising internally with the lab and externally with study sites.

2.3 Data collection, cleaning, and management

2.3.1 PANTS study cohort

Data submitted by sites from 120 UK hospitals for the PANTS study was held on a third-party server (pantsdb.co.uk). All data submitted regarding the the 1610 recruited patients were manually entered by research nurses and clinicians. The study was open to recruitment for three years and patients were followed-up for three years.

On submission of data, research staff from the Exeter IBD research team were required to review each datapoint and ensure standardisation of recording of data across the study. From my involvement of the study in 2018, until I finished my full-time role as a PhD student in 2022, I acted as lead for data review and analysis for the study. Therefore, for all demographic, clinical, biochemical, and outcome data submitted to the PANTS study, with particular relevance to the analyses performed in Chapters 3, 4, 5, and 6 of this thesis, I manually checked all data submitted to the central database by sites to ensure accuracy. I was responsible for sending and responding to data clarification queries with study sites directly.

To further verify data quality and to provide an enhanced level of data cleaning and review, I set up a REDCAP server to code specific datasets of the PANTS study (ie – loss of response visits, clinician action, and study subject response to clinician action). This allowed me to review the newly coded data at a study-wide level, rather than an individual subject level, and cross-check the output from the REDCap server (Vanderbilt University Medical Centre, Tennessee, US) against that of the originally-held third-party server. Where data was incongruent across

both servers, I was responsible for checking the source file and raising further data clarification queries with study sites.

2.3.2 IMSAT study cohort

To facilitate case identification for patients recruited to the IMSAT study (Chapter 7), I worked collaboratively alongside scientists from the department of biochemistry, Royal Devon University Healthcare NHS Foundation Trust (Exeter). To do this in line with our approved study protocol, I wrote the study methodology and coordinated the steps to be undertaken outlined in Figure 1. As study lead and coordinator, I was the key contact for the Exeter IBD research team.

Case identification was facilitated by independent NHS staff at the Royal Devon University Healthcare NHS Foundation Trust (Exeter) that were not part of the Exeter IBD research team and did not have a role in the analyses or write up of the project.

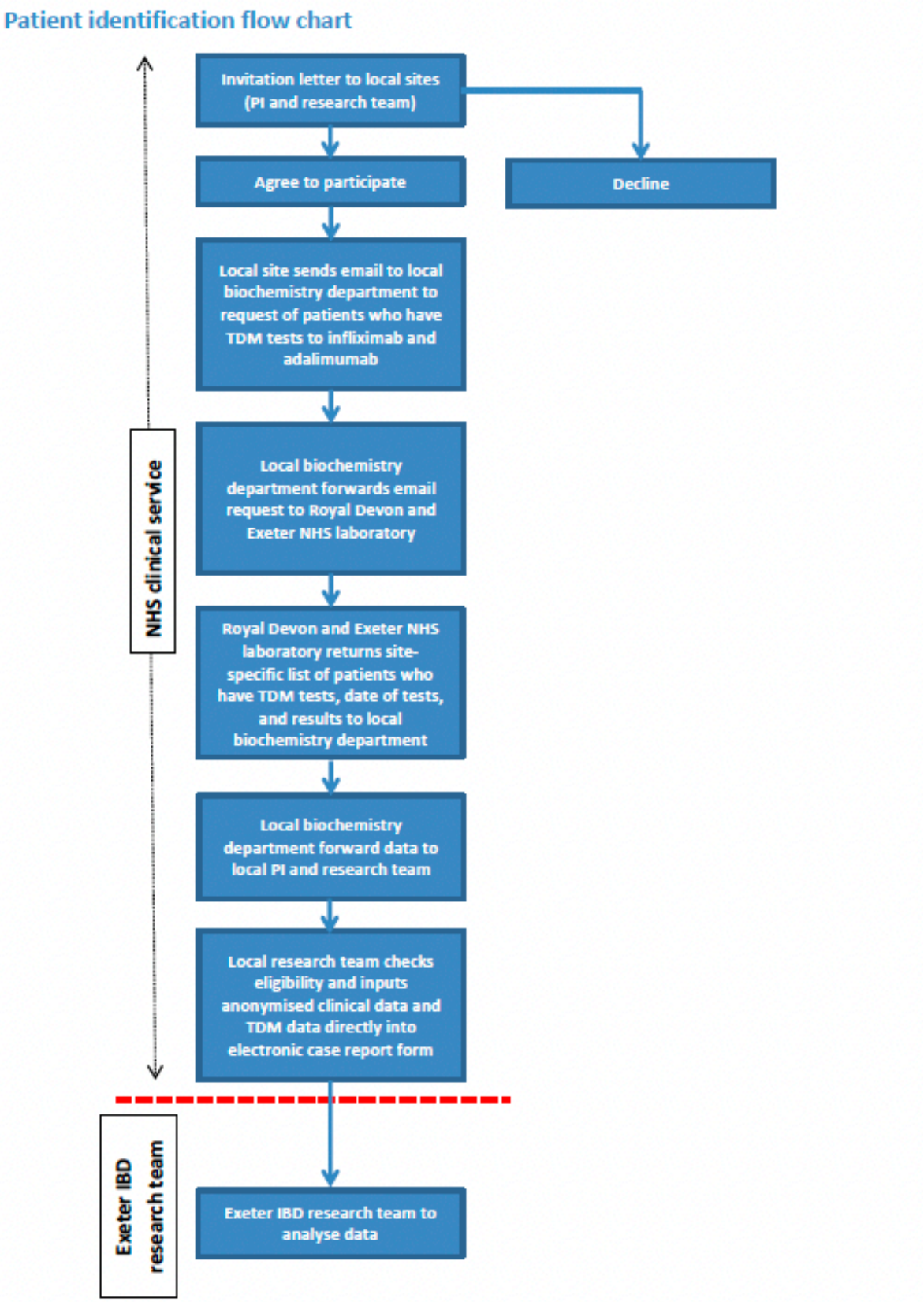
The Royal Devon University Healthcare NHS Foundation Trust (Exeter) department of biochemistry provided the Exeter IBD research team with a list of hospitals who use the Royal Devon University Healthcare NHS Foundation Trust (Exeter) anti-TNF therapeutic drug monitoring service. The research team wrote to UK users of anti-TNF TDM service to ask if they are willing to participate in this study.

The local principle investigator (PI) contacted their local biochemistry department and requested a list of patients who have had TDM tests carried out to both

infliximab and adalimumab at Royal Devon University Healthcare NHS Foundation Trust (Exeter) laboratory. This email request was forwarded by the local biochemistry department to named personnel at the Royal Devon University Healthcare NHS Foundation Trust (Exeter) department of biochemistry. Royal Devon University Healthcare NHS Foundation Trust (Exeter) department of biochemistry then generated a site-specific list of patients who have had tests carried out for both infliximab and adalimumab since 2013. This electronic file detailed the NHS numbers and therapeutic drug monitoring results. This file was then returned to the local biochemistry department who forwarded the information to the local PI. Secure nhs.net emails were used for all communication.

The local site then confirmed whether these patients met all the study inclusion criteria. Anonymised patient data was submitted to a secure web-based database (REDCap).

Figure 1: Patient identification flow chart for the IMSAT study



Abbreviations: IBD: inflammatory bowel disease, PI: principal investigator, TDM: therapeutic drug monitoring, IFX: infliximab, ADAL: adalimumab

2.3.3 Statistical analyses

Where possible, data were pseudonymized and entered either by the study site or myself into a purpose-designed electronic database in REDCap. For all statistical analyses in this thesis, I used the open source statistical computing environment R (R Foundation for Statistical Computing, Vienna, Austria).

I included patients with missing clinical data in analyses for which they had data and specified the denominator for each variable. All analyses were two tailed, unless otherwise stated, and $P < 0.05$ were considered significant. Summary descriptive statistics were presented as median and interquartile ranges for continuous variables and as numbers and percentages for categorical variables.

I performed univariable analyses using Fisher's exact, Mann–Whitney U, and Spearman's rank tests to identify differences in baseline characteristics between anti-TNF treated patients, and to determine categorical and continuous factors associated with exposures and the predefined clinical outcomes. Multivariable logistic regression analyses was used to confirm factors independently associated with outcome. Rates of immunogenicity and drug persistence were estimated using the Kaplan–Meier method, and comparative analyses were performed using univariable and multivariable Cox proportional hazards regression.

Youden's formula was used to determine the optimal ratio cut-off of exposure variables to predict pre-specified outcomes, and receiver operator characteristic curves and area under the curve analyses with bootstrapping were used to estimate the diagnostic accuracy of the model.

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Chapter 3: Validating the positivity thresholds of drug tolerant anti-infliximab and anti-adalimumab antibody assays

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Study aim

To validate the positivity threshold for the IDKmonitor drug-tolerant anti-infliximab and anti-adalimumab antibody assays, and to describe the relationship between drug and anti-drug antibody levels and clinical outcomes using these new positivity thresholds.

My role in the study

Alongside a senior biomedical scientist, Rachel Nice, I was responsible for designing the research study and determining the aims, objectives, and proposing the methodology. I obtained ethical approval for testing sera from healthy volunteers (EXTEND cohort). I performed the laboratory work and analysed the results. I wrote abstract for submission at the European Crohn's and Colitis Organisation annual conference which was accepted as an oral presentation, and I prepared the slides that were presented subsequently by the senior author of the study, Dr Nick Kennedy. I wrote up the study, and submitted it to multiple journals, revising it at each stage to align with multiple editors' and peer reviewers' comments.

Findings

The 80% one-sided lower confidence interval of the 99th centile concentration for anti-infliximab and –adalimumab antibodies were lower than the manufacturers threshold. Using these new thresholds in the TDM cohort, more adalimumab- than infliximab-treated patients were reclassified as antibody-positive. Adalimumab drug concentrations in this reclassified group were lower than those below the new threshold, but higher than at the manufacturer's threshold. In the PANTS cohort,

patients with anti-adalimumab antibody concentrations at or above the new threshold were more likely to be in primary non-response, and non-remission at week 54, than patients with anti-drug antibody concentrations in the group below the new threshold.

Relevance and impact on my learning

Conducting this study has led me to gain a deeper understanding not only in the theory underlying drug level and anti-drug antibody detection, but also the practical elements that underlie testing. This includes a more in-depth understanding of development and validation of assays, diagnostic accuracy of biochemical testing, and measures of performance including limit of blank, detection, and quantitation.

Working collaboratively alongside scientists from the department of biochemistry as well as in conjunction with researchers from the EXTEND study was a hugely educational experience and opportunity. Without their input, I would not have been able to apply the findings from each stage of the study to the next cohort, and generated as scientifically-robust results that I was able to.

Acknowledgements of co-authors and contributions to paper

Rachel Nice, myself, Tariq Ahmad, Timothy J McDonald, Mandy H Perry, and Nicholas A Kennedy conceived and designed the study. Rachel Nice and Timothy J McDonald obtained funding for the study. Rachel Nice, myself, Timothy J McDonald, Mandy H Perry, and Nicholas a Kennedy performed the biochemical experiments, analysis, and aspects of the work related to the central laboratory processing of samples. Rachel Nice, myself, Harry Green, and Nicholas A Kennedy acquired, analysed and interpreted the data. All authors reviewed the draft of the manuscript, contributed to the critical review, and final approval of the manuscript.

Abstract

Background

Used proactively, drug-tolerant anti-tumour necrosis factor (TNF) antibody assays provide early opportunity to suppress immunogenicity.

Aim

To validate positivity thresholds of IDKmonitor drug-tolerant anti-infliximab and -adalimumab antibody assays.

Methods

We applied positivity thresholds defined by testing sera from 498 anti-TNF naïve healthy adults from the Exeter Ten Thousand study to data from our therapeutic drug monitoring service and Personalised Anti-TNF Therapy in Crohn's disease (PANTS) cohort to explore associations with drug level and treatment outcomes.

Results

The 80% one-sided lower confidence interval of the 99th centile concentration for anti-infliximab and –adalimumab antibodies were lower than the manufacturers threshold of 10 (arbitrary units (AU)/mL; 9 AU/mL and 6 AU/mL, respectively. Using these new thresholds in the therapeutic drug monitoring cohort, more adalimumab- than infliximab- (11.1% [814/7,272] vs 3.1% [390/12,683] p<0.001) treated patients were reclassified antibody positive. Adalimumab drug concentrations in this reclassified group (median 8.1, interquartile range [IQR] 5.5 – 11 mg/L) were lower than those below the new threshold (median 9.9, IQR 7.1 – 13 mg/L; p<0.001), but higher than at the manufacturer's threshold (median 5.9 mg/L,

IQR 3.5 – 8.7 for anti-adalimumab 10-29 AU/ml; p<0.001). No difference in infliximab drug concentration was observed using the new or manufacturers positivity threshold (p=0.11).

In the PANTS cohort, patients with anti-adalimumab antibody concentrations at or above the new threshold were more likely to be in primary non-response (25/68 [37%] vs. 64/332 [19%], p=0.004), and non-remission at week 54 (51/62 [82%] vs. 168/279 [60%], p=0.001), than patients with anti-drug antibody concentrations in the group below the new threshold (0 – 5 AU/mL); this was not seen for anti-infliximab antibodies.

Conclusion

Laboratories should derive antibody positivity thresholds for assays they use. For adalimumab; low-concentration anti-drug antibodies were associated with lower drug levels and treatment failure.

Introduction

Biopharmaceuticals, or biologics, are large complex proteins manufactured in, or derived from, living sources. The anti-tumor necrosis factor (TNF) therapies, infliximab and adalimumab, are the most widely used biologics for treating immune-mediated diseases, including inflammatory bowel disease, and in 2018, they accounted for an expenditure in excess of $29 billion in the United States alone (1). Repeated administration, however, often induces the formation of anti-drug antibodies that lead to drug clearance and treatment failure (2 – 5).

Pharmacokinetic therapeutic drug monitoring (TDM) in patients with inflammatory bowel disease, improves durability of response, safety, and cost-effectiveness of anti-TNF therapy, compared to empirical dosing alone (6 – 9). Debate remains, however, how best to measure drug and anti-drug antibody levels and whether TDM is best undertaken proactively during routine follow-up, or whether reactive TDM at the time of loss of response is adequate (10). Recent data support proactive TDM because it allows optimization of drug levels and earlier detection of anti-drug antibodies, which provides a window of opportunity for clinicians to suppress immunogenicity by introducing an immunomodulator (9, 11 – 16).

Enzyme-linked immunosorbent assays (ELISAs) are the most commonly analytical methods for the measurement of anti-TNF drug and anti-drug antibody levels (17, 18). Most studies have reported results using ‘drug-sensitive’ or ‘free’ antibody assays. ‘Drug-tolerant’ or ‘total’ antibody assays, include a pre-analytical acid antibody-drug disassociation step. This allows antibodies to be detected earlier, at

a potentially reversible stage, when drug is still present. These assays are therefore ideally suited for proactive TDM (17).

The Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust uses the Immundiagnostik AG (IDKmonitor) drug-tolerant anti-infliximab and anti-adalimumab antibody assays for its national TDM service. The positivity threshold is defined by the manufacturer as 10 arbitrary units (AU)/mL. We sought to validate this positivity threshold for both assays and to describe the relationship between drug and anti-drug antibody levels and clinical outcomes using these new positivity thresholds.

Methods

Study design

We designed three related studies in mutually exclusive cohorts: -

1. To validate the positivity thresholds of the IDK drug-tolerant anti-TNF antibody assays, we tested sera from healthy individuals who had not been exposed to anti-TNF therapies (EXTEND cohort).
2. To explore the relationship between drug and anti-drug antibody levels and the impact on clinical reporting at the new positivity threshold we used paired drug and antibody data from our TDM Clinical Service (Exeter TDM cohort).
3. To test whether anti-drug antibody concentrations using the new positivity thresholds were associated with treatment failure, we reanalysed data from the prospective Personalised Anti-TNF Therapy in Crohn's disease study at the new positivity threshold (PANTS cohort).

Participants and outcome definitions

Validating the positivity threshold

The Exeter Ten Thousand (EXTEND) cohort is prospective cohort study with a recallable biorepository designed to understand genetic contributions to common diseases. To be included, adult volunteers, needed to live within 25 miles of the city of Exeter, in the South West of England, United Kingdom (EXTEND; www.exeter10000.org). Participants were invited to a single 30-minute appointment when they completed a short self-reported questionnaire about their health and lifestyle and provided urine and blood samples. We tested sera from a random sample of 498 healthy volunteers from this cohort for antibodies to infliximab and adalimumab, who were not taking regular medications and had

never been exposed to anti-TNF therapies. We validated the positivity thresholds as the 80% one-sided lower confidence interval of the 99thcentile of antibody concentration in the EXTEND cohort, as per United States Food and Drug Administration and European Medicines Agency guidelines for validating confirmatory assays (19, 20).

Exploring the relationship between drug and antibody levels

The Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust provides an anti-TNF TDM clinical service to hospitals throughout the UK. Requests come from physicians who work in a variety of disciplines; the majority are from gastroenterologists administering anti-TNF therapy for inflammatory bowel disease. Clinicians are asked to send trough drug levels, but no clinical data is linked to TDM test requests. We applied the new positivity thresholds to anti-infliximab and –adalimumab antibody results from the Exeter laboratory TDM cohort (21). We compared drug levels in antibody positive patients using the manufacturer's threshold and the new threshold.

In all patients with paired drug and anti-drug antibody results at the time of last testing, we assigned the proportion of patients who had *clearing* (antibody positive, drug negative; < 0.8mg/L) and *non-clearing anti-drug antibodies* (antibody positive, drug positive; ≥ 0.8mg/L) using the new thresholds compared to using the manufacturers threshold. In order to explore the effect of lowering the diagnostic positivity threshold on the prevalence of transient antibodies, in patients who had multiple anti-drug antibody tests, we classified the proportion of patients who had consistently *negative* (all antibody tests negative); *transient* (a single positive test

with subsequent negative test); *single last-test positive* (last test positive with no subsequent antibody measurements) and *persistent* (at least two positive tests) *anti-drug antibodies*.

Investigating antibody positivity and treatment failure

PANTS is a UK-wide, multicenter, prospective observational cohort reporting the treatment failure rates of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar CT-P13 [Celltrion, South Korea]), and adalimumab (Humira [Abbvie, USA]) in 1,610 anti-TNF naïve patients with active luminal Crohn's disease (3). Treatment failure endpoints were primary non-response at week 14 and non-remission at week 54. Primary non-response was defined as exit for resectional surgery or corticosteroid use at week 14. Patients who exhibited both a failure of C-reactive protein to fall to ≤3 mg/L or by 50% from baseline and a failure of Harvey Bradshaw Index (22) to fall to ≤4 or by 3 points were also classified as primary non-response. For children, a failure of short Pediatric Crohn's Disease Activity Index (23) to fall to <15 or by more than 12.5 points was used. Response and grey zone were intermediate categories based on improvements in symptoms and/or C-reactive protein, respectively. Remission was defined at week 14 and 54 as a C-reactive protein of ≤3 mg/L and Harvey Bradshaw Index of ≤4 points (short Pediatric Crohn's Disease Activity Index ≤15), without ongoing steroid therapy or exit for treatment failure.

Laboratory methods

All laboratory analyses were performed at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust. Anti-TNF drug

and anti-drug antibodies were measured on the Dynex Technologies (Chantilly, Virginia, USA) DS2 automated ELISA platform.

Drug tolerant anti-TNF antibody assays

The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor infliximab (K9654) and adalimumab (K9651) total anti-drug antibody assays allow semi-quantitative measurement of both 'free and bound anti-drug antibodies (24, 25). A pre-treatment acid dissociation step is used to separate anti-drug antibodies from the therapeutic antibody. The assay then follows a standard ELISA format using recombinant therapeutic antibody as a capture and detection antibody. For both assays, the manufacturer established a positivity threshold by linear dilution of sera with high concentrations of anti-TNF antibody until no further linear dilution was possible; 10 AU/ml for both assays. The manufacturer then validated the anti-TNF antibody threshold in sera from 40 anti-TNF naïve individuals.

The infliximab and adalimumab total anti-drug antibody assays have measuring ranges of 4.5-400 AU/mL and 5.5-200 AU/mL respectively. Based on analysis of pooled patient serum quality control, the intra-assay coefficient of variation is ≤ 8.7% at 11.8 AU/mL for the infliximab total anti-drug antibody assay (n=128), ≤13.16% at 12.7 AU/mL for adalimumab antibodies (n=130). The manufacturer's recommended positivity threshold for both total anti-TNF drug antibody assays is 10 AU/mL.

Anti-TNF drug level assays

The IDKmonitor free infliximab (K9655) and adalimumab (K9657) drug level assays

permit quantitative measurement of free therapeutic drug in serum. The assays follow a standard ELISA format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody. The measuring range for both assays is 0.8 - 45 mg/L, with absence of drug being defined using a cutoff of <0.8 mg/L.

Statistical analysis

Statistical analyses were undertaken in R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). All analyses were two tailed, unless otherwise stated, and p-values <0.05 were considered significant. Summary descriptive statistics are presented as median and interquartile ranges for continuous variables and as percentages for categorical variables.

Validating the positivity threshold

We constructed cumulative distribution plots of anti-drug antibody concentrations from the EXTEND cohort and used bootstrapping to calculate the 80% one-sided lower confidence interval of the 99th centile to define anti-infliximab and anti-adalimumab antibody assay threshold (19,20).

Exploring the relationship between drug and antibody levels

To visualize the relative effects of changing from the manufacturer's positivity thresholds to the newly validated thresholds, we also constructed cumulative distribution plots of anti-infliximab or –adalimumab antibody concentrations in all patients at the time of last testing in the Exeter TDM cohort. We used pairwise Mann-Whitney U tests to compare median drug concentrations in patients with

increasing anti-drug antibody concentrations. Anti-drug antibody levels for each drug were categorized as follows: positive using the new positivity threshold, positive using the manufacturer's threshold, and based on cut-offs established in the PANTS study; moderate and high antibody concentrations (30-99 AU/mL and ≥100 AU/ml respectively) (3). Differences between proportions of patients with clearing, non-clearing, transient and persistent anti-drug antibodies using the manufacturers and the newly validated positivity thresholds, were sought using chi-squared analyses.

Investigating antibody positivity and treatment failure

We collapsed the predefined treatment outcomes from the PANTS study – grey zone and response, into the remission category at week 14. We used chi-squared analyses to detect differences in rates of primary non-response at week 14 and non-remission at week 54 between patients with increasing antibody concentrations using the categories described above (3).

Ethical considerations

In line with Health Research Authority guidelines, formal ethical approval for our TDM service evaluation was not required (26). The sponsor of both the EXTEND and PANTS studies is the Royal Devon and Exeter NHS Foundation Trust. The South West Research Ethics Committee approved both studies (REC Reference: 14/SW/1089 for Exeter 10,000; November 2009, REC Reference: 12/SW/0323 for the PANTS study; January 2013). Patients were involved in the design of both the EXTEND and PANTS cohorts.

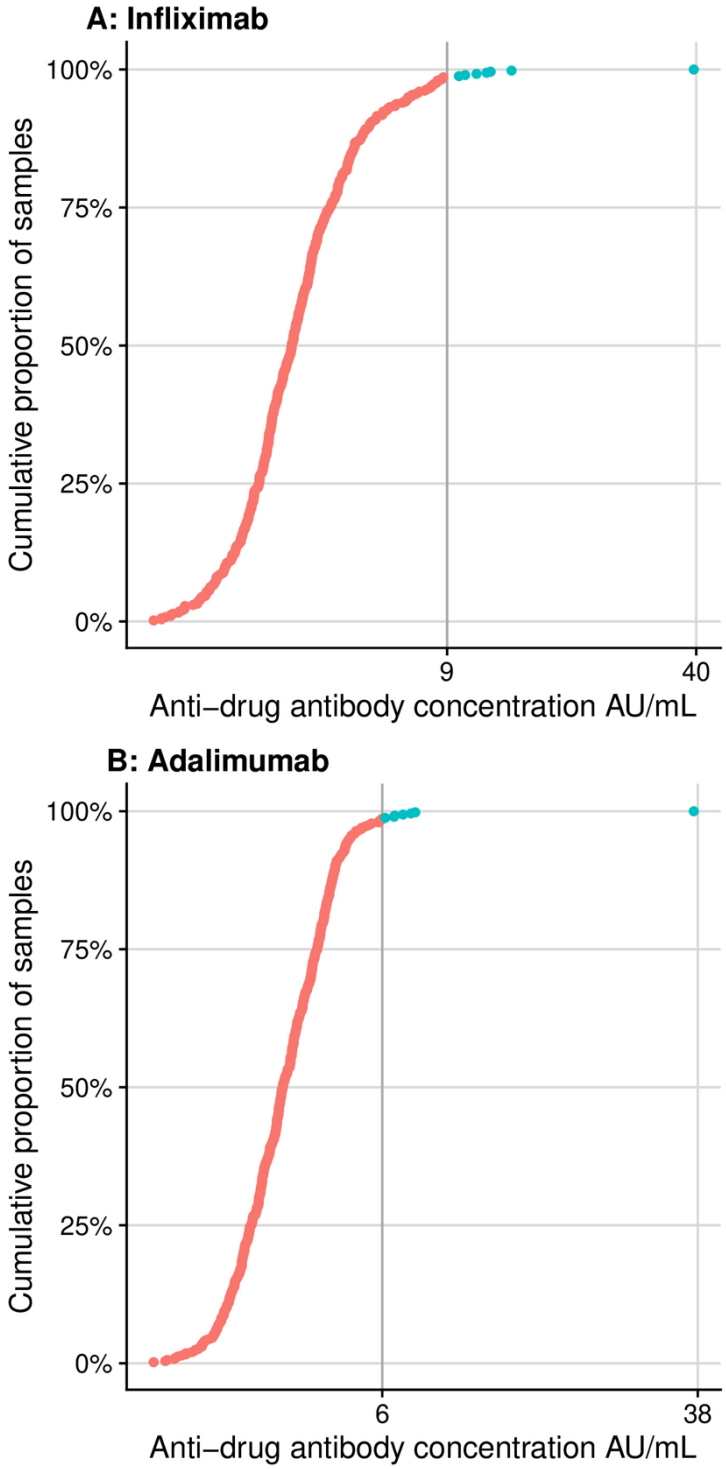
Results

Defining the positivity threshold

We obtained sera from 498 healthy volunteers who had not been exposed to anti-TNF therapies: 54% (269/498) were female, 91.3% (455/498) were white European, with a median age of 48 (interquartile range [IQR] 39-58) years. Overall, 5.2% (26/498) were current smokers. At inclusion 39.4% (196/498) individuals were overweight (Body Mass Index 25 – 29.9 kg/m^2) and 14.4% (72/498) were obese (Body Mass Index >30 kg/m^2).

Cumulative distribution plots for anti-TNF drug concentrations in the healthy volunteers from the EXTEND cohort are shown in Figure 1A and 1B. The 80% one-sided lower confidence interval of the 99th centile concentrations for anti-drug antibodies to infliximab and adalimumab were 9 AU/mL and 6 AU/mL, respectively, both lower than the manufacturers recommended threshold of 10 AU/mL (the point estimate of the 99% centiles were 10 AU/mL for antibodies to infliximab and 6 AU/mL for antibodies to adalimumab).

Figure 1A and 1B: Cumulative distribution plots of anti-drug antibody concentrations (on a log scale) in 498 biologic-naïve healthy volunteers, using our drug-tolerant anti-infliximab (A) and anti-adalimumab (B) assays, respectively. The vertical line denotes the 80% one-sided lower CI of the 99th centile.



Exploring the relationship between drug and antibody levels

Between January 2012 and December 2019, 32,490 paired infliximab and 11,830 adalimumab drug and anti-drug antibody assays in 12,683 and 7,272 patients, respectively were analyzed as part of the routine TDM service in Exeter.

At the time of last testing, immunogenicity was more common in infliximab- than adalimumab-treated patients, irrespective of whether we used the manufacturers or the newly validated positivity threshold (Figure 2A and 2B). Using the manufacturer's threshold of 10 AU/mL, anti-infliximab antibodies were detected in 47.8% (6,068/12,683) patients compared to 24.4% (1,771/7,272) adalimumab-treated patients ($p<0.001$). The proportion of patients reclassified as positive with anti-drug antibodies using the newly validated positivity thresholds (infliximab 9 AU/mL and adalimumab 6 AU/mL), was greater in adalimumab (11.1% [814/7,272]) than infliximab (3.1% [390/12,683]) treated patients ($p<0.001$). Reducing the positivity threshold resulted in more patients classified with non-clearing antibodies to both infliximab (manufacturer's threshold 26.7% (2,678/12,683) vs. newly validated threshold 29.4% (3,733/12,683) $p<0.001$) and adalimumab (manufacturer's threshold 15.8% (1,146/7,272) vs. newly validated threshold 26.7% (1,941/7,272) $p<0.001$): but had no effect on the proportions of patients with clearing antibodies, to either drug (Table 1).

Figure 2A and 2B: Cumulative distribution plots of anti-drug antibody concentrations (log scale) measured by the Exeter therapeutic drug monitoring service from January 2012 to December 2019 n= 32,940 samples from infliximab- (A) n= 12,683 and adalimumab- (B) n = 7,272 treated patients. Vertical lines indicate our newly validated positivity thresholds of 9 AU/mL and 6 AU/mL for infliximab and adalimumab, respectively, and the manufacturer's threshold of 10 AU/mL. Samples in pink are those less than the newly validated threshold, in green are those between the newly validated and manufacturer's threshold, and in blue are those above the manufacturer's threshold.

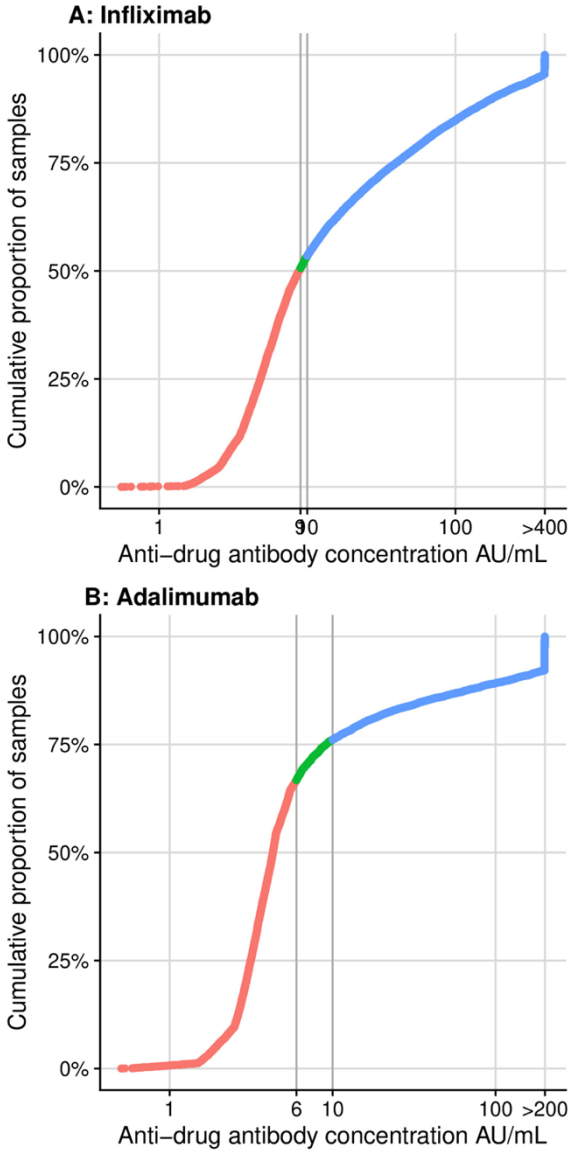


Table 1: Antibody status, stratified by type of drug and applied threshold, in patients tested in the Exeter therapeutic drug monitoring cohort

	Infliximab		Adalimumab	
Antibody status	**Manufacturer's threshold (10 AU/mL)**	**Newly validated threshold (9 AU/mL)**	**Manufacturer's threshold 10 AU/mL)**	**Newly validated threshold (6 AU/mL)**
Patients tested				
Clearing[1]	**21.1%** (2,678/12,683)	**21.5%** (2,725/12,683)	**8.6%** (625/7,272)	**8.9%** (644/7,272)
Non-clearing[2]	**26.7%** (3,390/12,683)	**29.4%** (3,733/12,683)	**15.8%** (1,146/7,272)	**26.7%** (1,941/7,272)
Patients with more than one sample				
Negative[3]	**40.8%** (2,515/6,170)	**36.9%** (2,278/6,170)	**70%** (1,872/2,673)	**53.6%** (1,434/2,673)
Transient[4]	**8.8%** (540/6,170)	**8.6%** (530/6,170)	**6.8%** (182/2,673)	**9.5%** (255/2,673)
Single last test positive[5]	**9.1%** (564/6,170)	**9.7%** (597/6,170)	**6.7%** (179/2,673)	**10.5%** (280/2,673)
Persistent[6]	**41.3%** (2,551/6,170)	**44.8%** (2,765/6,170)	**16.5%** (440/2,673)	**26.3%** (704/2,673)

[+]Chi-square test performed

[1] Positive anti-drug antibody result with an undetectable drug level

[2] Positive anti-drug antibody result with a detectable drug level

[3] All anti-drug antibody tests negative

[4] A single positive anti-drug antibody test with subsequent negative test

[5] Last anti-drug antibody test positive with no subsequent anti-drug antibody measurements

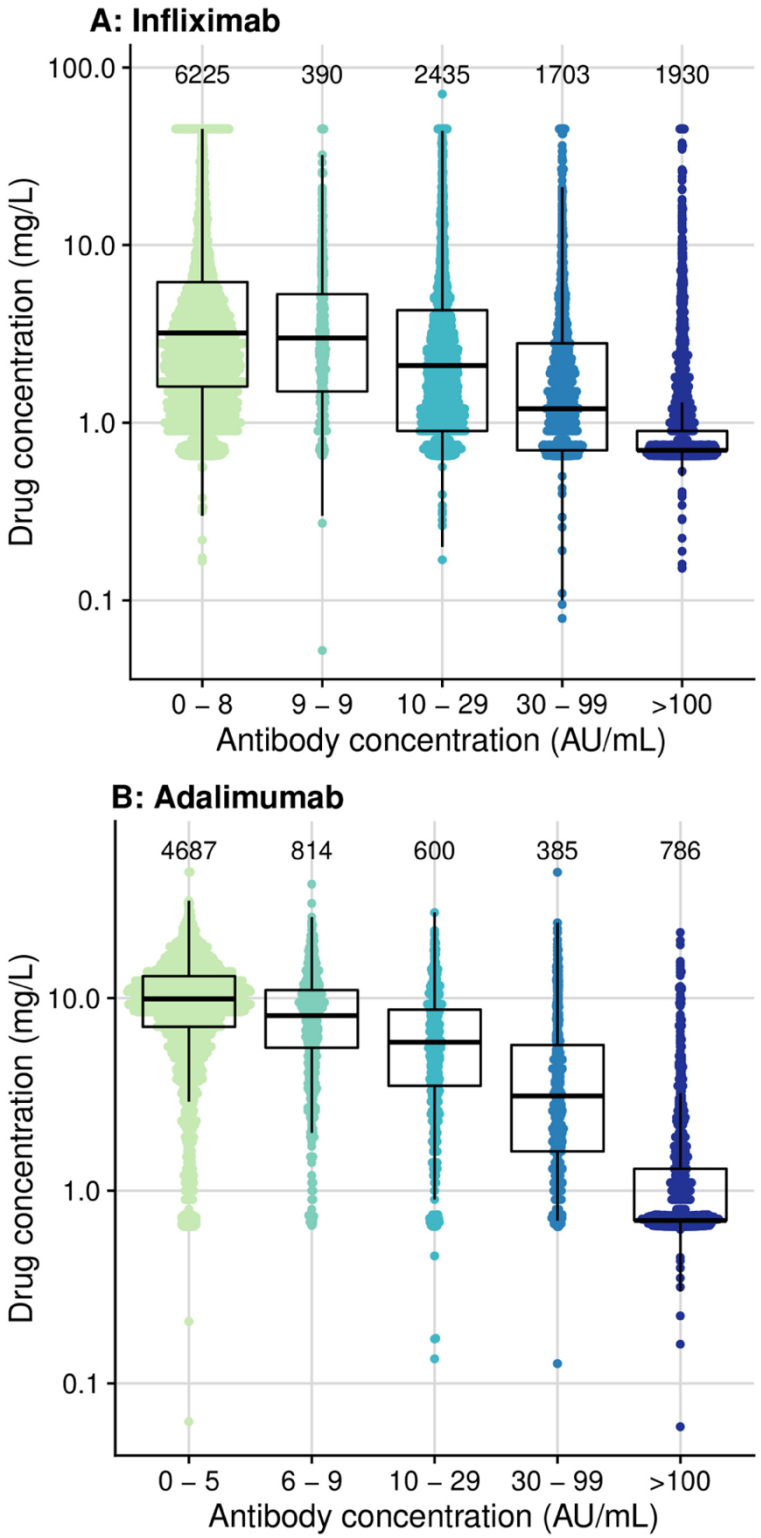
[6] At least two anti-drug antibody positive tests

In total, 6,170 and 2,673 patients had more than one anti-infliximab and anti-adalimumab antibody level tested, respectively. The median number of tests per patient was 3 (range: 2 – 4) for infliximab and 2 (range: 2 – 3) for adalimumab-treated patients.

Reducing the positivity threshold resulted in more patients classified with persistent anti-drug antibodies to both infliximab (manufacturer's threshold 41.3% [2,551/6,170]) vs. newly validated threshold 44.8% [2,765/6,170] p<0.001) and adalimumab (manufacturer's threshold 16.5% (440/2,673) vs. newly validated threshold 26.3% (704/2,673) p<0.001). The proportions of adalimumab-, but not infliximab-treated, patients whose last and only anti-drug antibody test was positive or who had transient antibodies increased following the reclassification of anti-drug antibody test results (Table 1).

The effect of progressively increasing anti-drug antibodies on infliximab and adalimumab drug concentrations is shown in Figures 3A and 3B, respectively. Adalimumab concentrations in the newly reclassified positive group (6 – 9 AU/mL) were lower (median adalimumab concentration 8.1, IQR 5.5 – 11 mg/L), than in the group below the new threshold (≤5 AU/mL) (median adalimumab concentration 9.9, IQR 7.1 – 13 mg/L; p<0.001) but were not as low as in the group above the manufacturer's threshold (10 - 29 AU/mL). There was not a significant difference between infliximab concentrations for patients with an anti-infliximab concentration of 9 AU/mL (the group reclassified with the lowered threshold) and those with an anti-infliximab concentration of <9 AU/mL (p=0.11).

Figure 3A and 3B: Bee-swarm box and whiskers plot showing anti-infliximab (A) and anti-adalimumab (B) antibody concentration plotted against drug concentration for samples received through the Exeter therapeutic drug monitoring service.



Investigating antibody positivity and treatment failure

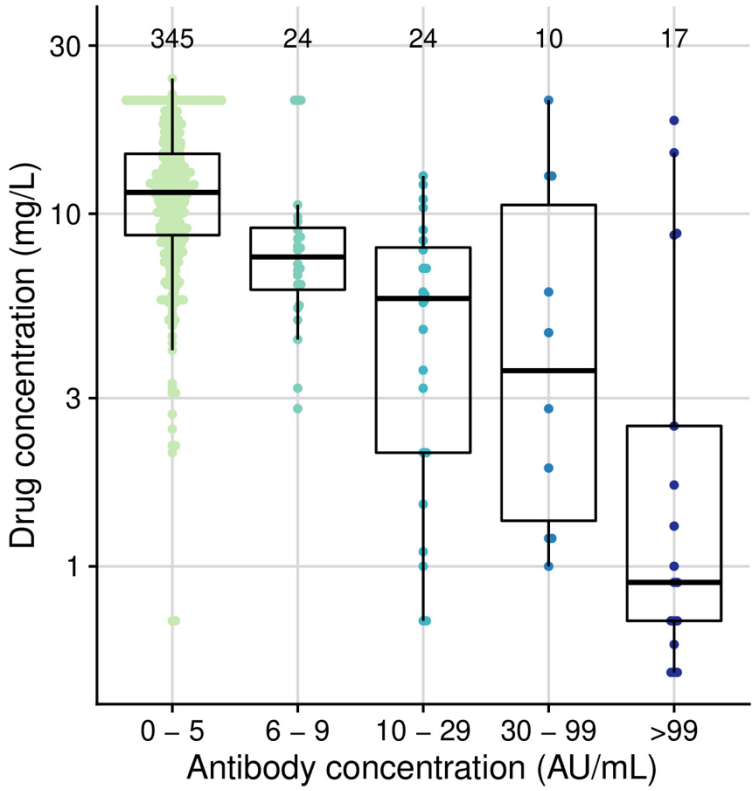
The difference between the new anti-infliximab antibody positivity threshold (9 AU/mL) and manufacturers threshold (10 AU/mL) is very small. When applied to the PANTS cohort only 1.7% (11/658) infliximab-treated patients would be reclassified as antibody positive at week 14 compared to 5.7% (24/420) for adalimumab-treated patients. In view of the small proportion of infliximab treated patients reclassified as positive in the PANTS cohort, the relationship between antibody positivity and treatment failure has not been investigated in this group. Adalimumab-treated patients on combination therapy with an immunomodulator were less likely to develop anti-drug antibodies above our new threshold of 6 AU/mL compared to patients on monotherapy with adalimumab only ($p<0.001$; Table 2).

Table 2: Anti-adalimumab antibody concentration, stratified at week 14 by immunomodulator use at baseline

Antibody concentration (AU/mL)	**Immunomodulator (n = 227)**	**No immunomodulator (n = 193)**
<6	205/227 (90%)	140/193 (73%)
6 - 9	6/227 (3%)	18/193 (9%)
10 - 29	9/227 (4%)	15/193 (8%)
30 - 99	3/227 (1%)	7/193 (4%)
>99	4/227 (2%)	13/193 (7%)

Week 14 adalimumab drug concentrations in the reclassified positive group (6-9 AU/mL), were lower (median 7.6, IQR 6.1-9.1 mg/L) than in the group below the new threshold (0 – 5 AU/mL) (median 11.5, IQR 8.7-14.8 mg/L, p<0.001), but were not as low as individuals above the manufacturer's threshold (10-29 AU/mL) (median 5.8, IQR 2.1-8.0 mg/L, p=0.04) (Figure 4).

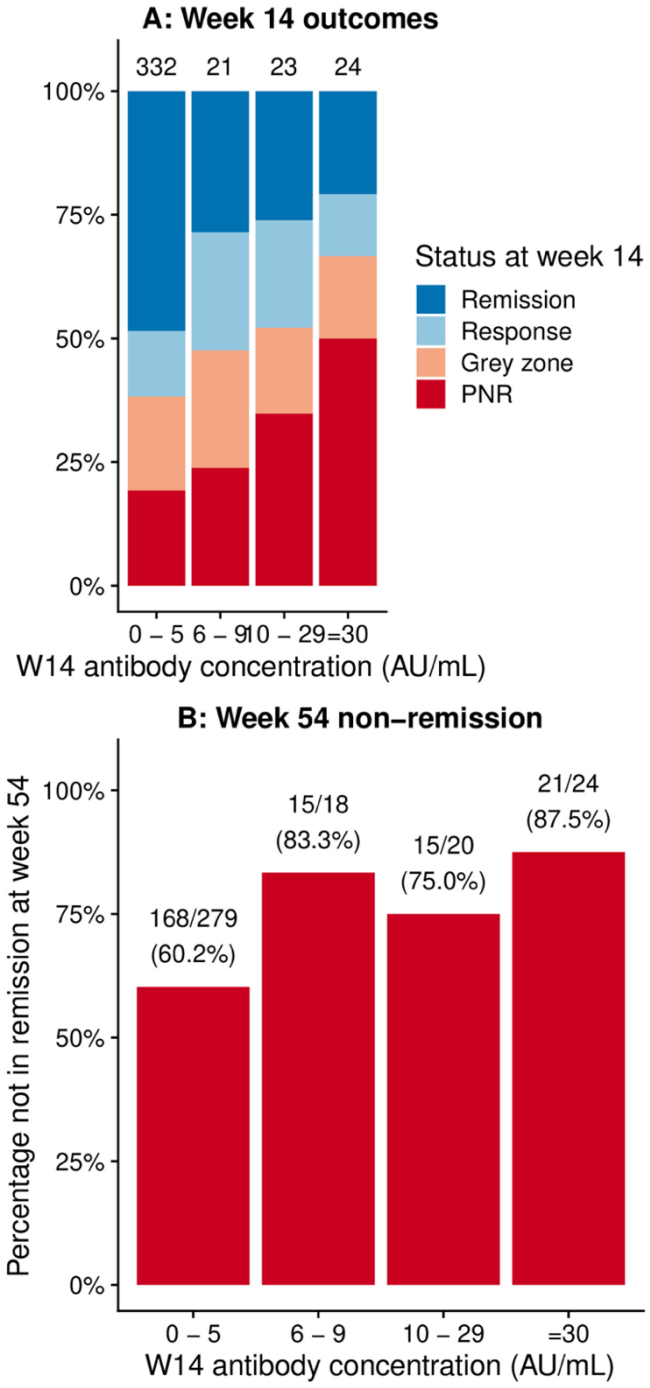
Figure 4: Bee-swarm box and whisker plot showing anti-drug antibody concentration against adalimumab concentration for 420 samples received in the first year of the PANTS study.



Using the prespecified outcome definitions from PANTS, at week 14, patients with anti-adalimumab antibody concentrations at or above the new threshold were more likely to be in primary non-response (25/68 [37%] vs. 64/332 [19%], p=0.004), and non-remission at week 54 (51/62 [82%] vs. 168/279 [60%], p=0.001), (Figures 5A

and B) than patients with anti-drug antibody concentrations in the group below the new threshold (0 – 5 AU/mL).

Figure 5: (A) Stacked barchart showing proportion of adalimumab-treated patients in the PANTS study meeting criteria for predefined treatment outcomes, stratified by week 14 anti-adalimumab antibody concentration and (B) barchart showing the proportion of adalimumab-treated patients in the PANTS study in remission at week 54, stratified by week 14 antibody concentration.



Discussion

Key findings

We have demonstrated that the positivity thresholds for the IDKmonitor drug tolerant anti-infliximab and anti-adalimumab antibody assays are lower than the manufacturer's suggested threshold of 10 AU/mL for both infliximab (9 AU/mL) and adalimumab (6 AU/mL). This was done in a cohort of almost 500 anti-TNF naïve individuals from the Exeter 10,000 study.

Immunogenicity was more common in infliximab than adalimumab-treated patients. The new anti-drug antibody thresholds, however, differentially increased rates of persistent, non-clearing anti-drug antibodies for adalimumab-treated patients. Anti-TNF anti-drug antibody concentrations above the newly validated, but below the manufacturer's recommended positivity thresholds were associated with intermediate drug concentrations for adalimumab. In the PANTS cohort this translated to higher rates of primary non-response and non-remission at week 54 in adalimumab-, but not infliximab-treated patients.

Interpretation

Because antibody responses are heterogeneous, there is a lack of standardized antibody testing material meaning manufacturers define positivity thresholds in small cohorts of healthy individuals (17, 18). There are several potential explanations to account for why the new positivity thresholds for both anti-TNF antibody assays were lower than the manufacturer's recommended thresholds. Most importantly, our sample was more than ten times larger than the manufacturer's original cohorts (24, 25), meaning we were able to report positivity

thresholds with greater precision: in particular, for the anti-adalimumab assay where the prevalence, and variance of anti-drug antibody concentrations were less than the anti-infliximab concentrations. Furthermore, compared to the manufacturer's original convenience cohorts, our selection of patients without any comorbidities from the Exeter 10,000 cohort were less likely to have had cross-reactive anti-allotype antibodies such as rheumatoid factor (27, 28).

The reasons why we see a larger difference between the manufacturer's and the new positivity thresholds for the anti-adalimumab than anti-infliximab antibody assays are less clear. One explanation may relate to differences in the prevalence of pre-formed antibodies to the drugs (29, 30). Because of recognition of xenotopes in the mouse variable domains of the chimeric antibody, as a result of environmental exposure to rodents, pre-formed antibodies are more commonly detected by anti-infliximab than anti-adalimumab antibody assays (31, 32).

Establishing the prevalence and clinical impact of transient anti-drug antibodies across studies is limited by a lack of standardized nomenclature and differences in type and drug-tolerance of the assays used (33 – 35). In this study, we have shown that lowering the positivity thresholds of the IDKmonitor anti-TNF antibody assays would not lead to a clinically meaningful increase in reporting of transient anti-drug antibodies. The significance of reporting a higher prevalence of persistent, non-clearing antibodies when lowering the positivity threshold is less clear. We recognise that there will always be a balance between test sensitivity and specificity; using the manufacturer's positivity thresholds, these were not well defined or validated. We benchmarked specificity on 99% based on international

guidelines, however, there is a potential for the newly classified group to be false positives. Equally, increasing test sensitivity by reducing the positivity threshold may allow detection of true positives earlier in their development before leading to drug clearance.

In the PANTS study (3), like in other studies (34, 36 – 38), immunogenicity only impacted clinical outcome if the antibodies led to drug clearance. Studying the function of non-clearing antibodies is hampered by analytical difficulties of excluding drug from ex-vivo samples whilst maintaining a functional antibody product (17, 18, 39). Further work is needed to understand their natural history; for example, asking do non-clearing antibodies eventually clear drug with further maturation; do they neutralize drug; or are they simply bystanders? For now, earlier detection of anti-drug antibodies may allow the introduction of an immunomodulator, or anti-TNF dose optimization, to mitigate immunogenicity. Because these antibodies may be false positives or transient, repeat testing should occur before treatment changes.

Limitations

Data submitted by participants recruited to the EXTEND cohort were self-reported and not externally validated against primary or secondary care records, potentially leading to information bias with respect to past medical history and previous anti-TNF exposure. This may account for differential rates of immunogenicity observed. The Exeter TDM cohort is a non-selected clinical referral cohort and although we recommend that blood sampling occurs just before the next dose, inevitably, some non-trough samples will have been processed. Because anti-drug antibody assays

are not completely drug tolerant, this is likely to bias the data by underestimating of rates of immunogenicity (40). This effect may be more important in adalimumab-treated patients where TDM testing is more often ad-hoc rather than immediately before administration of drug. In addition, we have only studied the IDKmonitor assays here: users of other assays should consider validating their positivity thresholds using similar methodologies. Finally, in the PANTS cohort, we used pragmatic definitions of remission closely aligned to routine treatment targets: we accept that our data would have been strengthened by endoscopic outcomes.

Generalisability

As over 90% of participants in both the Exeter 10,000 and PANTS studies were white European, it is highly likely that our findings using the IDKmonitor anti-TNF drug-tolerant antibody assays are generalizable to other cohorts of white European patients with inflammatory bowel disease. Whether our results are generalizable to other ethnicities, where rates of anti-drug antibody formation are lower, is less certain (41, 42). Furthermore, whether lower thresholds are clinically relevant in other immune-mediated disorders, such as rheumatoid arthritis, where autoantibodies frequently cross-react in anti-drug antibody ELISA assays, is also unknown (31). Manufacturers of other assays should consider validating their positivity thresholds using similar methodologies.

Conclusions

Laboratories should independently derive antibody positivity thresholds for assays they use as demonstrated here for the IDKmonitor drug-tolerant anti-drug antibody assays. Our findings suggest that lowering the positivity threshold of the anti-adalimumab antibody assay to 6 AU/mL may add value to the use of this test. Changing to the lower thresholds differentially increased the rates of persistent, non-clearing antibodies to both infliximab and adalimumab. Anti-drug antibody concentrations above the newly validated thresholds, but below the manufacturer's threshold, were associated with intermediate drug concentrations that were related to treatment failure in adalimumab- but not infliximab-treated patients.

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There was no external funding for this study (other than supply of assay kits as above). The sponsor for the study was the Royal Devon and Exeter NHS Foundation Trust.

Data availability statement:

The data underlying this article are available in the article.

Conflict of interest disclosures:

T.A reports grants from AbbVie and MSD, grants and other from NAPP, grants from Celltrion, grants from Pfizer, personal fees and non-financial support from Immunodiagnostik, grants from Celgene, during the conduct of the study; personal fees and non-financial support from NAPP, personal fees and non-financial support from AbbVie , personal fees and non-financial support from MSD , personal fees from Celltrion, personal fees from Pfizer, grants and personal fees from Takeda, grants from Janssen, grants and non-financial support from Tillotts, outside the submitted work; J.R.G received honoraria from Falk, Abbvie and Shield therapeutics for unrelated topics; N.A.K received personal fees from Falk, Takeda, Pharmacosmos and other from Janssen, and non-financial support from Janssen, AbbVie and Celltrion outside the submitted work;

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Immundiagnostik AG (Bensheim, Germany) provided the IDKmonitor total antibody assays for the experiment to determine the positivity thresholds. They did not have a role in any of the following: the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and decision to submit the manuscript for publication.

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Chapter 4: Understanding anti-TNF treatment failure: Does serum triiodothyronine-to-thyroxine (T3/T4) ratio predict outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease?

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Study aim

To assess whether baseline serum fT3/fT4 ratio predicted primary non-response and non-remission to infliximab and adalimumab in patients with Crohn's disease.

My role in the study

I was responsible for designing the research study and determining the aims, objectives. I determined the methodology used. I was responsible for sample preparation prior to measurement of serum thyroid-stimulating hormone, triiodothyronine and thyroxine levels by the department of biochemistry. I analysed the results. I prepared the abstract for submission at the European Crohn's and Colitis Organisation annual conference was accepted as a poster presentation, which I authored. I wrote up the study, and submitted it to for publication, revising it at each stage to align with multiple editors' and peer reviewers' comments.

Findings

Lower baseline serum free triiodothyronine/thyroxine (fT3/fT4) ratio was associated with female sex, corticosteroid use and disease activity and predicted primary non-response to anti-TNF treatment at week 14, but not non-remission at week 54.

Relevance and impact on my learning

I conceived this study, alongside the other first-named authors, Simeng Lin and Isabel Carbery, in response to a paper that was published in Alimentary Pharmacology & Therapeutics. My study found opposing results to the already published findings. Initially, I was going to publish the study as a letter of response, but instead conducted a broader, more comprehensive experiment that

necessitated a formal paper. Following publication of my study's results, the authors of the original published paper responded with a linked editorial contextualising my findings alongside theirs. This exercise taught me how to engage with published research within my field of interest, and how I can scientifically debate and challenge other groups' works using an academic process.

By conducting this experiment, I learnt about the importance of conducting research using homogenous cohorts, and how known and unknown confounders (ie - age, sex, disease behaviour, genetic risk, steroid use) can affect the association between exposure and outcome. Given that anti-TNF treatment response is multifactorial and occurs via different physiological pathways, understanding the effect of one exposure on outcome remains very challenging in clinical research. One way to overcome this is to aim to replicate findings via muti-omic analyes, which will help improve our understanding of different contribution of genetic variants to anti-TNF treatment response.

Acknowledgements of co-authors and contributions to paper

Simeng Lin, myself, Isabel Carbery, Nicholas A Kennedy, Tariq Ahmad, Christian Selinger, and James R Goodhand participated in the conception and design of the work. Christian Selinger and Tariq Ahmad obtained the funding for the study. Claire Bewshea was the project manager and coordinated recruitment of participants. Rachel Nice and Timothy J McDonald coordinated all biochemical analyses and central laboratory aspects of the project. Simeng Lin, myself, Isabel Carbery, Malik Janjua, Rachel Nice, Timothy J McDonald, Claire Bewshea, Nicholas A Kennedy, Tariq Ahmad, Christian Selinger, and James R Goodhand were involved in the acquisition, analysis or interpretation of data. The data analysis was performed by Simeng Lin and Nicholas A Kennedy. Drafting of the manuscript was conducted by Simeng Lin, myself, Isabel Carbery, Nicholas A Kennedy, Tariq Ahmad, Christian Selinger, and James R Goodhand. All the authors contributed to the critical review and final approval of the manuscript.

Abstract

Introduction

During illness, adaptations of the hypothalamic-pituitary-thyroid axis reduce energy expenditure, protein catabolism and modulate immune responses to promote survival. Lower serum free (f) triiodothyronine-to-thyroxine (fT3/fT4) ratio has been linked to non-response to treatment in a range of diseases, including in biologic-treated patients with inflammatory bowel disease.

Aim

We sought to assess whether baseline serum fT3/fT4 ratio predicted primary non-response and non-remission to infliximab and adalimumab in patients with Crohn's disease.

Methods

Thyroid function tests were undertaken in stored serum from biologic-naïve adult patients with active luminal Crohn's disease immediately prior to treatment with infliximab (427 Remicade; 122 biosimilar CT-P13) or adalimumab (448 Humira) in the Personalised Anti-TNF Therapy in Crohn's Disease study (PANTS).

Results

Baseline median [IQR] fT3/fT4 ratios were lower in women than men (0.30 [0.27 - 0.34] vs 0.32 [0.28 - 0.36], p<0.001), in patients with more severe inflammatory disease, and in patients receiving corticosteroids (0.28 [0.25 - 0.33] vs 0.32 [0.29 - 0.36], p<0.001). Multivariable logistic regression analysis demonstrated that fT3/fT4 ratio was independently associated with primary non-response at week 14

(odds ratio [OR] 0.51, 95% confidence interval [CI] 0.31 – 0.85, $p < 0.001$), but not non-remission or changes in faecal calprotectin concentrations at week 54. The optimal threshold to determine primary non-response was 0.31 (Area Under the Curve 0.57 [95% CI 0.54 - 0.61] sensitivity 0.62 [95% CI 0.41 - 0.74], specificity 0.53 [95% CI 0.42 - 0.73]).

Conclusions

Lower baseline serum fT3/fT4 ratio was associated with female sex, corticosteroid use and disease activity and predicted primary non-response to anti-TNF treatment at week 14, but not non-remission at week 54.

Background

Ulcerative colitis (UC) and Crohn's disease are archetypal relapsing and remitting immune-mediated inflammatory diseases of the gut that affect about 1% of western populations.[1,2] Active disease is characterised by gastrointestinal inflammation, malnutrition, reduced quality of life and increased rates of depression.

During acute illnesses, adaptations of the hypothalamic-pituitary-thyroid axis reduce energy expenditure, protein catabolism and modulate immune processes to promote survival[3,4]. Most, if not all, critically ill patients have low serum free triiodothyronine (fT3), and low-normal free thyroxine (fT4) levels without a compensatory rise in thyroid stimulating hormone (TSH).[3,4] This so called non-thyroidal illness-, sick euthyroid- or low T3- syndrome, has been consistently linked to illness severity and outcome, including with COVID-19.[4,5] Similar observations have also been made in patients with chronic diseases including heart failure, renal failure, neurological dysfunction, and inflammatory bowel disease (IBD).[6–9]

The anti-TNF monoclonal antibodies, infliximab and adalimumab, are the most frequently prescribed biologic medications and have transformed the management of IBD. In Crohn's disease, successful treatment leads to mucosal healing, reduced surgeries and improvements in quality of life.[10,11] Regrettably, however, anti-TNF treatment failure is common. About one-quarter of patients experience primary non-response and one-third of initial responders lose response, such that only one-third of patients are in remission at the end of a year.[12]

The biology of non-response is complex, but being able to predict who will fail anti-TNF therapy could help prompt concomitant immunomodulator use, anti-TNF dose optimisation and biologic sequencing. Multiple patient, disease, and drug related factors have been implicated in anti-TNF treatment failure, but few studies have been adequately powered to define their relative effects, interactions, and impact on drug and anti-drug antibody levels.[13] In the PANTS study, we showed that obesity, cigarette smoking, higher baseline markers of disease activity, anti-TNF monotherapy and the development of antidrug antibodies are associated with low drug levels and anti-TNF treatment failure.[12] Carriage of the HLADQA1*05 allele confers a two-fold risk of developing antibodies to anti-TNF treatment.[14]
Recent data reported by Bertani *et al.,* showed that low serum triiodothyronine-to-thyroxine ratios (fT3/fT4) at initiation of infliximab or vedolizumab therapy predicted poor endoscopic outcomes at 54 weeks in a mixed cohort of patients with UC and Crohn's disease.[15]

We sought to assess whether baseline serum fT3/fT4 ratio predicted primary non-response and non-remission to infliximab and adalimumab in patients with Crohn's disease.

Methods

Study design

The Personalised Anti-TNF Therapy in Crohn's Disease study (PANTS) is a UK-wide, multicentre, prospective observational cohort reporting the treatment failure rates of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar, CT-P13 [Celltrion, South Korea]) and adalimumab (Humira [Abbvie, USA]) in anti-TNF-naïve patients with active luminal Crohn's disease.[12]

Patients were recruited at the time of first anti-TNF exposure between February 2013 and June 2016 and studied for 12 months or until drug withdrawal (Supplemental Table 1). Eligible patients were aged ≥ 6 years with objective evidence of active luminal Crohn's disease involving the colon and/or small intestine. Exclusion criteria included prior exposure to, or contraindications for the use of, anti-TNF therapy.

The choice of anti-TNF was at the discretion of the treating physician and prescribed according to the licensed dosing schedule. Study visits were scheduled at first dose, post-induction (week 14), and at weeks 30 and 54. Additional visits were planned for infliximab-treated patients at each infusion and for both groups at treatment failure or exit.

For this analysis, we included adult patients over the age of 17 years only because of limited or exhausted stored serum in paediatric patients. Patients who were

treated with endocrine-related medications that may have affected the hypothalamic-pituitary-thyroid axis, including thyroxine, carbimazole, growth hormone, and testosterone, or who had evidence of possible primary hypothyroidism or Grave's disease, were excluded.

Treatment failure endpoints were primary non-response at week 14, non-remission at week 54, and adverse events leading to drug withdrawal. We used composite endpoints defined using the Harvey Bradshaw Index (HBI)[16], corticosteroid use, and C-reactive protein (CRP).

Primary non-response (PNR): exit prior to week 14 for treatment failure (including resectional IBD surgery) or corticosteroid use at week 14 (new prescriptions or failure to taper). Patients who exhibited both a failure of CRP to fall to ≤3 mg/L or by 50% from baseline (week 0) and failure of HBI to fall to ≤4 or by 3 points were also classified as PNR.

Grey zone (intermediate between PNR and response): CRP falls to ≤3mg/L or by 50% from baseline (Week 0) or HBI falls to ≤4 or by 3 points from baseline (but not both).

Response: both CRP falls to ≤3mg/L or by 50% from baseline (Week 0) and HBI falls to ≤4 or by 3 points from baseline.

Remission: CRP of ≤3 mg/L and HBI of ≤4 points, no ongoing corticosteroid therapy, and no exit for treatment failure.

Non-Remission was assessed at week 54 and defined as either CRP of > 3mg/L or HBI of >4 points, ongoing corticosteroid therapy, or exit for treatment failure.

We defined corticosteroid therapy for the purposes of non-remission and PNR as any systemic therapy, including prednisolone and budesonide, either oral or intravenous. We included use of corticosteroids for other conditions, but excluded use single pre-biologic infusion dosing with hydrocortisone.

Patients excluded from effectiveness analysis: Three groups of patients were excluded from our effectiveness analyses.[12] First, patients with stomas, because the HBI is not validated in this patient group; second, patients that were recruited into the study with normal calprotectin and CRP concentrations at pre-screening and during the first visit; third, patients for whom the only indication for anti-TNF treatment was perianal disease.

Exit: Patients exited the study when they stopped anti-TNF therapy or had an intestinal resection. Patients who exited the study for treatment failure were deemed to be in non-remission for subsequent time points. Patients who exited the study for loss to follow-up, withdrawal of consent, or elective withdrawal of drug, including for pregnancy, were censored at the time of study exit and excluded from the denominator for subsequent analyses.

Clinical and laboratory variables

At baseline, sites recorded demographic data (sex, ethnicity, body mass index [BMI]), smoking status, age at diagnosis, disease duration, Montreal

classification[17], prior medical and drug history, and previous Crohn's disease-related surgeries. At every visit, disease activity score, weight, current therapy and adverse events were recorded.

Blood and stool samples were processed through the central laboratory at the Royal Devon & Exeter NHS Foundation Trust (https://www.exeterlaboratory.com/) for haemoglobin, white cell count, platelets, serum albumin, CRP, anti-TNF drug and anti-drug antibody concentrations, and faecal calprotectin, respectively. All analysis were carried out on the Cobas 801 module of the Cobas 8000 automated platform (Roche Diagnostics).

Serum thyroid stimulating hormone (TSH), triiodothyronine and thyroxine levels were measured on stored baseline samples between 30th November 2021 and 7th January 2022. The Roche Elecsys TSH immunoassay is an electrochemiluminescence assay that sandwiches TSH between biotinylated and ruthenium-complexed TSH-specific monoclonal antibodies.[18] The local reference range is 0.27 – 4.2 μIU/ml. The Roche Elecsys T3 and T4 electrochemiluminescence assays are competitive immunoassays. The reference ranges are 3.1 - 6.8 pmol/L and 1.2 - 22 pmol/L, respectively.

Study size & statistical methods

The assumptions underlying the PANTS sample size calculation have been reported previously[12]. Herein we included all adult patients who had sufficient stored serum at baseline for analysis who had outcome data at week 14.

Statistical analyses were undertaken in R 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two tailed and p-values <0.05 were considered significant. We included patients with missing clinical variables in analyses for which they had data and have specified the denominator for each variable. Continuous data are reported as median and interquartile range, and discrete data as numbers and percentages.

We performed univariable analyses using Fisher's exact and Mann-Whitney U tests to identify differences in baseline characteristics between infliximab- and adalimumab-treated patients, and to determine categorical factors associated with fT3/fT4 ratio and predefined outcomes. Spearman's rank correlation was used to determine continuous factors, including faecal calprotectin, associated with fT3/fT4 ratio. Multivariable logistic regression models were used to identify factors independently associated with primary non-response at week 14 and remission at week 54. Variables identified in the PANTS study as associated with each outcome were included in the model.[12] For infliximab-treatment, this included older age, smoking, immunomodulator use at baseline, and albumin, and for adalimumab-treatment, older age and BMI.

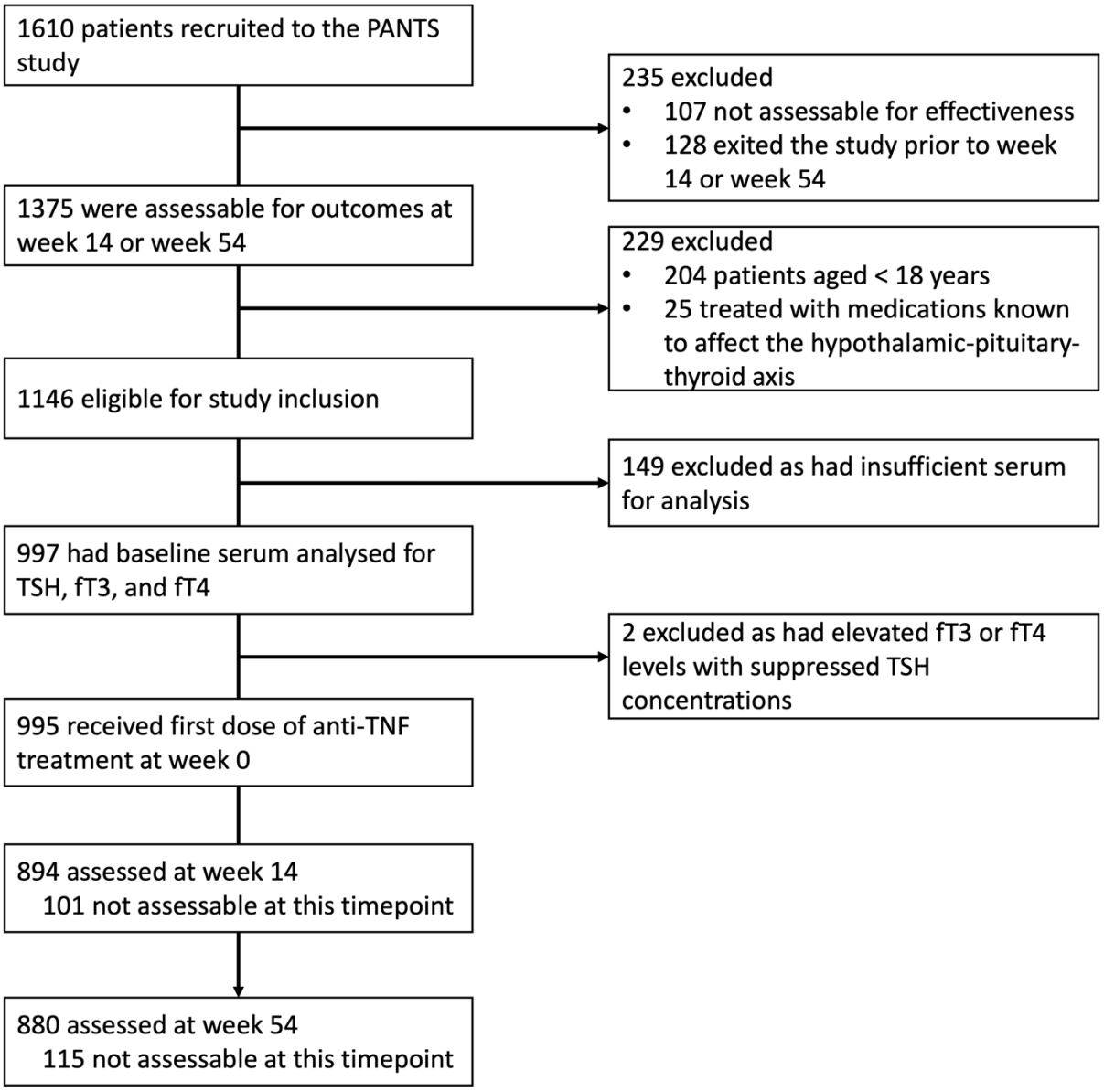
Youden's formula[19] was used to determine the optimal fT3/fT4 ratio cut-off to predict primary non-response, and receiver operator characteristic curves and area under the curve analyses with bootstrapping were used to estimate the diagnostic accuracy of the model. We performed sensitivity analyses restricting the cohort to patients not treated with corticosteroids at baseline and in line with the inclusion criteria in the study by Bertani *et al.,* to those over 60 years.

Results

Participants

Overall, 86.9% (997/1146 [86.9%]) adult patients who participated in PANTS were included: 549 (55.1%) were treated with infliximab (427 [42.8%] with originator infliximab, and 122 [12.2%] with biosimilar CT-P13) and 448 (44.9%) treated with adalimumab (Figure 1). We excluded 1.8% (25/1375) patients who were treated with medications known to affect the hypothalamic-pituitary-thyroid axis, including thyroxine, carbimazole, growth hormone, and testosterone. 0.2% (2/997) patients had elevated fT3 or fT4 levels with suppressed TSH concentrations, suggestive of hyperthyroidism, and were excluded. No patients had hypothyroidism. No differences were seen in baseline characteristics between patients who were included in the study and in whom we did not have sufficient serum for analysis.

Figure 1: Study profile. Patients were not assessable when one of more key data items were missing. *TSH=thyroid stimulating hormone, fT3=free triiodothyronine, fT4=free thyroxine.*



Differences between demographic and clinical characteristics of infliximab- and adalimumab-treated patients are shown in Table 1. Similar to the whole cohort[12], there were significant demographic differences at baseline between the infliximab- and adalimumab-treated patients, including in sex, age, ethnicity, disease behaviour and activity. At the initiation of anti-TNF treatment, no differences were seen in the proportion of patients treated with immunomodulators or corticosteroids.

Table 1: Baseline demographic and clinical characteristics, stratified by anti-TNF

Variable	Level	Adalimumab	Infliximab	Overall
n		448	549	997
Sex	Female	49.3% (221/448)	55.7% (306/549)	52.9% (527/997)
	Male	50.7% (227/448)	44.3% (243/549)	47.1% (470/997)
Ethnicity	White	95.5% (428/448)	92.3% (507/549)	93.8% (935/997)
	South Asian	2.7% (12/448)	2.9% (16/549)	2.8% (28/997)
	Other	1.8% (8/448)	4.7% (26/549)	3.4% (34/997)
Anti-TNF	Adalimumab	100% (448/448)	0% (0/549)	44.9% (448/997)
	Remicade	0% (0/448)	77.8% (427/549)	42.8% (427/997)
	CT-P13	0% (0/448)	22.2% (122/549)	12.2% (122/997)
Age at first dose of anti-TNF		38.6 (28.8 - 51)	34.6 (26 - 47.3)	36.3 (27.3 - 49.2)
Disease duration		3.1 (0.8 - 11.6)	3 (0.7 - 10.1)	3 (0.7 - 10.7)

Montreal disease location	L1	32.1% (141/440)	31.7% (173/545)	31.9% (314/985)
	L2	21.4% (94/440)	27.2% (148/545)	24.6% (242/985)
	L3	45.9% (202/440)	40.4% (220/545)	42.8% (422/985)
	L4	0.7% (3/440)	0.7% (4/545)	0.7% (7/985)
Montreal L4 modifier		3.9% (17/440)	5% (27/545)	4.5% (44/985)
Montreal disease behaviour	B1	56.9% (252/443)	56.8% (309/544)	56.8% (561/987)
	B2	36.8% (163/443)	31.1% (169/544)	33.6% (332/987)
	B3	6.3% (28/443)	12.1% (66/544)	9.5% (94/987)
Smoking history	Current	20.1% (89/443)	19.7% (107/542)	19.9% (196/985)
	Ex	35.7% (158/443)	32.5% (176/542)	33.9% (334/985)
	Never	44.2% (196/443)	47.8% (259/542)	46.2% (455/985)

Body mass index (kg /m^2)		24.3 (21.5 - 28.3)	24 (20.9 - 28.2)	24 (21 - 28.3)
Baseline immunomodulator use	TRUE	52% (233/448)	55.6% (305/549)	54% (538/997)
Baseline steroid use	TRUE	28.8% (129/448)	29.9% (164/549)	29.4% (293/997)
C-reactive protein (mg/L)		7 (2 - 14)	9 (3 - 22)	7 (3 - 18)
Faecal calprotectin (µg/g)		317 (140 - 644)	404 (164 - 799)	351 (151 - 727)
Haemoglobin (g/L)		131 (121 - 142)	127 (116 - 138)	129 (118 - 139)
Albumin (g/L)		39 (35 - 43)	39 (34 - 42)	39 (35 - 42)
Harvey Bradshaw Index		5 (3 - 8)	6 (3 - 9)	5 (3 - 9)

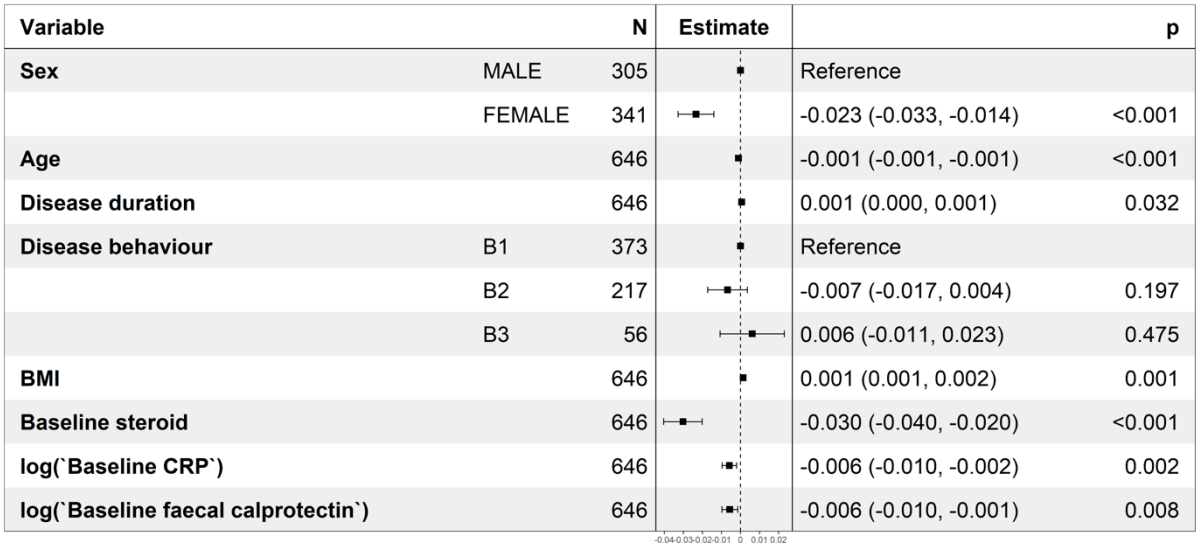
Factors associated with lower fT3/fT4 ratio

Serum fT3, fT4 and TSH concentrations were similar in infliximab- and adalimumab-treated patients (fT3: infliximab 4.8 pmol/L [4.2 – 5.4] vs adalimumab 4.9 pmol/L [4.4 – 5.5], p = 0.10; fT4: infliximab 15.6 pmol/L [14.1 – 17.1] vs adalimumab 15.6 [14.3 – 17.5], p = 0.16; TSH: infliximab 1.3 [0.91 – 1.9] vs adalimumab 1.4 [0.9 – 2], p = 0.29). Univariable analyses demonstrated that female sex, older age, higher BMI, disease duration, CRP, faecal calprotectin, and corticosteroid use at baseline, but not anti-TNF type, smoking, or immunomodulator use, were associated with lower fT3/fT4 ratio (Table 2). Multivariable linear regression analysis confirmed that female sex, higher CRP and higher faecal calprotectin concentrations, and corticosteroid use were independently associated with lower fT3/fT4 ratio (Figure 2).

Table 2: Baseline demographic and clinical characteristics associated with fT3/fT4 ratio. Variables were log-transformed for analysis. *CRP=C-reactive protein.*

Categorical variables				
Variable	**Level**	**n**	**fT3/fT4 ratio**	**p**
Sex	Female	526	0.30 (0.27 - 0.34)	<0.001
	Male	469	0.32 (0.28 - 0.36)	
Drug	Adalimumab	448	0.31 (0.28 - 0.35)	0.74
	Infliximab	547	0.31 (0.27 - 0.35)	
Smoker	Current smoker	196	0.31 (0.27 - 0.34)	0.36
	Non-current smoker	787	0.31 (0.27 - 0.35)	
Corticosteroid use at baseline	Yes	293	0.28 (0.25 - 0.33)	<0.001
	No	702	0.32 (0.29 - 0.36)	
Immunomodulator use at baseline	Yes	537	0.31 (0.27 - 0.36)	0.16
	No	458	0.31 (0.27 - 0.35)	
Continuous variables				
Variable			**Spearman's Rho (R)**	**p**
Age			-0.12	<0.001
Disease duration			0.06	0.05
BMI			0.12	<0.001
CRP*			-0.10	0.01
Faecal calprotectin*			-0.12	0.01

Figure 2: Forest plot showing the coefficients from a multivariable linear regression model of associations with fT3/fT4 ratio. The resultant values represent the change of fT3/fT4 ratio associated with each variable. *CRP=C-reactive protein.*



Association between T3/T4 ratio and clinical outcomes

Overall, 89.8% (894/995) and 88.4% (880/995) patients included in the effectiveness analysis of the PANTS study at weeks 14 and 54 respectively, were included here. Of 894 patients who were assessable at week 14, 25.5% (228/894) patients experienced PNR, 21% (188/894) patients were classified as grey zone, 13.6% (122/894) patients as having had a response, and 39.8% (356/894) patients were in remission. PNR occurred in 22.7% (113/497, 95% CI 19.3-26.6%) of infliximab-treated and 29% (115/397, 95% CI 24.7-33.6%) of adalimumab-treated patients. Of 880 patients who were assessable at week 54, 65.1% (573/880) were classified as being in non-remission, with no significant difference between infliximab- and adalimumab-treated patients (p = 0.29).Univariable analyses across both anti-TNF treated groups demonstrated that a lower fT3/fT4 ratio was associated with PNR (PNR: 0.30 [0.27 – 0.34] vs no PNR 0.32 [0.28 – 0.36], p

<0.001) (Figure 3a and 4). Lower fT3/fT4 ratio remained significantly associated with primary non-response, when stratified by anti-TNF drug (Figure 3b and 3c). No association was seen for baseline fT3/fT4 ratio and non-remission at week 54.

Figure 3: Beeswarm plot of fT3/fT4 ratio at baseline and primary non-response at week 14, a) combined cohort b) infliximab-treated patients, c) adalimumab-treated patients. The number of individuals tested for each group are shown in black at the top of each panel.

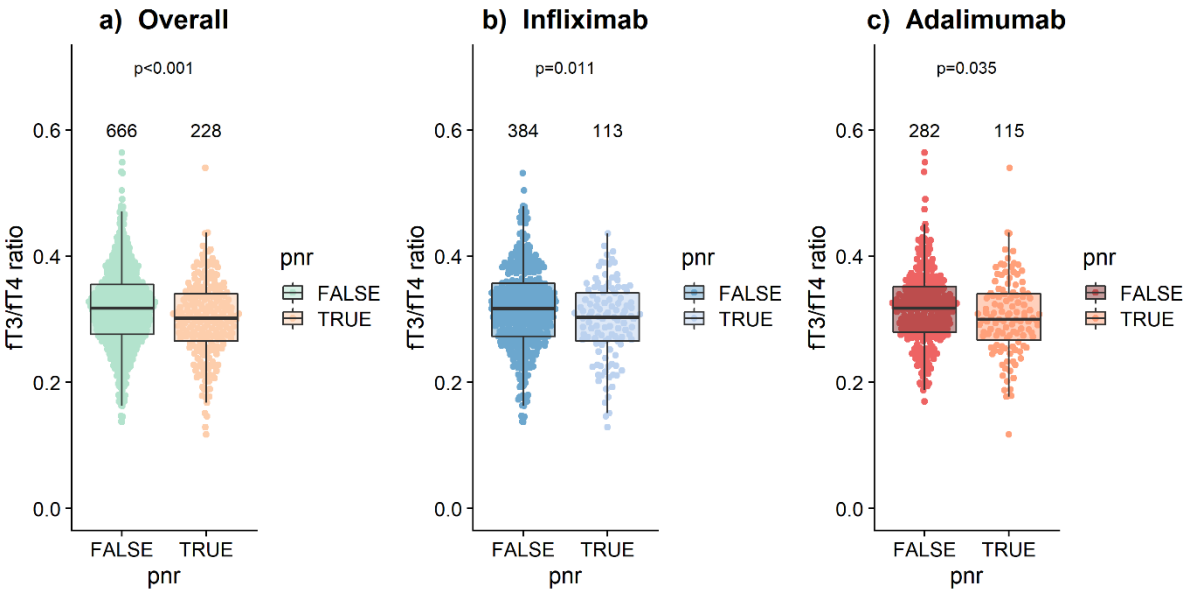
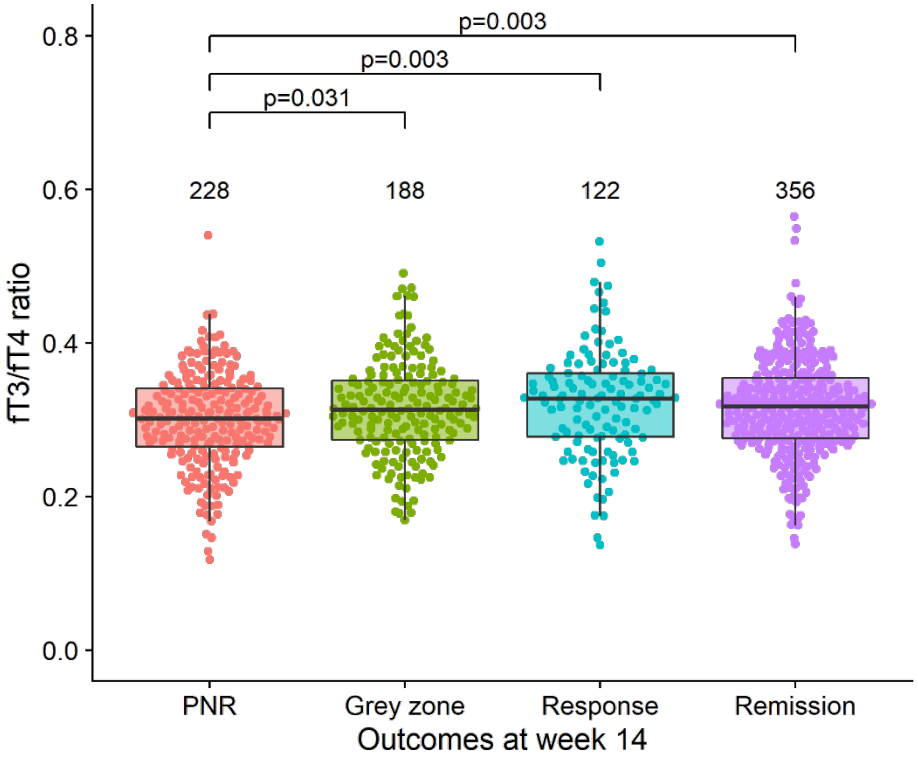
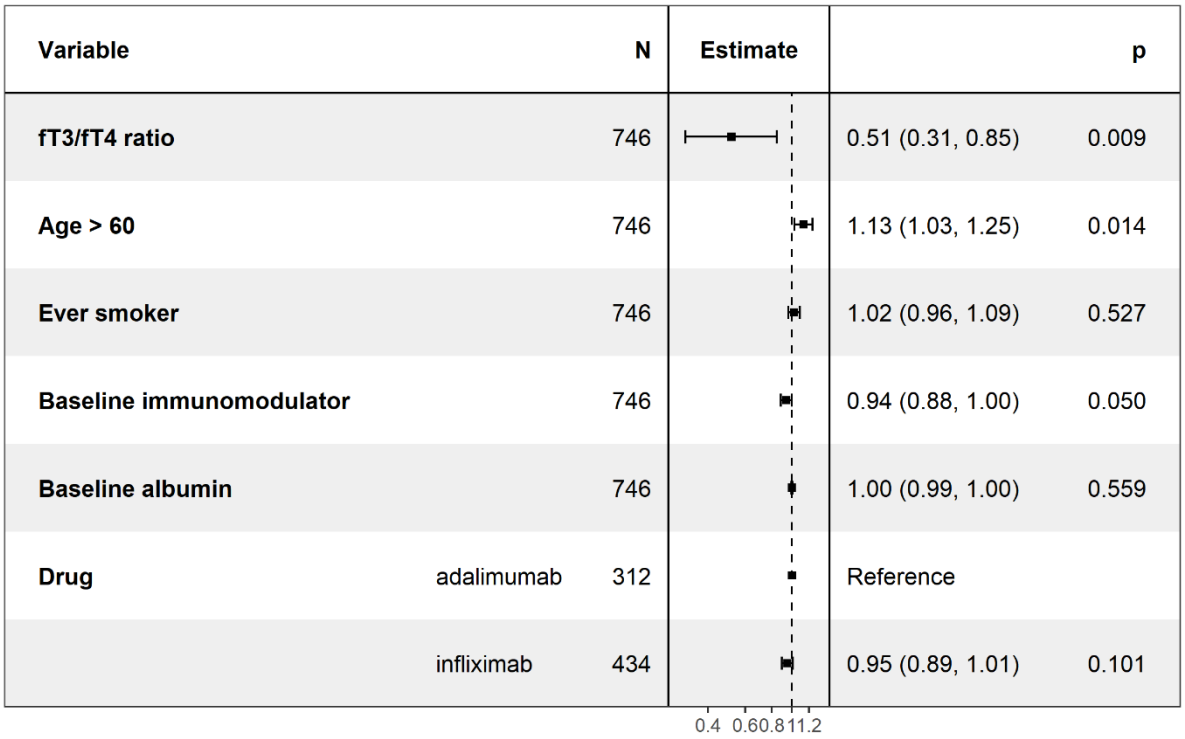


Figure 4: Beeswarm plot of fT3/fT4 ratio at baseline, stratified by outcome at week 14. The number of individuals tested for each group are shown in black at the top of each panel.



Multivariable logistic regression analyses confirmed that fT3/fT4 ratio was independently associated with PNR (odds ratio (OR) 0.51 [95% confidence interval (CI) 0.31 – 0.85, p = 0.01) (Figure 5). When stratified by anti-TNF and adjusted for variables known to be associated with PNR, low fT3/fT4 ratio remained associated with primary non-response for adalimumab-, but not infliximab-, treated patients (Supplementary Figures 1 and 2).

Figure 5: Forest plot showing the coefficients from a multivariable logistic regression model of associations with primary non-response



Youden's method demonstrated that the optimal cut-off threshold for baseline fT3/fT4 ratio to determine PNR at week 14 was 0.31, with an area under the curve of 0.57 (95% CI 0.54 – 0.61). The sensitivity and specificity were 0.62 (95% CI 0.41 – 0.74), specificity 0.53 (95% CI 0.42 – 0.73), respectively. When incorporating anti-TNF drug concentrations at week 14, in addition to fT3/fT4 ratio, we observed a marginal increase in AUC to 0.60 (95% CI 0.55 - 0.65).

Sensitivity analyses

We performed a sensitivity analysis assessing the association between fT3/fT4 ratio at baseline and faecal calprotectin at week 54. In a subset of 51% (451/880) of patients who had week 54 faecal calprotectin data, we found no correlation between fT3/fT4 ratio at baseline and, when assessing as a continuous variable, concentrations at week 54 (Rho = -0.08, p = 0.09). Furthermore, we found no difference in fT3/fT4 ratio at baseline between patients who had faecal calprotectin concentration > 250ug/g, representing active inflammation, and those who did not (0.31 [0.27 - 0.34] vs 0.31 [0.27 - 0.35], p = 0.39).

As steroid use was associated with fT3/fT4 ratio and is known to affect the hypothalamic-pituitary-thyroid axis, we performed a sensitivity analysis excluding patients treated with corticosteroids at baseline. Amongst 630 patients, there was no difference in fT3/fT4 ratio in those who experienced primary non-response compared to those who did not (PNR: [128/630] 0.31 [0.28 – 0.35] vs no PNR: [502/630] 0.32 [0.29 – 0.36], p = 0.10). Lastly, we performed a sensitivity analysis restricting the cohort to those 60 years or over only. Amongst 105 patients, there was no difference in fT3/fT4 ratio in those who experienced primary non-response compared to those who did not (PNR: [42/105] 0.27 [0.23 – 0.31] vs no PNR: [63/105] 0.29 [0.24 – 0.33], p = 0.19).

Discussion

Key results

Lower baseline serum fT3/fT4 ratio was independently associated with female sex, higher inflammatory burden at baseline, and baseline corticosteroid use, and predicted PNR to anti-TNF therapy at week 14, but not non-remission, or change in faecal calprotectin concentrations, at week 54. Overall, however, the diagnostic accuracy of baseline fT3/fT4 ratio to predict primary non-response to anti-TNF treatment was modest, limiting its clinical utility.

Interpretation

This is the first large scale effort to examine the association between fT3/T4 ratio and clinical outcomes in patients with IBD initiated on anti-TNF therapy. Few previous studies have reported the prevalence of thyroid dysfunction or if serum fT3/fT4 levels influence the response to anti-TNF therapy in patients with IBD.

Our observation that 1.3% and 0.2% patients were being treated for or had occult hypothyroidism and hyperthyroidism respectively, is consistent with previous estimates of thyroid dysfunction in patients with IBD.[20,21] Up to 3.7% and 8.3% patients reportedly have concomitant hypothyroidism and hyperthyroidism, respectively, and rates are broadly similar to the background population.[22]

The pathophysiology underlying the non-thyroidal illness syndrome is slowly being elucidated. Relevant to patients with active IBD, proinflammatory cytokines and leptin reportedly have a critical role.[4] They act centrally to reset release of thyroid

releasing hormone from the paraventricular nucleus of the hypothalamus. Peripherally, they modulate serum thyroid hormone binding protein levels and receptor expression and influence the activity of tissue deiodinases, which deactivate fT3 to 3,5-diiodo-L-thyronine (T2) and fT4 to reverse triiodothyronine (rT3).[4,23]

Bertani *et al,* showed that low serum fT3/fT4 ratio at initiation of infliximab or vedolizumab therapy predicted endoscopic outcomes at 54 weeks in a mixed cohort of patients with UC and Crohn's disease.[15] Whilst we replicated the association with PNR to anti-TNF therapies, we did not demonstrate an association between lower fT3/fT4 ratio and non-remission, or changes in faecal calprotectin concentrations, after 1 year. Moreover, despite using a similar threshold, in our data the fT3/fT4 ratio lacked diagnostic accuracy to be clinically useful to predict PNR to anti-TNF therapies at week 14.

There are a number of important differences in study design which may account for these discordant findings. Bertani *et al.* studied a mixed cohort of patients aged over 60 years with UC or Crohn's disease who were treated with either infliximab or vedolizumab, whereas we examined adults over 17 years with Crohn's disease and treated with an anti-TNF drug only. When we restricted our analyses to patients over the age of 60 years, there was no longer an association between fT3/fT4 ratio on any of our predefined outcomes. Corticosteroid use at baseline is not reported in, or adjusted for, in the Bertani *et al* study. Here, we have shown a negative association between corticosteroid use and fT3/fT4 ratio, like others have suggested.[24,25] Isolating the independent effect of corticosteroids on thyroid

metabolism, however, remains challenging, largely due to underpowered studies with lack of adjustment for known confounders.[25–27] Importantly, in our sensitivity analyses, we were unable to show an association between fT3/fT4 ratio and PNR in patients who were not treated with steroids at baseline. We acknowledge that corticosteroid use may reflect more active disease, and indeed was part of our definition of PNR, so the association between fT3/fT4 ratio and corticosteroids may be a combination of the direct effect of the corticosteroids on the hypothalamic-pituitary-thyroid axis and more severe IBD, as evidenced by raised CRP and higher faecal calprotectin concentrations.

Whether anti-TNF treatment influences serum thyroid hormone levels is largely unknown. In a case series of 55 patients with IBD, Paschou *et al* reported that fT4 concentrations reduced during anti-TNF therapy, whilst fT3 and TSH levels were unchanged.[28] Interestingly, they also observed higher than expected levels of thyroid autoimmunity. It is not clear whether this was due to a true increased risk of autoimmunity in patients with IBD, or whether they were detecting an excess of false positives because the anti-TNF drugs interfered with the antithyroid antibody assay.

Limitations and generalizability

We acknowledge some important limitations of our work. We used pragmatic definitions of treatment failure combining corticosteroid use with clinical and biochemical markers of disease activity that are closely aligned to routine treatment targets. We accept that our data would have been strengthened by endoscopic

outcomes as used by Bertani *et al.* However, in PANTS[12] we observed a significant association between clinical outcomes at weeks 14 and week 54 and faecal calprotectin which corelates closely with endoscopic findings; sensitivity analysis did not demonstrate an association between fT3/T4 levels at baseline predicting changes in week 54 faecal calprotectin concentrations. We accept there was some missingness in our cohort, in particular, we were only able to include the adults enrolled in the PANTS study because of limited or exhausted stored serum in the paediatric patients who had lower volumes collected at each blood draw. Lastly, we analysed our stored serum several years after it was collected, against this having biased our results, median thyroid hormone levels were similar to the Bertani study.

Our findings are likely to be generalisable to patients with Crohn's disease, and based on the Bertani report, to patients with UC. Anti-TNF medications are used to treat a number of other immune-mediated inflammatory diseases, which together affect about 5 - 7% of Western populations including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, hidradenitis suppurativa, and uveitis.[29] Whether our findings are generalisable to other anti-TNF drugs including adalimumab, certolizumab, golimumab and etanercept and other biologicals, across these other disease indications is unknown.

Predicting treatment response in patients with IBD is complex. Few of the so-called precision medicine biomarkers to facilitate the right drug, to the right patient, at the right time have translated to clinical care.[14,30–32] In part, this is because of their relatively modest effect size and the challenges of clinical translation of the basic science. The initial findings of the Bertani *et al.* study were exciting, not least

because the fT3/fT4 ratio is a physiological barometer of the complex adaptations that occur as a consequence of inflammation in patients with IBD and because thyroid hormone testing is inexpensive and already set-up in most hospitals. Based on our findings however, further work using endoscopic outcomes by disease and biologic type is needed to confirm or refute the usefulness of the fT3/T4 ratio to predict anti-TNF treatment outcomes. Our results do not suggest fT3/fT4 ratio as a predictor of anti-TNF response is clinically useful, however, it may have a role in a larger panel including pharmacokinetic variables such as drug concentration, and emergent molecular biomarkers that may be clinically significant, such as Oncostatin M.[31,32]

Conclusions

Lower baseline serum fT3/fT4 ratio was associated with female sex, higher inflammatory burden at baseline, and corticosteroid use, and predicted PNR to anti-TNF treatment at week 14, but not non-remission, or changes in faecal calprotectin concentrations, at week 54. Overall, serum fT3/fT4 ratio to predict primary non-response lacked diagnostic accuracy and is unlikely to be a clinically useful predictor.

Role of the funding source

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Data availability statement

Individual participant de-identified data that underlie the results reported in this article will be available immediately after publication for a period of 5 years. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be directed to james.goodhand@nhs.net. To gain access data requestors will need to sign a data access agreement.

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Competing interests

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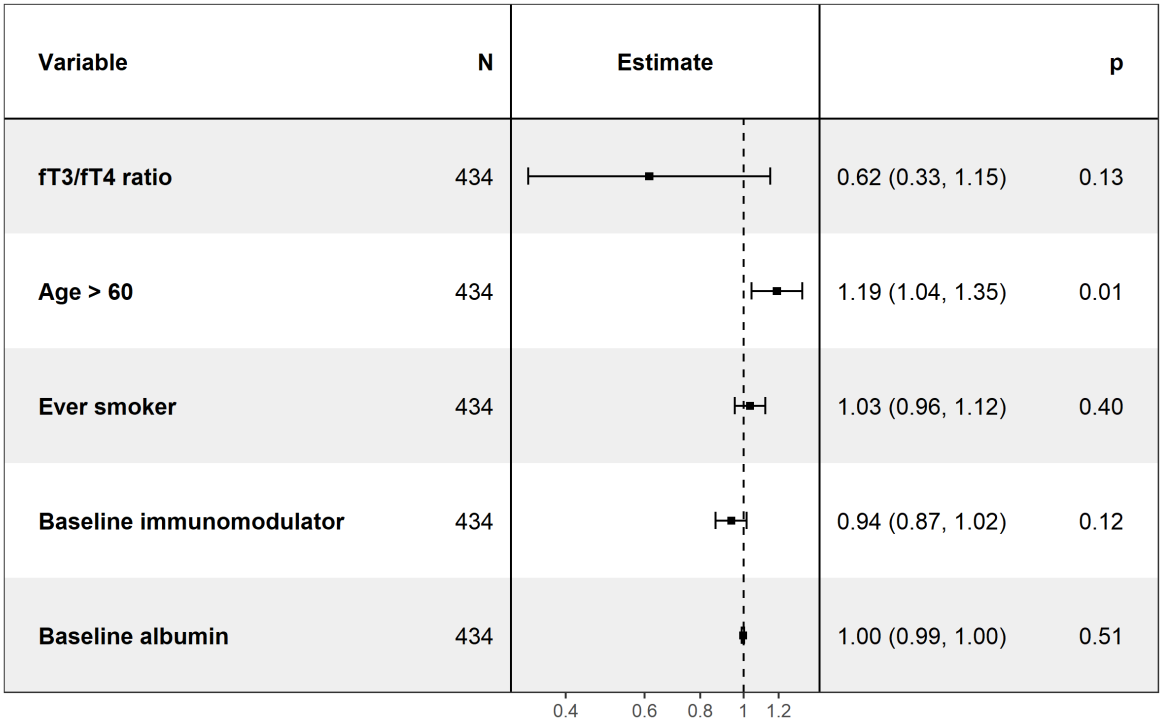
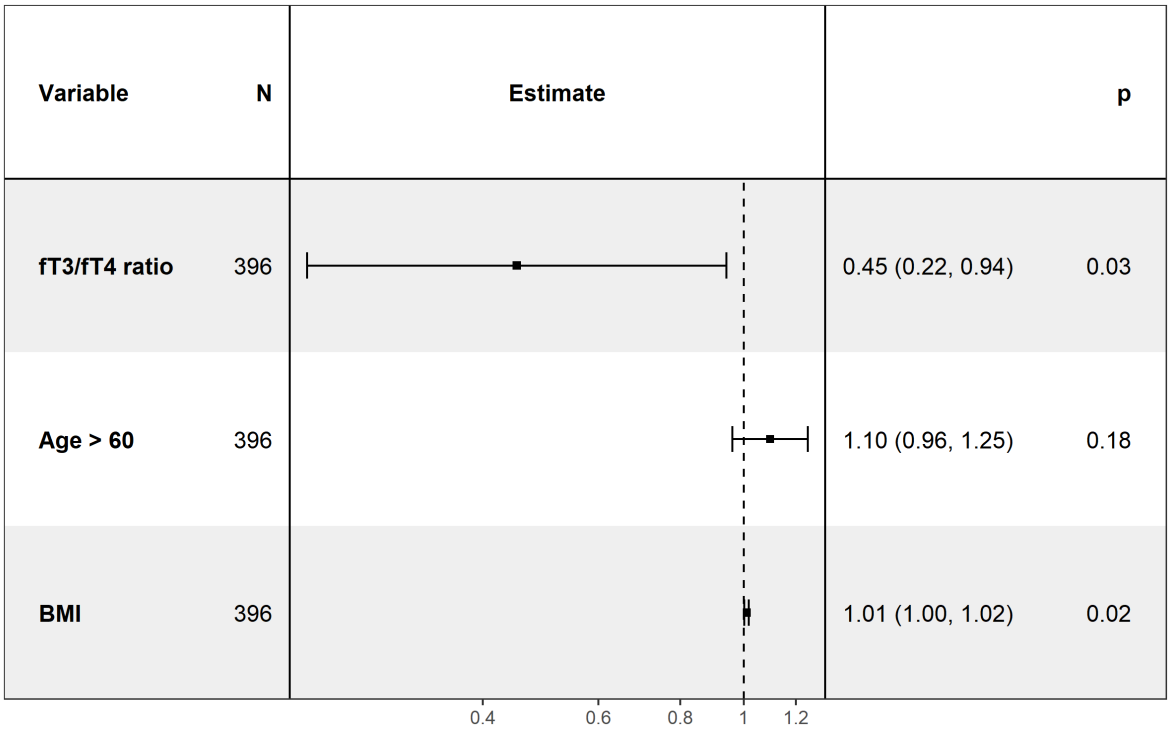
Supplementary Figure 1: Forest plot showing the coefficients from a multivariable logistic regression model of associations with primary non-response, and adjusted for variables known to be associated, stratified by anti-TNF (infliximab)



Supplementary Figure 2: Forest plot showing the coefficients from a multivariable logistic regression model of associations with primary non-response, and adjusted for variables known to be associated, stratified by anti-TNF (adalimumab)



Chapter 5: Pre-treatment vitamin D concentrations do not predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease

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Study aim

To assess whether pretreatment 25-hydroxyvitamin D concentrations predicted primary non-response and non-remission to infliximab and adalimumab in patients with Crohn's disease.

My role in the study

I was responsible for designing the research study and determining the aims, objectives. I determined the methodology used. I was responsible for sample preparation prior to measurement of 25-hydoxyvitamin D concentrations by the department of biochemistry. I analysed the results. I prepared the abstract for submission at the European Crohn's and Colitis Organisation annual conference was accepted as a poster presentation, which I authored. I wrote up the study, and submitted it to multiple journals, revising it at each stage to align with multiple editors' and peer reviewers' comments.

Findings

About 17% and 48% of patients with Crohn's disease in the PANTS study had vitamin D deficiency and insufficiency, respectively. Multivariable analysis confirmed that sampling during non-summer months, South Asian ethnicity, lower serum albumin concentrations, and non-treatment with vitamin D supplementation were independently associated with lower vitamin D concentrations. Pretreatment vitamin D status did not predict response or remission to anti-TNF therapy at week 14 or non-remission at week 54. Vitamin D deficiency was, however, associated with a longer time to immunogenicity in patients treated with infliximab, but not adalimumab.

Relevance and impact on my learning

Conducting this study allowed me to gain an in-depth, broad understanding of the role of vitamin D in inflammatory bowel disease, not only with respect to outcome of anti-TNF treatment, but also as a diagnostic and prognostic predictor of disease and clinical outcomes.

I analysed the results of this study question independently, and sought review of my code/analysis at a later stage compared to work carried out earlier in my PhD, thereby demonstrating increased confidence and competency in using R and performing statistical analyses.

My study's conclusions were different to the conclusions from smaller, mostly retrospective studies that investigated the same research question, demonstrating to me the benefit of using large, well-powered, prospective cohorts to replicate results from smaller cohorts. It would seem unlikely that this question needs to be further investigated, and resources can therefore be re-allocated to research questions for which uncertainty remains.

Acknowledgements of co-authors and contributions to paper

Myself, Simeng Lin, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad participated in the conception and design of the work. Claire Bewshea was the project manager and coordinated recruitment. Rachel Nice and Timothy J McDonald coordinated all biochemical analyses and central laboratory aspects of the project. Myself, Simeng Lin, Rebecca Smith, Christopher Roberts, Rachel Nice, Timothy J McDonald, Benjamin Hamilton, Maria Bishara, Claire Bewshea, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad were involved in the acquisition, analysis or interpretation of data. The data analysis was performed by Simeng Lin and Nicholas A Kennedy. Drafting of the manuscript was conducted by myself, Simeng Lin, Rebecca Smith, Christopher Roberts, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad. All the authors contributed to the critical review and final approval of the manuscript. Myself and Tariq Ahmad obtained the funding for the study. Tariq Ahmad is the guarantor of the article.

Abstract

Background and Aims

Vitamin D has a regulatory role in innate and adaptive immune processes. Previous studies have reported that low pre-treatment vitamin D concentrations are associated with primary non-response (PNR) and non-remission to anti-TNF therapy. This study aimed to assess whether pre-treatment 25-hydroxyvitamin D concentrations predicted PNR and non-remission to infliximab and adalimumab in patients with active luminal Crohn's disease.

Methods

25-hydroxyvitamin D concentrations were measured in stored baseline samples from 659 infliximab- and 448 adalimumab-treated patients in the Personalised Anti-TNF Therapy in Crohn's disease (PANTS) study. Cut-offs for vitamin D were: deficiency < 25nmol/L, insufficiency 25-50nmol/L and adequacy/sufficiency > 50nmol/L.

Results

17.1% (189/1107; 95% confidence interval [CI] 15 - 19.4%) and 47.7% (528/1107; 95% CI 44.8 - 50.6%) patients had vitamin D deficiency and insufficiency, respectively. 22.2% (246/1107) patients were receiving vitamin D supplementation.

Multivariable analysis confirmed that sampling during non-summer months, South Asian ethnicity, lower serum albumin concentrations and nontreatment with vitamin

D supplements were independently associated with lower vitamin D concentrations.

Pre-treatment vitamin D status did not predict response or remission to anti-TNF therapy at week 14 (infliximab $P_{pnr} = 0.89$, adalimumab $P_{pnr} = 0.18$) or non-remission at week 54 (infliximab $p = 0.13$, adalimumab $p = 0.58$). Vitamin D deficiency was, however, associated with a longer time to immunogenicity in patients treated with infliximab, but not adalimumab.

Conclusion

Vitamin D deficiency is common in patients with active Crohn's disease. Unlike previous studies, pre-treatment vitamin D concentration did not predict PNR to anti-TNF treatment at week 14 or non-remission at week 54.

Key words: Vitamin D, IBD, Crohn's disease, PANTS

Background

By binding to the vitamin D receptor expressed on most immune cells, vitamin D has a key regulatory role in innate and adaptive immune processes. Relevant to the pathogenesis of inflammatory bowel disease (IBD), in animal and *in vitro* experimental models, vitamin D modulates tight junctions, maintaining intestinal epithelial integrity and regulating host-microbiota interactions.[1]

Patients with IBD have multiple risk factors for vitamin D deficiency including: chronic diarrhoea, bile salt malabsorption, dietary restrictions and reduced sunlight exposure. Consequently, vitamin D deficiency is more common than in the general population[2–4], and whilst it does not cause IBD[5–8], because of the link with active disease[9–12], there is considerable interest in the role of vitamin D as an adjunct to IBD therapies.[13]

Over the last three decades, the anti-TNF monoclonal antibodies, infliximab and adalimumab have become the most frequently prescribed biologics for immune mediated inflammatory diseases (IMIDs). Unfortunately, anti-TNF treatment failure in patients with Crohn's disease is common: one-quarter of patients experience primary non-response, one-third of responders lose response and only 40% patients are in remission at the end of a year.[14] Anti-TNF monotherapy, obesity, smoking, disease severity and the development of antidrug antibodies are associated with low drug levels and subsequent anti-TNF treatment failure.[15,16] Carriage of the HLADQA1*05 allele confers a two-fold risk of developing antibodies to anti-TNF treatment.[17,18]

In small retrospective studies, vitamin D deficiency has been associated with primary non-response, non-remission and durability of anti-TNF therapy.[19–21] We sought to assess whether pre-treatment 25-hydroxyvitamin D concentrations predicted primary non-response and non-remission to infliximab and adalimumab in patients with Crohn's disease.

Methods

Study design

The Personalised Anti-TNF Therapy in Crohn's Disease study (PANTS) is a UK-wide, multicentre, prospective observational cohort reporting the treatment failure rates of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar, CT-P13 [Celltrion, South Korea]) and adalimumab (Humira [Abbvie, USA]) in anti-TNF-naïve patients with active luminal Crohn's disease.[15]

Patients were recruited between February 2013 and June 2016 at the time of first anti-TNF exposure and studied for 12 months or until drug withdrawal. After 12 months, patients were invited to continue follow-up for a further two years. Eligible patients were aged ≥ 6 years with objective evidence of active luminal Crohn's disease involving the colon and/or small intestine. Exclusion criteria included prior exposure to, or contraindications for the use of, anti-TNF therapy. The choice of anti-TNF was at the discretion of the treating physician and prescribed according to the licensed dosing schedule. Study visits were scheduled at first dose, week 14, and at weeks 30 and 54. Additional visits were planned for infliximab-treated patients at each infusion and for both groups at treatment failure or exit.

For this analysis, we included all patients who had stored serum available from baseline visits and effectiveness outcomes. Patients were excluded from our effectiveness analysis if they had a stoma as HBI and spCDAI scores used in the effectiveness analysis have not been validated in these patient group. Moreover, patients who were recruited into the study with normal pre-screening and visit 1

calprotectin and C-reactive protein (CRP) levels and where the only indication for anti-TNF was perianal disease.

Outcomes

Treatment failure endpoints were primary non-response at week 14, non-remission at week 54, and adverse events leading to drug withdrawal.

We used composite endpoints using the Harvey Bradshaw Index (HBI) in adults and the short paediatric Crohn's disease activity index (sPCDAI) in children, corticosteroid use, and CRP to define primary non-response (Supplementary Figure 1). Remission was defined as CRP of ≤3 mg/L and HBI of ≤4 points (sPCDAI ≤15 in children), without corticosteroid therapy or exit for treatment failure.

Secondary outcomes included anti-TNF drug concentration measured at weeks 14 and 54 and the time to development of anti-TNF antibodies. Drug persistence was defined as the duration of time from initiation of anti-TNF therapy to exit from the study due to treatment failure.

Patients exited the study when they stopped anti-TNF therapy or had an intestinal resection regardless of surgical outcome. They were deemed to be in non-remission for subsequent time points. Patients who declined to participate in the two-year extension or who exited the study for loss to follow-up, withdrawal of consent, or elective withdrawal of drug, including for pregnancy, were censored at the time of study exit and excluded from the denominator for subsequent analyses.

Clinical variables and laboratory analyses

Variables recorded at baseline by sites were demographics (age, sex, ethnicity, comorbidities, height and weight and smoking status) and IBD phenotype and its treatments (age at diagnosis, disease duration, Montreal classification, prior medical and drug history, and previous Crohn's disease-related surgeries). At every visit, disease activity score, weight, current therapy and adverse events were recorded.

Blood and stool samples were processed through the central laboratory at the Royal Devon University Healthcare NHS Foundation Trust (https://www.exeterlaboratory.com/) for hemoglobin, white cell count, platelets, serum albumin, CRP, anti-TNF drug and anti-drug antibody concentrations, and faecal calprotectin, respectively. Genotyping methods and the genetic analysis have been reported previously.[17]

25-hydoxyvitamin D concentrations

Serum 25-hydoxyvitamin D was measured in baseline samples between 22nd January 2020 and 20th March 2020 using the Elecsys 25-hydoxyvitamin electrochemiluminescence immunoassay (Roche) using the Cobas 801 module on the Cobas 8000 analyser.[22] This competitive electrochemiluminescence assay uses a ruthenium-complexed vitamin D binding protein to capture vitamin D3 (25-OH) and vitamin D2 (25-OH). The local reference range defines vitamin D deficiency as < 25nmol/L and insufficiency as 25 – 50nmol/L and adequacy/sufficiency > 50nmol/L. Pre-analytical stability of serum 25-

hydroxyvitamin D following long-term sample storage at up to -40°C has been demonstrated previously.[23,24]

TNF drug levels assays

The IDKmonitor free infliximab (K9655) and adalimumab (K9657) drug level assays permit quantitative measurement of free therapeutic drug in serum.[15] The assays follow a standard ELISA format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody. The measuring range for both assays is 0.8 - 45 mg/L, with absence of drug being defined using a cutoff of <0.8 mg/L.

Drug-tolerant anti-TNF antibody assays

Total anti-drug antibody concentrations were measured with IDKmonitor® ELISA assays (Immundiagnostik AG, Bensheim, Germany) performed on the Dynex DS2 ELISA robot (Dynex technologies, Worthing, UK). The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor infliximab (K9654) and adalimumab (K9651) total anti-drug antibody assays allow semi-quantitative measurement of both free and bound anti-drug antibodies.[15] A pre-treatment acid dissociation step is used to separate anti-drug antibodies from the therapeutic antibody. The assay then follows a standard ELISA format using recombinant therapeutic antibody as a capture and detection antibody. The positivity thresholds for the infliximab and adalimumab assays are 9 AU/ml and 6 AU/ml.[25]

Study size & statistical methods

The sample size calculation for the PANTS study has been reported previously.[15] Here we included all patients who had sufficient stored serum from their baseline visit and had outcome data at week 14.

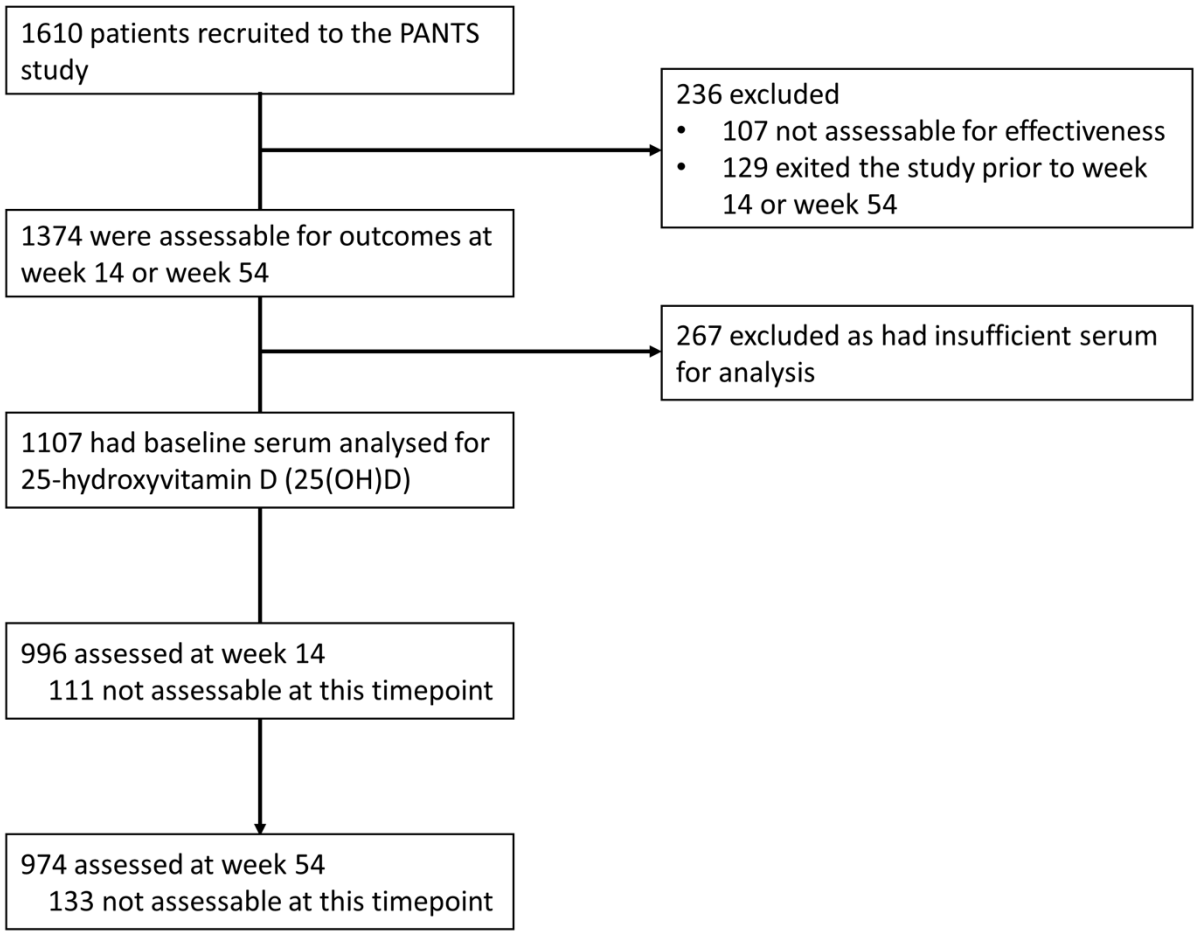
Statistical analyses were undertaken in R 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two tailed and p-values <0.05 were considered significant. We included patients with missing clinical variables in analyses for which they had data and have specified the denominator for each variable. Continuous data are reported as median and interquartile range, and discrete data as numbers and percentages. We performed univariable analyses using Fisher's exact, Mann-Whitney U and Spearman's rank tests to identify differences in baseline characteristics between infliximab- and adalimumab-treated patients, and to determine categorical and continuous factors associated with vitamin D levels and the predefined clinical outcomes above. Multivariable logistic regression analyses were used to confirm factors independently associated with vitamin D deficiency. Rates of immunogenicity and drug persistence were estimated using the Kaplan-Meier method, and comparative analyses were performed using univariable and multivariable Cox proportional hazards regression. Further sensitivity analyses using calprotectin at week 54 as an outcome and stratifying the cohort by vitamin D supplements and/or corticosteroid treatments at baseline were undertaken.

Results

Participants

Overall, 80.6% (1107/1374) patients who participated in the PANTS study who were assessable for effectiveness were included: 659 (59.5%) were treated with infliximab (526 [47.5%] with originator infliximab, and 133 [12%]) with biosimilar CT-P13) and 448 (40.5%) were treated with adalimumab (Figure 1).

Figure 1: Study profile. Patients were not assessable when 1 or more of the key data items were missing.



At baseline, 22.2% (246/1107) patients were receiving a form of vitamin D supplementation, of whom 52.9% were prescribed corticosteroids. Differences between demographic and clinical characteristics of infliximab- and adalimumab-treated patients are shown in Table 1.

Table 1: Baseline demographic and clinical characteristics, stratified by anti-TNF

Variable	Level	Infliximab	Adalimumab	Overall
n		659	448	
Sex	Female	50.7% (334/659)	53.6% (240/448)	51.9% (574/1107)
	Male	49.3% (325/659)	46.4% (208/448)	48.2% (533/1107)
Ethnicity	White	89.5% (590/659)	96.7% (433/448)	92.4% (1023/1107)
	South Asian	5% (33/659)	1.8% (8/448)	3.7% (41/1107)
	Other	5.5% (36/659)	1.6% (7/448)	3.9% (43/1107)
Anti-TNF	Adalimumab	0 % (0/659)	100% (448/448)	41% (448/1107)
	CT-P13	20.2% (133/659)	0% (0/448)	12% (133/1107)
	Remicade	79.8% (526/659)	0% (0/448)	47.5% (526/1107)
Age at first dose of anti-TNF		30 (19 - 44.7)	38.6 (28.5 - 50.5)	33.3 (22.8 - 47.3)
Age at first dose of anti-TNF < 18		22.9% (151/659)	3.1% (14/448)	14.9% (165/1107)

Disease duration		2.1 (0.6 - 7.4)	2.8 (0.7 - 10.5)	2.3 (0.7 - 8.8)
Montreal disease location	L1	28.1% (184/655)	33.3% (147/442)	30.2% (331/1097)
	L2	24.1% (158/655)	21.7% (96/442)	23.2% (254/1097)
	L3	46.7% (306/655)	44.3% (196/442)	45.8% (502/1097)
	L4	1.1% (7/655)	0.7% (3/442)	0.9% (10/1097)
Montreal L4 modifier		13.3% (87/655)	4.5% (20/442)	9.8% (107/1097)
Montreal disease behaviour	B1	63.6% (417/656)	57.8% (256/443)	61.2% (673/1099)
	B2	25.9% (170/656)	36.8% (163/443)	30.3% (333/1099)
	B3	10.5% (69/656)	5.4% (24/443)	8.5% (93/1099)
Perianal disease		14.4% (95/659)	7.8% (35/448)	11.7% (130/1107)
Smoking history	Current	14.2% (92/649)	21.4% (95/444)	17.1% (187/1093)
	Ex	25.7% (167/649)	35.6% (158/444)	29.7% (325/1093)

	Never	61% (390/649)	43% (191/444)	53.2% (581/1093)
Body mass index (kg /m^2)		22.5 (19.6 - 27.1)	24.3 (21.5 - 28.3)	23.3 (20.3 - 27.7)
Baseline immunomodulator use	TRUE	61.9% (408/659)	51.8% (232/448)	57.8% (640/1107)
Baseline steroid use	TRUE	29.3% (193/659)	27% (121/448)	28.4% (314/1107)
C-reactive protein (mg/L)		9 (3 - 23)	7 (2 - 14)	8 (3 - 19)
Faecal calprotectin (µg/g)		458 (187 - 899)	318 (142 - 629)	373 (164 - 762)
Haemoglobin (g/L)		125 (114 - 136)	131 (120 - 142)	127 (117 - 139)
Albumin (g/L)		39 (34 - 42)	40 (36 - 43)	39 (34 - 42)
Harvey Bradshaw Index		6 (3 - 9)	5 (3 - 8)	5 (3 - 9)
Short paediatric Crohn’s disease activity index		25 (15 - 50)	NA	25 (15 - 50)

Similar to the whole cohort, there were significant differences at baseline between the infliximab- and adalimumab-treated patients, including in age, ethnicity, smoking, body-mass index, disease duration and disease behaviour. Patients treated with infliximab had more active disease at baseline than patients treated with adalimumab, as evidenced by higher serum CRP and faecal calprotectin concentrations. At the initiation of anti-TNF treatment, immunomodulator use was higher in patients treated with infliximab compared to those treated with adalimumab, but there was no difference in the proportion of patients treated with corticosteroids.

Baseline factors associated with vitamin D concentrations

Median [IQR] vitamin D concentrations were lower in patients subsequently treated with infliximab than adalimumab (39 nmol/L [29 - 56] vs 44 nmol/L [3 - 59], p = 0.02). The other univariable factors associated with vitamin D concentrations are shown in Table 2. Multivariable linear regression analysis confirmed that baseline sampling during non-summer months (Supplementary Figure 2), South Asian ethnicity, lower serum albumin concentrations and nontreatment with vitamin D supplements were independently associated with lower vitamin D concentrations (Figure 2).

Table 2: Baseline demographic and clinical characteristics associated with vitamin D concentrations. †Sampling during the summer was defined as a blood sample obtained for vitamin D analysis in the months of June, July and August. ‡Variables were log-transformed for analysis. *CRP=C-reactive protein.*

Categorical variables				
Variable	**Level**	**n**	**Vitamin D (nmol/L)**	**p**
Month of sampling	Non-summer	849	38 (27 - 54)	<0.001
	Summer†	258	51 (39 - 65)	
Ethnicity	South Asian	41	30 (22 - 44)	0.001
	White/Others	1066	42 (30 - 58)	
Pre-treatment vitamin D supplementation	No	861	39 (28 - 55)	
	Yes	246	50 (36 - 64)	<0.001
Drug	Infliximab	659	39 (29 - 56)	0.02
	Adalimumab	448	44 (31 - 59)	
Continuous variables				
Variable			**Spearman's Rho (R)**	**p**
Serum albumin			0.18	<0.001
Harvey Bradshaw Index			-0.07	0.04
Short paediatric Crohn's disease activity index			-0.33	0.003
CRP‡			-0.12	<0.001
Faecal calprotectin‡			-0.11	0.003
Haemoglobin			0.10	0.002

Figure 2: Forest plot showing the coefficients from a multivariable linear regression model of associations with pre-treatment vitamin D concentrations. The resultant values represent the change in vitamin D concentrations associated with each variable. *HBI=Harvey Bradshaw Index, sPCDAI=short paediatric Crohn's disease activity index, CRP=C-reactive protein.* † Sampling during the summer was defined as a blood sample obtained in the months of June, July and August

Variable		N	Estimate	p
Season †	Summer	143	Reference	
	Non-summer months	470	-10.60 (-14.31, -6.90)	<0.001
Ethnicity	White	576	Reference	
	South Asian	14	-12.12 (-22.58, -1.65)	0.02
	Other	23	-5.82 (-14.05, 2.41)	0.17
Vitamin D supplementation		613	7.72 (4.06, 11.39)	<0.001
Drug	infliximab	378	Reference	
	adalimumab	235	-0.65 (-3.91, 2.62)	0.70
Baseline serum albumin		613	0.62 (0.32, 0.93)	<0.001
Baseline HBI/sPCDAI non-remission		613	-1.78 (-5.05, 1.50)	0.29
Baseline CRP		613	-0.03 (-0.10, 0.04)	0.42
Baseline calprotectin		613	-0.00 (-0.00, 0.00)	0.61
Baseline haemoglobin		613	-0.02 (-0.13, 0.10)	0.78

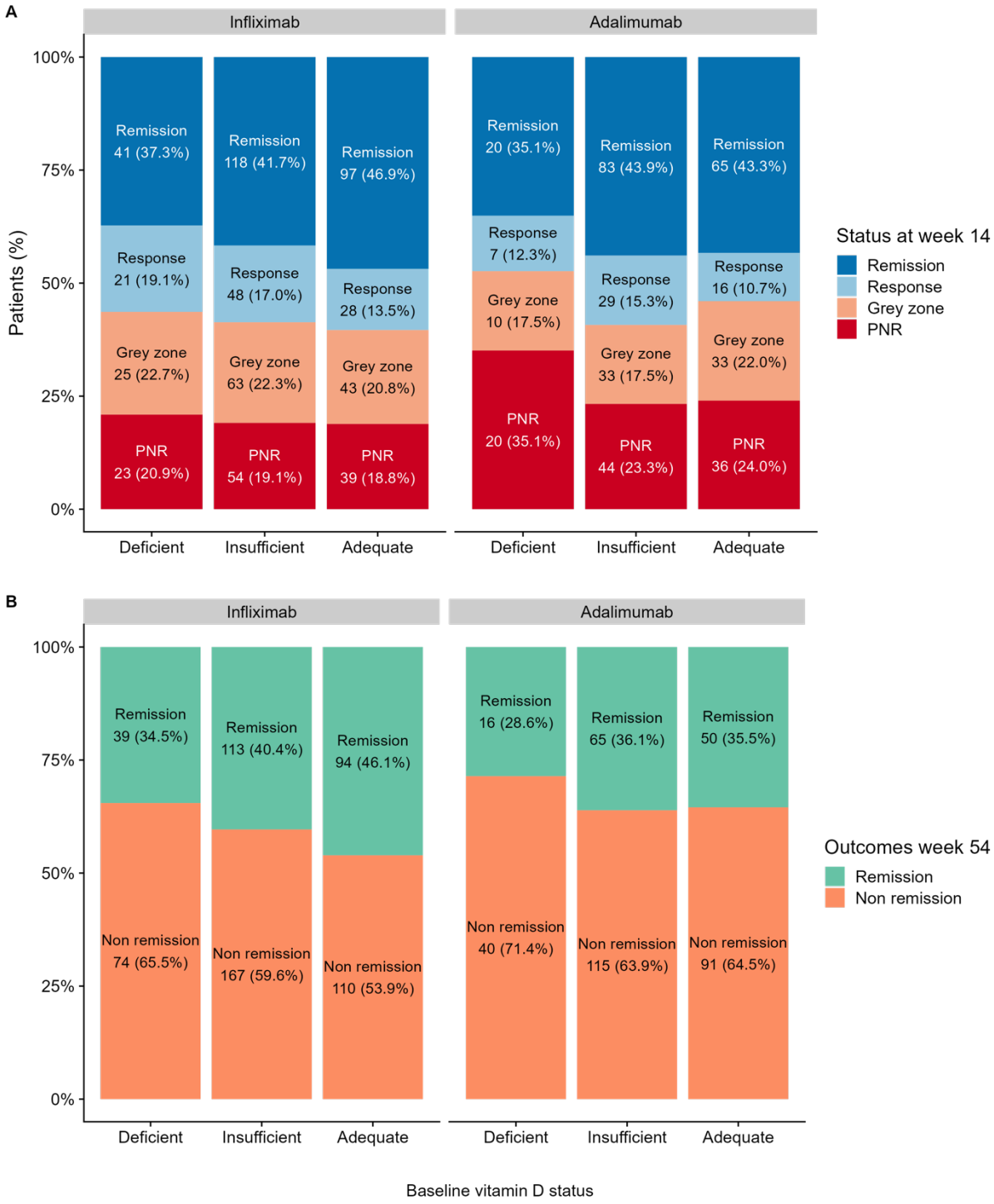
Baseline vitamin D status and clinical outcomes

Overall, 17.1% (189/1107; 95% confidence interval [CI] 15 - 19.4%) and 47.7% (528/1107; 95% CI 44.8 - 50.6%) patients had vitamin D deficiency and insufficiency, respectively. Primary non-response at week 14 and non-remission at week 54 occurred in 19.3% (116/600; 95% CI 16.4 - 22.7%) and 58.8% (351/597;

95% CI 54.8- 62.7%) patients treated with infliximab and 25.3% (100/396; 95% CI 21.2- 29.8%) and 65.3% (246/377; 95% CI 60.3- 69.9%) of patients treated with adalimumab, respectively.

Pre-treatment vitamin D status did not predict response or remission status to anti-TNF therapy at week 14 (primary non-response: infliximab p = 0.89, adalimumab p = 0.18; remission: infliximab p = 0.19, adalimumab p = 0.38) or non-remission at week 54 (infliximab p = 0.13, adalimumab p = 0.58) (Figure 3). Overall, there were no differences in median (IQR) vitamin D levels at baseline according to response or remission status at weeks 14 and 54, respectively (Supplementary Figure 3).

Figure 3: Proportion of patients stratified by their pre-treatment vitamin D status and outcomes to anti-TNF at A) week 14 and B) week 54. Infliximab-treated patients on the left panel, and adalimumab-treated patients on the right panel. The number of patients experiencing each outcome is annotated in the plot, with the proportion in brackets (%). *PNR = primary non-response.*



In patients who continued in the study beyond week 14, there was no difference in drug persistence between patients with vitamin D deficiency (hazard ratio [HR] 0.98 [95% confidence interval 0.71 - 1.34], p = 0.89), or insufficiency (HR 1.08 [95% CI 0.85 - 1.36], p = 0.54) at baseline compared to those with adequate concentrations.

Sensitivity analyses

In a subset of 47.1% (520/1107) of patients who had week 54 faecal calprotectin data, we found a weak negative correlation between vitamin D concentrations at baseline and faecal calprotectin concentrations at week 54 (Rho = -0.09, p = 0.04). Of the 28.4% (314/1107) patients treated with corticosteroids at baseline, 41.4% (130/314) were receiving concurrent vitamin D supplementation. Vitamin D concentrations were higher in those receiving vitamin D supplementation (50 nmol/L [36.3 - 64.8] vs 36 [25.8 - 48], p < 0.001), however, there was no difference in primary non-response rates at week 14 (35.3% vs 32.7%, p = 0.70), or non-remission at week 54 (65.5% vs 63.1%, p = 0.71).

We then excluded from the whole cohort, all patients who were receiving vitamin D supplementation at baseline. Of 773 patients remaining, pre-treatment vitamin D status was not associated with primary non-response at week 14 (p = 0.15) or non-remission at week 54 (p = 0.26).

Anti-TNF drug concentrations and time to immunogenicity

We observed a weak positive correlation between pre-treatment vitamin D concentration and anti-TNF drug concentrations at week 14 (infliximab: Rho = 0.1,

p = 0.03; adalimumab: Rho = 0.2, p < 0.001): however, when we included the factors previously associated with week 14 drug level, vitamin D concentrations were not independently associated in our multivariable models (Supplementary Figure 4). We did not demonstrate associations with infliximab or adalimumab drug concentrations at week 54.

The estimated proportion of patients who developed anti-drug antibodies for the first, second and third year was 64.4% [95% CI 60 - 68.4], 69.6% [95% CI 64.9 - 73.6] and 78.4% [95% CI 69.1 - 84.9] in infliximab-treated patients, and 36.9% [95% CI 31.5 - 41.8], 45.8% [95% CI 38.7 - 52.1], 55.1% [95% CI 45.6 - 62.9] in adalimumab-treated patients, respectively. Time to immunogenicity was longer in patients with vitamin D deficiency in infliximab- (hazard ratio [HR] 0.69 [95% CI 0.52 - 0.91], p = 0.01], but not adalimumab-treated (HR 1.29 [95% CI 0.85 - 1.95], p = 0.23) patients (Figure 4). Multivariable analysis, including drug concentration at week 14, immunomodulator use, smoking, and carriage of the HLAD1A1*05 variant that we have previously reported to be associated with time to immunogenicity in this cohort, confirmed that vitamin D deficiency was independently associated with a longer time to immunogenicity in infliximab-treated patients (Figure 5).

Figure 4: Kaplan-Meier estimates of time to the development of anti-TNF antibodies in patients stratified by pre-treatment vitamin D status. Infliximab-treated patients are shown in A) and adalimumab-treated patients in B). p values calculated using the log-rank test. Shaded regions represent the 95% confidence interval

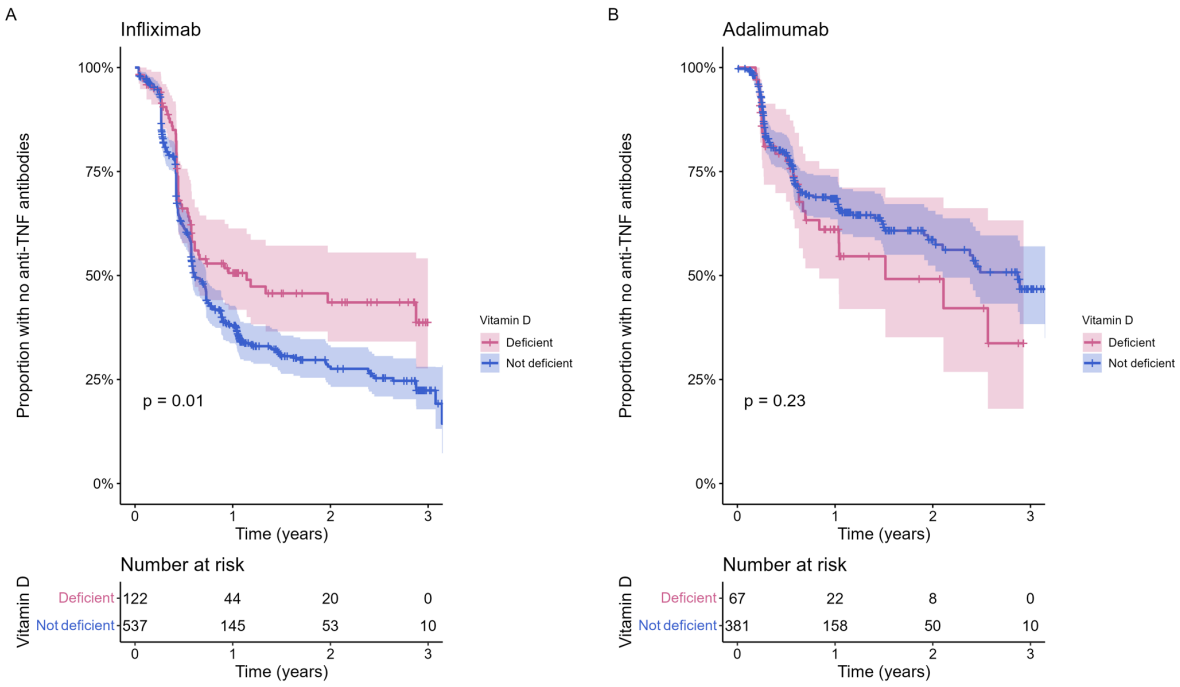
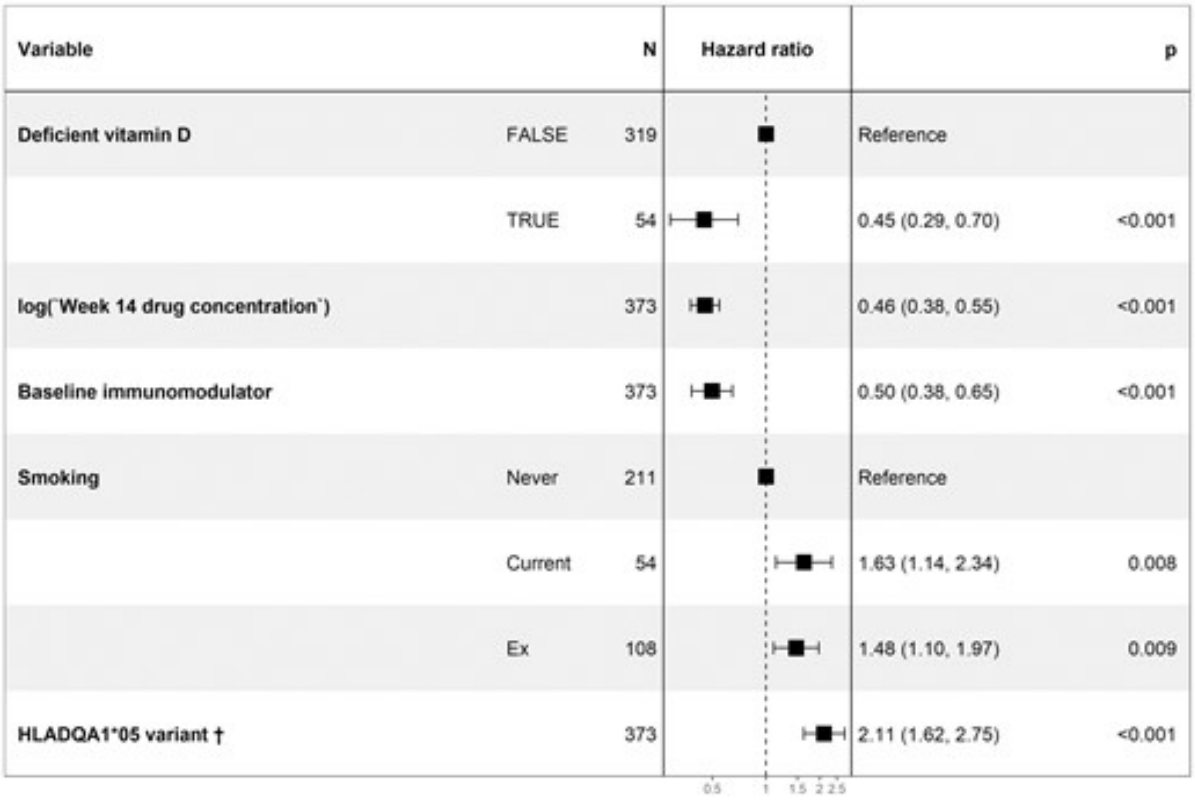


Figure 5: Forest plot showing the hazard ratio of the factors associated with time to the development of anti-TNF antibodies in infliximab-treated patients. † Patients with either 1 or 2 copies of the allele were considered to have carriage of the HLADQA1*05 variant



Discussion

Key results

Vitamin D deficiency is common in patients with active Crohn's disease. Unlike previous studies, pre-treatment serum 25-hydroxyvitamin D concentrations did not predict primary non-response, non-remission or anti-TNF drug persistence. Vitamin D deficiency was, however, associated with a longer time to immunogenicity in patients treated with infliximab.

Interpretation

Our observation that 17.1% (95% CI 15 - 19.4%) and 47.7% (95% CI 44.8 - 50.6%) patients had vitamin deficiency and insufficiency respectively, is consistent with previous estimates in patients with active IBD and almost double that compared of healthy controls.[3–5] Moreover, our model of 25-hydroxyvitamin D concentration confirmed independent associations with baseline sampling during non-summer months, South Asian ethnicity, lower serum albumin concentrations and nontreatment with vitamin D supplements further validates our findings against clinical outcome. Unlike Winter *et al.*[19], Zator *et al.*[20], and Xia *et al.*[21], in their small mixed cohorts we did not see associations with primary non-response, durability of IBD therapy or remission at week 54, respectively. The major criticisms of these studies are: measurement bias (to be included patients needed to have had vitamin D measured); the retrospective assessment of remission; how disease severity was controlled for, and the lack of data relating to concomitant corticosteroid therapy or vitamin D supplements.[26] This is the first prospective study with a large enough sample size to adjust for potential confounders to examine the association between pre-treatment vitamin D status/concentration and clinical outcomes in

patients with Crohn's disease initiated on anti-TNF therapy. Our negative findings are consistent with the response findings from a small (56 Crohn's disease and 12 UC) prospective study reported by Santos-Antunes *et al.*[27] In our sensitivity analyses we did not observe any association between pre-treatment vitamin D concentrations and clinical outcomes when we stratified our data by vitamin D supplements in patients who were treated with corticosteroids at baseline. Our weak association with calprotectin suggests that vitamin D supplementation might have, at best, a modest immunoregulatory role in anti-TNF therapy. Overall, however, and unlike previous reports our data provides no additional justification for use of vitamin D supplementation in anti-TNF treatment over current indications.

The finding that vitamin D deficiency was lower in infliximab-treated patients is likely to be explained by more active disease at baseline evidenced by a higher serum CRP and faecal calprotectin observed in infliximab- compared to adalimumab-treated patients in this real-world study.

Our observation that the time to immunogenicity was longer in patients with vitamin D deficiency is of interest. Vitamin D has a central role in antigen presentation and T cell function, with effects on immune tolerance in adaptive immune responses.[28] Whilst vitamin D deficiency did not predict primary non-response to anti-TNF treatment, whether low vitamin D levels protect against the development of anti-drug antibodies requires further study.

Limitations and generalizability

We acknowledge the following limitations. We accept that our data would have been strengthened by endoscopic outcomes or cross-sectional imaging. However, in PANTS[15] we observed a significant association between clinical outcomes at weeks 14 and week 54 with faecal calprotectin, which correlates closely with endoscopic findings. In our sensitivity analysis, we did not observe a clinically useful correlation (Rho = -0.09, p = 0.04) between pre-treatment vitamin D concentrations and calprotectin. The addition of cross-sectional imaging would have strengthened our data in those with disease affecting the small bowel, but less so those with disease affecting the large bowel only. Because our stored samples are slowly being exhausted, in particular, in children[16], we accept there was some missingness in our cohort. We may have been underpowered to detect associations between vitamin D concentrations and time to the development of anti-adalimumab antibodies, because immunogenicity events were less common and fewer in adalimumab- than infliximab-treated patients were vitamin D deficient at baseline.

Our findings are likely to be generalisable to other patients with Crohn's disease at least at similar latitudes to the United Kingdom. It is possible that vitamin D deficiency in patients with IBD at different latitudes will be less prevalent and whether our findings are generalisable in these populations remain unknown. It is perhaps less likely that vitamin D deficiency influences anti-TNF treatment responses in rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, hidradenitis suppurativa, and uveitis because these conditions are not associated with intestinal malabsorption of dietary vitamin D. Whether our findings

are generalisable to other anti-TNF drugs including certolizumab, golimumab and etanercept and other biologicals is unknown.

Conclusions

Vitamin D deficiency is common in patients with active Crohn's disease. Unlike previous studies, pre-treatment serum 25-hydroxyvitamin D concentration did not predict primary non-response to anti-TNF treatment at week 14 or non-remission at week 54.

Ethical and role of the funding source

The sponsor of the study was the Royal Devon University Healthcare NHS Foundation Trust. The South West Research Ethics committee approved the study (REC Reference: 12/SW/0323) in January 2013. PANTS is an investigator-led study funded by the research charities CORE, CCUK, and C3, and by unrestricted educational grants from Abbvie (USA), Merck Sharp & Dohme (UK), NAPP Pharmaceuticals (UK), Pfizer (USA), and Celltrion Healthcare (South Korea). Our vitamin D study was funded by grants from the European Crohn's Colitis Organisation and the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition. No funding bodies had any role in study design, data collection or analysis, writing or decision to submit for publication.

Data availability statement

Individual participant de-identified data that underlie the results reported in this article will be available immediately after publication for a period of 5 years. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be directed to tariq.ahmad1@nhs.net. To gain access data requestors will need to sign a data access agreement.

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management. The South West Research Ethics committee approved the study (REC Reference: 12/SW/0323) in January 2013. I acknowledge support from Crohn's & Colitis UK. S.L. is supported by a Wellcome GW4-CAT fellowship (222850/Z/21/Z). . C.B. is supported by the National Institute for Health and Care Research Exeter Biomedical Research Centre. We thank the patients who participated in the PANTS study, the Inflammatory Bowel Disease Pharmacogenetic Study Group, and research nurses who collected clinical data and biological samples at each study visit. We acknowledge the study coordinators Marian Parkinson and Helen Gardner-Thorpe for their ongoing administrative support to the study. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

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Supplementary Table 1: PANTS consortium

All UK gastroenterologists were invited to participate in the PANTS study which was promoted through the UK National Institute for Health Service Research (NIHR) and the British Society of Gastroenterology (BSG).

Hospital or Trust name	**City**	**Name**	**Job Title**
Tameside Hospital NHS Foundation Trust	Ashton U Lyne	Dr Vinod Patel	Consultant Gastroenterologist
Basildon and Thurrock University Hospitals NHS Foundation Trust	Basildon	Dr Zia Mazhar	Consultant Gastroenterologist
Hampshire Hospitals NHS Foundation Trust	Basingstoke	Dr Rebecca Saich	Consultant Gastroenterologist
Royal United Hospital	Bath	Dr Ben Colleypriest	Consultant Gastroenterologist
Ulster Hospital	Belfast	Dr Tony C Tham	Consultant Gastroenterologist
University Hospital's Birmingham NHS Foundation Trust	Birmingham	Dr Tariq H Iqbal	Consultant Gastroenterologist

East Lancashire NHS Teaching Trust	Blackburn	Dr Vishal Kaushik	Consultant Gastroenterologist
Blackpool Teaching Hospitals NHS Foundation Trust	Blackpool	Dr Senthil Murugesan	Consultant Gastroenterologist
Bolton NHS Trust	Bolton	Dr Salil Singh	Consultant Gastroenterologist
Royal Bournemouth Hospital	Bournemouth	Dr Sean Weaver	Consultant Gastroenterologist
Bradford Teaching Hospitals Foundation Trust - (St Lukes Hospital &Bradford Royal Infirmary)	Bradford	Dr Cathryn Preston	Consultant Gastroenterologist
Brighton and Sussex University Hospitals NHS Trust	Brighton	Dr Assad Butt	Paediatric Consultant Gastroenterologist
Brighton and Sussex University Hospitals NHS Trust	Brighton	Dr Melissa Smith	Consultant Gastroenterologist

University Hospitals Bristol NHS Foundation Trust	Bristol	Dr Dharamveer Basude	Consultant Paediatric Gastroenterologist
University Hospitals Bristol NHS Foundation Trust	Bristol	Dr Amanda Beale	Consultant Gastroenterologist
Frimley Park Hospital NHS Foundation Trust	Camberley	Dr Sarah Langlands	Consultant Gastroenterologist
Frimley Park Hospital NHS Foundation Trust	Camberley	Dr Natalie Direkze	Consultant gastroenterologist
Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Miles Parkes	Consultant Gastroenterologist
Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Franco Torrente	Consultant Paediatric Gastroenterologist
Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Juan De La Revella Negro	Research fellow

North Cumbria University Hospitals NHS Trust	Carlisle	Dr Chris Ewen MacDonald	Consultant Gastroenterologist
Ashford & St Peter's Hospitals NHS Foundation Trust	Chertsey	Dr Stephen M Evans	Consultant Gastroenterologist
St Peter's Hospital	Chertsey	Dr Anton V J Gunasekera	Consultant Gastroenterologist
Ashford & St Peter's Hospitals NHS Foundation Trust	Chertsey	Dr Alka Thakur	Paediatric Consultant
Chesterfield Royal NHS Foundation Trust	Chesterfield	Dr David Elphick	Consultant Gastroenterologist
Colchester Hospital University NHS Foundation Trust	Colchester	Dr Achuth Shenoy	Consultant Gastroenterologist
University Hospitals Coventry and Warwickshire NHS Trust	Coventry	Prof Chuka U Nwokolo	Consultant Gastroenterologist
County Durham and Darlington NHS Foundation Trust	Darlington	Dr Anjan Dhar	Consultant Gastroenterologist &

			Hon. Clinical Lecturer
Derby Hospital NHS Foundation NHS Trust	Derby	Dr Andrew T Cole	Consultant Gastroenterologist
Doncaster and Bassetlaw Hospitals NHS Foundation Trust	Doncaster	Dr Anurag Agrawal	Consultant Gastroenterologist
Dorset County Hospital NHS Foundation Trust	Dorchester	Dr Stephen Bridger	Consultant Gastroenterologist
Dorset County Hospitals Foundation Trust	Dorchester	Dr Julie Doherty	Paediatric Consultant
Dudley Group NHS Foundation Trust	Dudley	Dr Sheldon C Cooper	Consultant Gastroenterologist
Russells Hall Hospital, The Dudley Group NHS Foundation Trust	Dudley	Dr Shanika de Silva	Consultant Gastroenterologist
Ninewells Hospital & Medical School	Dundee	Dr Craig Mowat	Consultant Gastroenterologist

East Sussex Healthcare Trust	Eastborne	Dr Phillip Mayhead	Consultant Gastroenterologist
NHS Lothian	Edinburgh	Dr Charlie Lees	Consultant Gastroenterologist and Honorary Senior Lecturer
NHS Lothian	Edinburgh	Dr Gareth Jones	Research fellow
Royal Devon and Exeter NHS Foundation Trust	Exeter	Dr Tariq Ahmad	Consultant Gastroenterologist
Royal Devon and Exeter NHS Foundation Trust	Exeter	Dr James W Hart	Consultant Paediatrician
Glasgow Royal Infirmary	Glasgow	Dr Daniel R Gaya	Consultant Gastroenterologist
Royal Hospital for Children	Glasgow	Prof Richard K Russell	Consultant Paediatric Gastroenterologist
Royal Hospital for Children	Glasgow	Dr Lisa Gervais	Research fellow
Gloucestershire Hospitals NHS Trust	Gloucester	Dr Paul Dunckley	Consultant Gastroneterologist

United Lincolnshire Hospitals NHS Trust	Grantham	Dr Tariq Mahmood	Consultant Gastroenterologist
James Paget University Hospitals NHS Foundation Trust	Great Yarmouth	Dr Paul J R Banim	Consultant Gastroneterologist
Calderdale and Huddersfield NHS Trust	Halifax	Dr Sunil Sonwalkar	Consultant Gastroenterologist
Princess Alexandra Hospital NHS Trust	Harlow	Dr Deb Ghosh	Consultant Gastroenterologist
Princess Alexandra Hospital NHS Trust	Harlow	Dr Rosemary H Phillips	Consultant Gastroenterologist
Hull and East Yorkshire NHS Trust	Hull	Dr Amer Azaz	Paediatric Consultant Gastroenterologist
Hull and East Yorkshire NHS Trust	Hull	Dr Shaji Sebastian	Consultant Gastroenterologist
Airedale NHS Foundation Trust	Keighley	Dr Richard Shenderey	Consultant Gastroenterologist
Crosshouse Hospital	Kilmarnock	Dr Lawrence Armstrong	Consultant Paediatrician

Crosshouse Hospital	Kilmarnock	Dr Claire Bell	Research fellow
The Queen Elizabeth Hospital NHS Foundation Trust	Kings Lynn	Dr Radhakrishnan Hariraj	Consultant Gastroenterologist
Kingston Hospital NHS Trust	Kingston upon Thames	Dr Helen Matthews	Consultant Gastroenterologist
NHS Fife	Kirkcaldy	Dr Hasnain Jafferbhoy	Consultant Gastroenterologist
Leeds Teaching Hospitals NHS Trust	Leeds	Dr Christian P Selinger	Consultant Gastroenterologist
Leeds Teaching Hospitals NHS Trust	Leeds	Dr Veena Zamvar	Paediatric Consultant Gastroenteorlogist
University Hospitals of Leicester NHS Trust	Leicester	Prof John S De Caestecker	Consultant Gastroenterologist
University Hospitals of Leicester NHS Trust	Leicester	Dr Anne Willmott	Paediatric Consultant Gastroenterologist

Mid Cheshire Hospitals NHS Foundation Trust	Leighton	Mr Richard Miller	Research Nurse
United Lincolnshire Hospitals NHS Trust	Lincoln	Dr Palani Sathish Babu	Consultant Gastroenterologist
Alder Hey Childrens Hospital	Liverpool	Dr Christos Tzivinikos	Consultant Paediatric Gastroenterologist
University College London Hospitals NHS Foundation Trust	London	Dr Stuart L Bloom	Consultant Gastroenterologist
Kings College Hospital NHS Foundation Trust	London	Dr Guy Chung-Faye	Consultant Gastroenterologist
Royal London Childrens Hospital, Barts Health NHS Trust	London	Prof Nicholas M Croft	Paediatric Consultant Gastroenterologist
Chelsea & Westminster Hospital	London	Dr John ME Fell	Consultant Paediatric Gastroenterologist

Chelsea and Westminster Hospital NHS Foundation	London	Dr Marcus Harbord	Consultant Gastroenterologist
North West London Hospitals NHS Trust	London	Dr Ailsa Hart	Consultant Gastroenterologist
Kings College Hospital NHS Foundation Trust	London	Dr Ben Hope	Consultant Paediatrician
Guys & St Thomas' NHS Foundation Trust	London	Dr Peter M Irving	Consultant Gastroenterologist
Barts and The London NHS Trust	London	Prof James O Lindsay	Consultant Gastroenterologist
Guy's and St Thomas' NHS trust	London	Dr Joel E Mawdsley	Gastroenterology Consultant
Lewisham and Greenwich Healthcare NHS Trust	London	Dr Alistair McNair	Consultant Gastroenterologist
Chelsea and Westminster	London	Dr Kevin J Monahan	Consultant Gastroenterologist

Hospital NHS Foundation			
Royal Free London NHS Foundation Trust	London	Dr Charles D Murray	Consultant Gastroenterologist
Imperial College Healthcare NHS Trust	London	Prof Timothy Orchard	Consultant Gastroenterologist
St George's Healthcare NHS Trust	London	Dr Thankam Paul	Paediatric Consultant Gastroenterologist
St George's Healthcare NHS Trust	London	Dr Richard Pollok	Reader and Consultant Gastroenterologist
Great Ormond Street Hospital for Children NHS Foundation Trust	London	Dr Neil Shah	Consultant Gastroenterologist
North West London Hospitals NHS Trust	London	Dr Sonia Bouri	Research fellow
The Luton & Dunstable University Hospital	Luton	Dr Matt W Johnson	Consultant Gastroenterologist

Luton and Dunstable Hospital Foundation Trust	Luton	Dr Anita Modi	Paediatric Consultant with Allergy and Gastroenterology interest
The Luton & Dunstable University Hospital	Luton	Dr Kasamu Dawa Kabiru	Research fellow
Maidstone and Tunbridge Wells NHS Trust	Maidstone	Dr B K Baburajan	Consultant Gastroenterologist
Maidstone and Tunbridge Wells NHS Trust	Maidstone	Prof Bim Bhaduri	Paediatric Consultant Gastroenterologist
Manchester University Hospitals NHS Foundation Trust	Manchester	Dr Andrew Adebayo Fagbemi	Consultant Gastroenterologist
Central Manchester University Hospitals NHS Foundation Trust	Manchester	Dr Scott Levison	Consultant Gastroenterologist

The Pennine Acute Hospitals NHS Trust	Manchester	Dr Jimmy K Limdi	Consultant Gastroenterologist
Manchester University NHS Foundation Trust, Wythenshawe Hospital	Manchester	Dr Gill Watts	Consultant Gastroenterologist
Sherwood Forest Hospitals NHS Foundation Trust	Mansfield	Dr Stephen Foley	Consultant Gastroenterologist
South Tees Hospital NHS Foundation Trust	Middlesbrough	Dr Arvind Ramadas	Consultant Gastroenterologist
Milton Keynes Hospital NHS Foundation Trust	Milton Keynes	Dr George MacFaul	Consultant Gastroenterologist
Newcastle Upon Tyne Hospital Trust	Newcastle	Dr John Mansfield	Consultant Gastroenterologist
Isle of Wight NHS Foundation Trust	Newport	Dr Leonie Grellier	Consultant Gastroenterologist
Norfolk & Norwich University Hospital	Norwich	Dr Mary-Anne Morris	Consultant Paediatric Gastroenterologist

NHS Foundation Trust			
Norfolk & Norwich University Hospital NHS Foundation Trust	Norwich	Dr Mark Tremelling	Consultant Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Prof Chris Hawkey	Consultant Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Dr Sian Kirkham	Consultant Paediatric Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Dr Charles PJ Charlton	Consultant gastroenterologist
Oxford University Hospitals NHS Foundation Trust	Oxford	Dr Astor Rodrigues	Paediatric Consultant Gastroenterologist
Oxford University Hospitals NHS Trust	Oxford	Prof Alison Simmons	Consultant Gastroenterologist
Plymouth Hospitals NHS Trust	Plymouth	Dr Stephen J Lewis	Consultant Gastroenterologist

Poole Hospital NHS Foundation Trust	Poole	Dr Jonathon Snook	Consultant Gastroenterologist
Poole Hospital NHS Foundation Trust	Poole	Dr Mark Tighe	Paediatric Consultant with interest in Oncology and Gastroenterology
Portsmouth Hospitals NHS Trust	Portsmouth	Dr Patrick M Goggin	Consultant Gastroenterologist
Royal Berkshire NHS Foundation Trust	Reading	Dr Aminda N De Silva	Consultant Gastroenterologist
Salford Royal NHS Foundation Trust	Salford	Prof Simon Lal	Consultant Gastroenterologist
Shrewsbury and Telford Hospital NHS Trust	Shrewsbury	Dr Mark S Smith	Consultant Gastroenterologist
South Tyneside NHS Foundation Trust	South Shields	Dr Simon Panter	Consultant Gastroenterologist
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East and North Herts NHS Trust	Stevenage	Dr Martyn Carter	Consultant Gastroenterologist
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Stockport NHS foundation Trust	Stockport	Dr Zahid Mahmood	Consultant Gastroenterologist
North Tees and Hartlepool NHS Foundation Trust	Stockton	Dr Bruce McLain	Paediatric Consultant Gastroenterologist
University Hospitals of North Staffordshire	Stoke-on Trent	Dr Sandip Sen	Consultant Gastroenterologist
University Hospitals of North Midlands NHS Trust	Stoke-on-Trent	Dr Anna J Pigott	Consultant Paediatric Gastroenterologist
City Hospitals Sunderland NHS Foundation Trust	Sunderland	Dr David Hobday	Consultant Gastroenterologist
Taunton and Somerset NHS Foundation Trust	Taunton	Dr Emma Wesley	Consultant Gastroenterologist

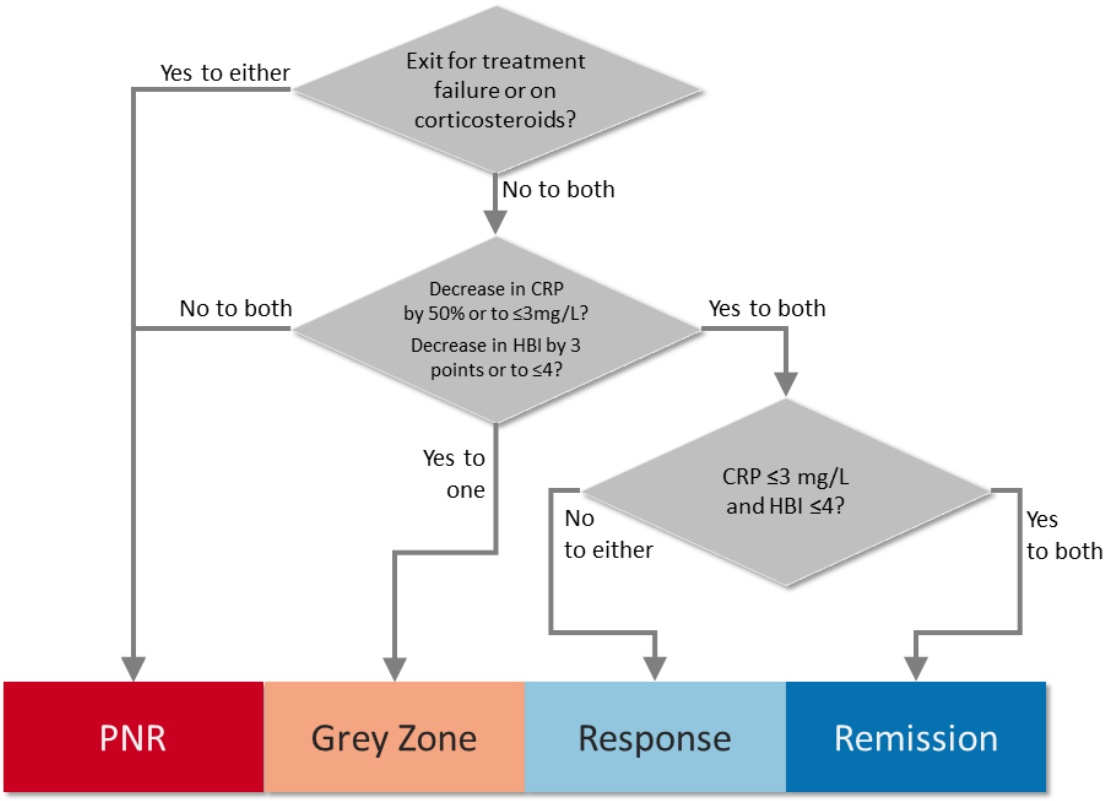
South Devon Healthcare NHS Foundation Trust	Torquay	Dr Richard Johnston	Consultant Gastroenterologist
South Devon Healthcare NHS Foundation Trust	Torquay	Dr Cathryn Edwards	Consultant gastroenterologist
Royal Cornwall Hospitals NHS Trust	Truro	Dr John Beckly	Consultant Gastroenterologist
Mid Yorkshire Hospitals NHS Trust	Wakefield	Dr Deven Vani	Consultant Physician & Gastroenterologist
Warrington& Halton NHS Foundation	Warrington	Dr Subramaniam Ramakrishnan	Consultant Gastroenterologist
West Hertfordshire Hospitals NHS Trust	Watford	Dr Rakesh Chaudhary	Consultant Gastroenterologist
Sandwell and West Birmingham Hospitals NHS Trust	West Bromwich	Dr Nigel J Trudgill	Consultant Gastroenterologist
Sandwell and West Birmingham	West Bromwich	Dr Rachel Cooney	Consultant gastroenterologist

Hospitals NHS Trust			
Weston Area Health NHS Trust	Weston-Super-Mare	Dr Andy Bell	Consultant Gastroenterologist
Royal Albert Edward Infirmary, Wrightington, Wigan & Leigh NHS Foundation Trust	Wigan	Dr Neeraj Prasad	Consultant Gastroenterologist
Hampshire Hospitals NHS Foundation Trust	Winchester	Dr John N Gordon	Consultant Gastroenterologist
Royal Wolverhampton Hospitals NHS Trust	Wolverhampton	Prof Matthew J Brookes	Consultant Gastroenterologist
Western Sussex Hospitals NHS Trust	Worthing	Dr Andy Li	Consultant Gastroenterologist
Yeovil District Hospital NHS Foundation Trust	Yeovil	Dr Stephen Gore	Consultant Gastroenterologist

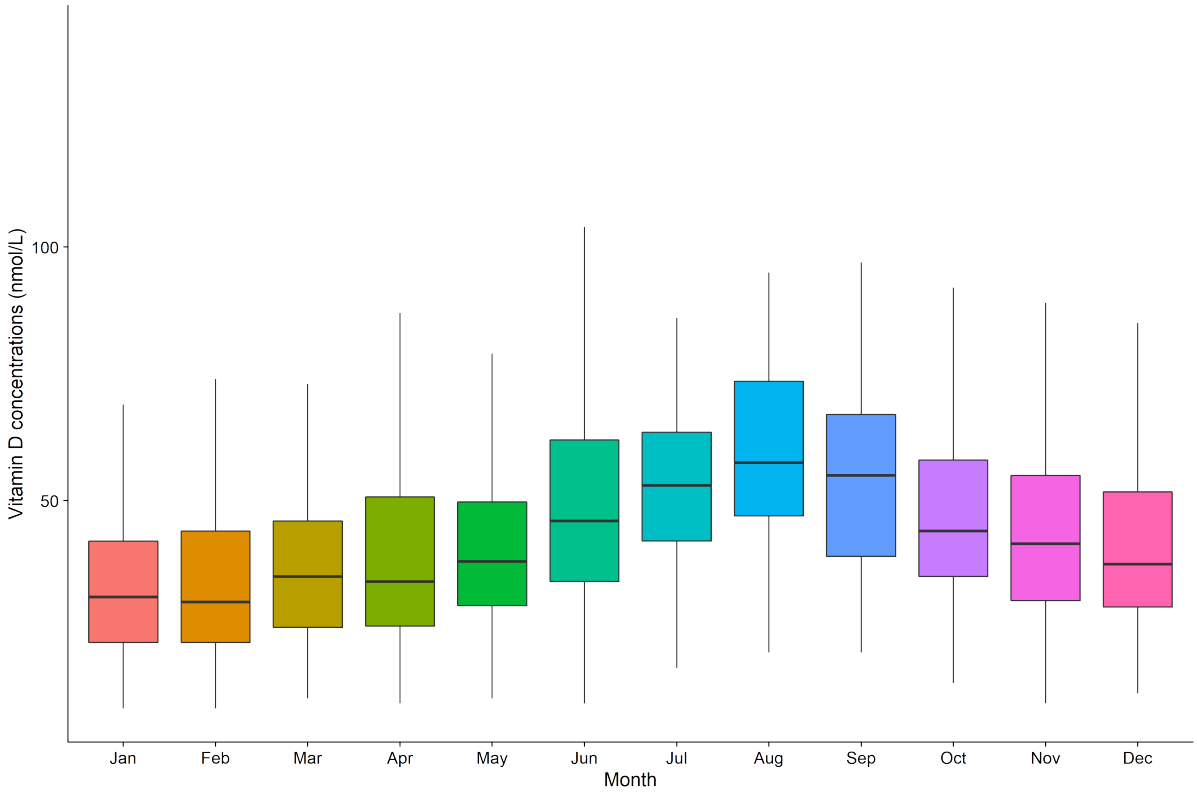
Supplementary Table 2: Baseline demographic and clinical factors and their pre-treatment vitamin D concentrations

Categorical variables				
Variable	**Level**	**n**	**Vitamin D (nmol/L)**	**p**
Sex	Female	574	41 (30 – 58)	0.96
	Male	533	42 (29 – 57)	
Age at first dose < 18 (Paediatric)	True	165	39 (29 – 55)	0.26
	False	942	42 (29 – 58)	
Montreal disease location	L1 only	319	44 (30 – 61)	0.07
	L2 or L3 only	671	41 (29 – 56)	
	L4 modifier	107	39 (28 – 56)	
Smoking history	Current smoker	187	39 (29 – 56)	0.34
	Non-current smoker	906	42 (30 – 58)	
Baseline immunomodulator use	Yes	640	42 (30 – 58)	0.65
	No	467	40 (29 – 57)	
Baseline steroid use	Yes	314	41 (30 – 55)	0.79
	No	793	41 (29 – 58)	
Continuous variables				
Variable			**Spearman's Rho (R)**	**p**
Age at first dose			0.06	0.05
Disease duration			0.01	0.69

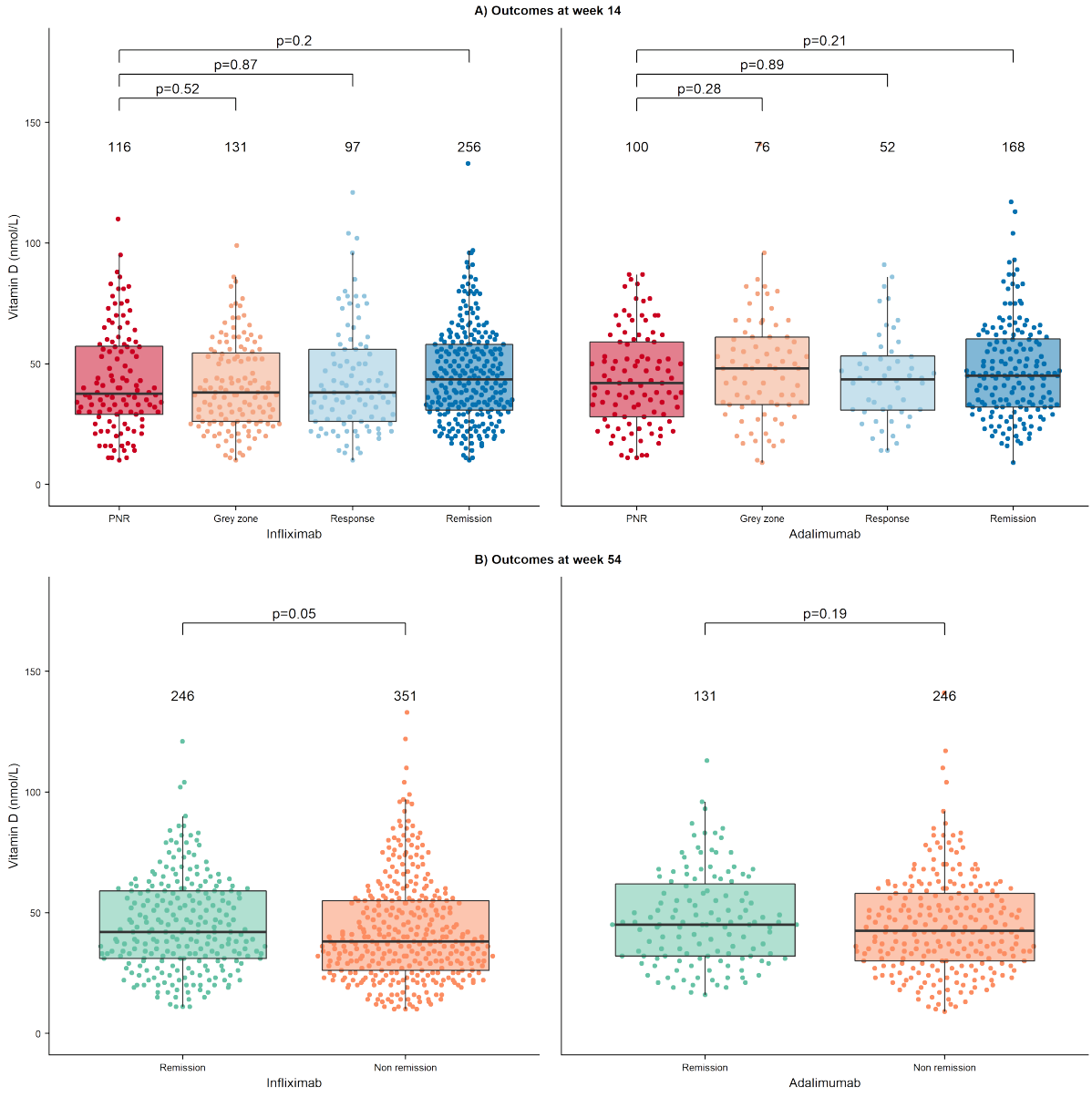
Supplementary Figure 1: Outcome definitions in the PANTS study. Primary non-response was defined using the Harvey Bradshaw Index in adults and the short paediatric Crohn's disease activity index in children, in combination with corticosteroid use, and CRP. *HBI=Harvey Bradshaw Index, sPCDAI=short paediatric Crohn's disease activity index, CRP=C-reactive protein, PNR=primary non-response.*



Supplementary Figure 2: Vitamin D concentrations stratified by month of sampling



Supplementary Figure 3: Pre-treatment vitamin D concentrations at baseline stratified by outcomes to anti-TNF. Outcomes to week 14 shown in A) and outcomes to week 54 shown in B). Patients treated with infliximab shown on the left and adalimumab on the right. The horizontal bars represent the tests undertaken between the individual groups, and p values generated using the Wilcoxon test displayed above each bar. PNR=primary non-response.



Supplementary Figure 4: Forest plot showing the exponentiated coefficients from a multivariable linear regression model of associations with anti-TNF drug concentrations at week 14. Model to infliximab drug concentrations shown in A) and to adalimumab drug concentrations shown in B). *CRP=C-reactive protein, BMI=body mass index, HBI=Harvey Bradshaw index.*

A) Infliximab

Variable		N	Fold change (95% CI)	p
Vitamin D (nmol/L)		218	1.00 (1.00, 1.01)	0.062
log(`Baseline calprotectin`)		218	0.94 (0.87, 1.02)	0.13
Smoking status	Never/ex-smoker	189	Reference	
	Current smoker	29	0.82 (0.64, 1.05)	0.12
log(`Anti-drug antibody at week 14`)		218	0.82 (0.76, 0.88)	<0.0001
log(`CRP at week 14`)		218	0.91 (0.84, 0.98)	0.018
Albumin at week 14		218	1.02 (1.00, 1.04)	0.026
log(`Calprotectin at week 14`)		218	0.91 (0.85, 0.98)	0.0082

B) Adalimumab

Variable		N	Fold change (95% CI)	p
Vitamin D (nmol/L)		293	1.00 (1.00, 1.00)	0.42
Smoker	Never/ex-smoker	238	Reference	
	Current smoker	55	0.87 (0.76, 1.01)	0.073
log(`Anti-drug antibody at week 14`)		293	0.68 (0.64, 0.71)	<0.0001
log(`Calprotectin at week 14`)		293	0.88 (0.84, 0.92)	<0.0001
BMI at study entry		293	0.97 (0.96, 0.98)	<0.0001
HBI at study entry		293	0.98 (0.97, 1.00)	0.025
log(`Baseline CRP`)		293	0.97 (0.93, 1.02)	0.21

Chapter 6: Understanding the mechanisms and management of loss of response to anti-TNF therapy: three-year data from the PANTS study

Neil Chanchlani, Simeng Lin, Claire Bewshea, Benjamin Hamilton, Amanda Thomas, Rebecca Smith, Christopher Roberts, Maria Bishara, Rachel Nice, Charlie W Lees, Shaji Sebastian, Peter M Irving, James Lindsay, Richard K Russell, Timothy J McDonald, James R Goodhand, Tariq Ahmad, Nicholas A Kennedy, on behalf of the PANTS Consortium

Accepted and in press at *The Lancet Gastroenterology and Hepatology.*

Study aim

To report the effectiveness of infliximab and adalimumab up to three years of treatment, the factors associated with anti-TNF treatment failure, and the effective strategies to prevent and mitigate loss of response.

My role in the study

The PANTS study recruited participants from 2013 – 2016, and follow-up took place until 2019. From 2019 until publication of study results in 2023, I was study lead, responsible for data collection, cleaning, and management (described in Chapter 2 in detail). I analysed the loss of response data, and verified all data published as part of the study. As lead author, I wrote the abstract, and submitted to multiple conferences, where it was published both oral and poster presentations. I wrote the paper, which has been accepted by the *Lancet Gastroenterology and Hepatology.*

Findings

Only about one-third of patients with active luminal Crohn's disease treated with an anti-TNF drug were estimated to be in remission at the end of two and three years. This was predicted by remission status at the end of treatment induction and year one. For both infliximab and adalimumab, low week 14 anti-TNF concentrations and presence of immunogenicity, were predictive of lower year two and year three remission rates. Two-thirds of initial responders experienced loss of response events by the end of year three. Loss of response events, for both infliximab and adalimumab-treated patients, were predicted by low anti-TNF concentrations at week 14, and for infliximab-treated patients, lower thiopurine dose quartiles. Drug-

clearing anti-drug antibodies, detected in almost a half of infliximab-treated patients and one fifth of adalimumab-treated patients by three years, were associated with loss of response or treatment failure. Concomitant use of an immunomodulator, started prior to, or on the day of the first infliximab infusion, was associated with increased survival without the development of drug-clearing antibodies. Infliximab dose intensification in the setting of immune-mediated pharmacokinetic failure was associated with low rates of drug persistence.

Relevance and impact on my learning

This project is my most impactful work to date, and has been accepted for publication in the highest-ranking gastroenterology journal worldwide. I reviewed data submitted for 1610 patients from 120 UK sites, across nine years of the study, which was a difficult task, but one that allowed me to exercise multiple research skills and learn a great deal with respect to study design and implementation. One of the most important lessons I learnt was that coordinating multi-site research requires clear aims, objectives, and standardised operating procedures to be cemented *a priori*. Personnel involved in submitting data for studies work most efficiently when they have clear direction and plan to follow. In order to obtain high-quality data, it is best to design platforms by which data is submitted to be user-friendly, with examples, and to minimise free text that can be entered.

Fostering good relationships is essential to achieving success within a study, particularly with respect to long-term cohort studies that rely on continued engagement from researchers, patients, and funders.

Acknowledgements of co-authors and contributions to paper

Tariq Ahmad conceived the design of this study. Claire Bewshea was project manager for the PANTS study. Rachel Nice and Timothy J Macdonald coordinated all biochemical analyses and central laboratory aspects of the project. All authors and collaborators were involved in the acquisition, analysis, or interpretation of data. Data analysis was done by myself, Simeng Lin, and Nicholas A Kennedy. Drafting of the manuscript was done by myself, Simeng Lin, James R Goodhand, Tariq Ahmad, and Nicholas A Kennedy. Tariq Ahmad obtained the funding for the study. Myself, Simeng Lin, and Nicholas A Kennedy have verified the underlying data.

Abstract

Background

Anti-TNF drugs are effective treatments for the management of Crohn's disease but treatment failure is common. We aimed to report the effectiveness of infliximab and adalimumab up to three years of treatment, the factors associated with anti-TNF treatment failure, and the effective strategies to prevent and mitigate loss of response.

Methods

The personalised anti-TNF therapy in Crohn's disease study (PANTS) is a prospective observational UK-wide study. We enrolled anti-TNF-naive patients (aged ≥6 years) with active luminal Crohn's disease at the time of first exposure to infliximab or adalimumab between March 7, 2013, and July 15, 2016. At the end of first year, sites were invited to take part in the PANTS-extension (PANTS-E) that extended follow-up to three years, where disease activity score, weight, therapy, adverse events, and drug and total anti-drug antibody concentrations were recorded at six-monthly visits.

Treatment failure endpoints were non-remission at years one, two, and three, and adverse events leading to drug withdrawal. Loss of response (LOR) was defined in patients who initially responded to anti-TNF therapy but subsequently developed symptomatic IBD activity. We used regression analyses to identify which factors were associated with treatment failure, and modified survival technique with permutation testing to estimate remission rates.

Findings

In PANTS, we enrolled 955 patients treated with infliximab and 655 treated with adalimumab. Of these, 594 patients entered PANTS-E (387 [65%] infliximab -and 207 [35%] adalimumab-treated patients).

Across all patients recruited to the PANTS, the estimated proportions of patients in remission at the end of years one, two, and three were, for infliximab: 40.1% (95%CI 36.7 – 43.7), 34.4% (95%CI 29.9 – 39), and 34.7% (95%CI 29.8 – 39.5), respectively, and for adalimumab: 36% (95%CI 31.4 – 50.6), 32.9% (95%CI 26.8 – 39.2), and 28.9% (95%CI 21.9 – 36.3), respectively. Optimal drug concentration thresholds at week 14 to predict remission at years one, two, and three were about 6.1 - 10 mg/L for infliximab and 10 - 12 mg/L for adalimumab.

The estimated proportion of patients who had LOR events by years one, two, and three were, for infliximab: 34.5% (30.5–38.2), 54.5% (49.5 – 59), and 60.1% (54.2 – 65.2), respectively, and for adalimumab: 32.5% (27–37.5), 47.6% (40.5 – 53.7), and 68.6% (51.2 – 79.8), respectively. For both drugs, week 14 drug concentration was the major independent risk factor associated with LOR after that timepoint. Additionally, female sex, obesity, and lower thiopurine drug quartiles were associated with LOR in infliximab-treated patients. Treatment with an immunomodulator prior to, or at the time of, starting infliximab, but not adalimumab, was associated with increased survival without the development of drug-clearing immunogenicity.

Amongst infliximab-treated patients who were dose-intensified at the point of LOR, those who experienced immune-mediated pharmacokinetic failure had the lowest rates of drug persistence compared to other treatment failure groups.

Interpretation

Only about one-third of patients with active luminal Crohn's disease treated with an anti-TNF drug are in remission at the end of three years of treatment. Low drug concentrations at the end of induction predict loss of response and non-remission to year 3 of treatment, suggesting higher drug concentrations during the first year of treatment, particularly during induction, may lead to better long-term outcomes. Drug-clearing anti-drug antibodies are associated with long-term loss of response and treatment failure, and can be mitigated by an immunomodulator, started prior to, or on the day of the first infliximab infusion.

Background

Over the last three decades, the anti-TNF monoclonal antibodies infliximab and adalimumab have become the most frequently prescribed and cost-effective biologics for immune-mediated inflammatory diseases worldwide.[1,2]

Unfortunately, anti-TNF treatment failure is common, with a quarter of patients experiencing primary non-response, and one-third of initial responders losing response by the end of the first year. In the first year of the Personalising Anti-TNF Therapy in Crohn's Disease (PANTS) study, we observed a complex multi-directional relationship between disease activity, anti-TNF drug concentrations, and the development of anti-drug antibodies.[3] Individuals with the most active disease had the highest risk of sub-optimal drug concentrations and subsequent immunogenicity, leading to drug clearance and treatment failure. The major modifiable factor in this disease-drug-immune response relationship was drug concentration.

Estimated anti-TNF response rates beyond a year are scarce.[4–8] However, they are increasingly important when weighing up the long-term risks and benefits of multiple new medical and surgical options.[1,2,9] Observational studies have largely been retrospective in nature, from single-centres, and have rarely reported pharmacokinetic data or explored the utility of therapeutic drug monitoring (TDM) in the setting of loss of response.[10–12] Consequently, the factors associated with longer-term anti-TNF treatment failure remain poorly elucidated.

Guidelines recommend strategies to manage loss of response informed by TDM, but data to support these actions are limited.[13,14] Stratifying loss of response episodes based on clinical symptoms, anti-TNF drug concentration, and the development of anti-drug antibodies are used by clinicians to adjust anti-TNF dose or frequency, optimise concomitant immunomodulator, or switch to another targeted therapy.

Here we report data from the two-year extension to the PANTS study, including the effectiveness of infliximab and adalimumab at two and three years, the factors associated with anti-TNF treatment failure, and the effective strategies to prevent and mitigate loss of response.

Methods

Study design and participants

PANTS is a UK-wide, multicentre, prospective observational cohort study reporting the rates of treatment failure of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar, CT-P13 [Celltrion, South Korea]) and adalimumab (Humira [Abbvie, USA]).[3,15–18]

In brief, patients were recruited at the time of first anti-TNF exposure between March 2013 and July 2016 (Supplementary Table 1).[3] Eligible patients were aged ≥ 6 years with objective evidence of active luminal Crohn's disease involving the colon and/or small intestine. Exclusion criteria included prior exposure to, or contraindications for the use of, anti-TNF therapy. The choice of anti-TNF was at the discretion of the treating physician and prescribed according to the licensed dosing schedule. All patients were followed-up for one year or until drug withdrawal. At the end of first year, sites were invited to take part in the PANTS-extension (PANTS-E) that extended follow-up to three years.

Study visits were scheduled at first dose, post-induction (week 14), and at weeks 30 and 54. In PANTS-E the visits occurred 6-monthly and at treatment failure or exit. Exit occurred when patients stopped anti-TNF therapy or had an intestinal resection. In cases where the visit did not exactly occur on the day delineated by the protocol, the following windows of eligibility were specified: week 0 (week -4 to 0), week 14 (week 10 to 20), week 30 (week 22 to 38), week 54 (week 42 to 66), week 78 (week 66 to 90), week 102 (week 90 to 114), week 126 (week 114 to 138), and week 150 (week 138 to 162).

Outcomes

We used composite endpoints using the Harvey Bradshaw Index (HBI) in adults and the short paediatric Crohn's disease activity index (sPCDAI) in children, corticosteroid use, and serum C-reactive protein (CRP) to define primary non-response (Supplementary Figure 1).

Remission at any time point was defined as CRP of ≤3 mg/L and HBI of ≤4 points (sPCDAI ≤15 in children), without corticosteroid therapy or exit for treatment failure. Non-remission was defined as CRP of >3mg/L and/or HBI of >4 points (sPCDAI >15), corticosteroid therapy, or exit for treatment failure. For the purposes of non-remission and loss of response we defined corticosteroid therapy as any systemic therapy, either oral or intravenous (excluding use of steroids for other conditions), but not including single pre-infusion dosing with hydrocortisone.

Loss of response was defined in patients who initially responded to anti-TNF therapy at the end of induction who subsequently developed symptomatic IBD activity that warranted an escalation of steroid, immunomodulatory or anti-TNF therapy, resectional surgery, or exit from study due to treatment failure, including adverse events. Anti-TNF dose intensification was defined as an increase in anti-TNF dose and/or shortening of the time interval between anti-TNF doses. Time to loss of response was defined as the time from initiation of an anti-TNF to treatment escalation, drug withdrawal, or surgery.

Drug persistence was defined as the length of time from initiation of anti-TNF to discontinuation of therapy.[19]

Adverse events were coded centrally according to the Medical Dictionary for Regulatory Activities (MedDRA) version 23.1. Serious adverse events included those that required hospitalization, were life-threatening, or resulted in persistent/permanent or significant disability/incapacity. Causality was graded according to the Good Clinical Practice framework guidelines as ‘not related’, ‘unlikely’, ‘possibly’, ‘probably’, or ‘definitely’ by the local research sites.[20]

Clinical and laboratory variables

Variables recorded at baseline by sites were demographics (age, sex, ethnicity, comorbidities, height and weight, and smoking status), Crohn’s disease phenotype (age at diagnosis, disease duration, Montreal classification), prior medical history and its treatments (drug history and previous Crohn’s disease-related surgeries). Blood and stool samples were processed through the central laboratory at the Royal Devon University Healthcare NHS Foundation Trust (exeterlaboratory.com) for haemoglobin, white cell count, platelets, serum albumin, CRP, anti-TNF drug and anti-TNF antibody concentrations, and faecal calprotectin, respectively. For all infliximab-treated patients we measured trough drug concentrations. For adalimumab-treated patients, we asked research sites to take blood samples as near as possible to trough whilst minimising inconvenience to patients.

The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor free infliximab (K9655) and adalimumab (K9657) drug concentration assays permit quantitative measurement of a free therapeutic drug in serum. The assays follow a standard ELISA format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody.

Since our previous publication reporting immunogenicity outcomes to the end of first year[3], the infliximab drug concentration assay has been recalibrated to an international standard. The measuring range for infliximab is now 1.88 - 45 mg/L, with absence of drug defined using a cutoff <1.88 mg/L. For adalimumab, the measuring range remains 0.8 – 45 mg/L, with absence of drug defined using a cutoff of <0.8 mg/L.

The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor infliximab (K9654) and adalimumab (K9651) total anti-drug antibody assays allow semi-quantitative measurement of both free and bound anti-drug antibodies.[21,22] A pre-treatment acid dissociation step is used to separate anti-drug antibodies from the therapeutic antibody. The assay then follows a standard ELISA format using recombinant therapeutic antibody as a capture and detection antibody. Positivity thresholds for anti-infliximab antibodies is 9 AU/ml and for anti-adalimumab antibodies is 6 AU/mL.[16]

Results of TDM tests were made available to clinicians in real-time once participants had completed 12 months in the study. Management of treatment failure was decided by the treating clinicians and not mandated by TDM results. We evaluated the impact of drug and antidrug antibody concentrations at the time of loss of response using internationally-recommended definitions:[14,19,23,24]

1. **Immune-mediated pharmacokinetic failure** was defined as treatment failure with undetectable anti-TNF drug concentrations (infliximab <1.88 mg/L, adalimumab <0.8 mg/L), and the presence of anti-TNF antibodies (infliximab ≥9 AU/mL, adalimumab ≥6 AU/ml).

2. **Non-immune-mediated pharmacokinetic failure** was defined as treatment failure with undetectable or subtherapeutic anti-TNF drug concentrations (infliximab ≤10.25 mg/L, adalimumab ≤12 mg/L), and absence of anti-TNF antibodies (infliximab <9 AU/ml, adalimumab <6 AU/mL).
3. **Pharmacodynamic failure in the presence of antibodies ('double positive' status)** was defined as treatment failure with subtherapeutic anti-TNF drug concentrations (infliximab 1.88 – 10.25 mg/L, adalimumab 0.8 - 12 mg/L), and the presence of anti-TNF antibodies (infliximab ≥9 AU/ml, adalimumab ≥6 AU/mL).
4. **Pharmacodynamic failure in the absence of antibodies** was defined as treatment failure with adequate anti-TNF drug concentrations (infliximab >10.25 mg/L, adalimumab >12 mg/L), and the absence of anti-TNF antibodies (infliximab <9 AU/ml, adalimumab <6 AU/mL).

Statistical analysis

Sample size considerations and statistical analysis

The sample size calculation for the PANTS study was based on identifying a genetic marker of primary non-response to anti-TNF therapy.[3] It was adjusted for the introduction of the infliximab biosimilar CT-P13 that occurred during the study. In brief, 1610 patients, 955 [59.3%] treated with infliximab (Remicade 753 [46.8%], CT-P13 202 [12.5%]) and 655 [40.7%] treated with adalimumab were included.

Statistical analyses were undertaken in R 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two tailed and p-values <0.05 were

considered significant. We included patients with missing clinical variables in analyses for which they had data and have specified the denominator for each variable. Continuous data are reported as median and interquartile range, and discrete data as numbers and percentages.

Because of differences in drug formulation, route of delivery, dosing interval and rates of immunogenicity infliximab and adalimumab treatment outcomes were analysed separately. We performed univariable analyses using Fisher's exact and Mann-Whitney U tests to identify differences in characteristics between infliximab- and adalimumab-treated patients.

We included the whole cohort to estimate remission over the course of the first three years of treatment. Patients who exited the study because of treatment failure were deemed to be in non-remission for every subsequent timepoint. Patients who exited the study because of loss to follow-up, patient withdrawal of consent, or elective withdrawal of drug by their physician, including for pregnancy, were censored at the time of study exit and were excluded from the denominator for subsequent analyses.

To account for variable length of follow up, including the requirement to consent separately for the extension phase, we used a modified survival technique to estimate remission rates at later timepoints. We used permutation testing to determine statistical significance for comparisons using estimates of remission. This was done by permuting the values for the independent covariate of interest and determining the proportion of repetitions where we observed results at least

extreme as the one we observed in the real data. We used the comparisons of the absolute of the log odds ratio, and therefore p-values are two-tailed. We used bootstrapping to calculate 95% confidence intervals of the estimates.

Only patients who had responded to anti-treatment at week 14 were used to assess rates of and the factors predictive of loss of response thereafter. Rates of loss of response and immunogenicity were estimated using the Kaplan-Meier method, and comparative analyses were performed using univariable and multivariable Cox proportional hazards regression. In our analyses of time to loss of response and immunogenicity patients were censored if they exited for reasons other than treatment failure, after their last drug and antibody measurement or at week 150.

We used multivariable Cox proportional hazards analyses to confirm which factors were independently associated with loss of response. We included variables with univariable $p<0.05$ in the model and used Akaike Information Criterion (AIC) and backward stepwise variable selection. We also built predictive models, using backwards stepwise model selection starting from the null model, again using AIC. We used leave-one-out cross validation to test the model, firstly to ensure the model was not overfitted and secondly to estimate the diagnostic accuracy of the model. For prediction testing, a probability threshold was determined by maximising the sum of sensitivity and specificity. We explored associations with drug concentration using linear regression, using the same variable selection methods as those detailed above for logistic regression.

Optimal thresholds for drug concentrations were determined graphically by plotting outcome against intervals of drug concentration and looking for the threshold beyond which further increases were not associated with improvement in outcome. Non-inferiority for biosimilar infliximab was assessed by determining whether the one-sided 95% confidence interval of the absolute difference in proportions was ≥10%. The confidence interval was calculated using the prop.test function in R.

Role of the funding source and ethical consideration

PANTS is an investigator-led study funded by the research charities CORE, CCUK, and C3, and by unrestricted educational grants from Abbvie (USA), Merck Sharp & Dohme (UK), NAPP Pharmaceuticals (UK), Pfizer (USA), and Celltrion Healthcare (South Korea). No funding bodies had any role in study design, data collection or analysis, writing or decision to submit for publication.

The South West Research Ethics committee approved the study (REC reference: 12/SW/0323) in January 2013. Patients were included after providing informed, written consent. The protocol is available online [ClinicalTrials.gov number: NCT03088449].

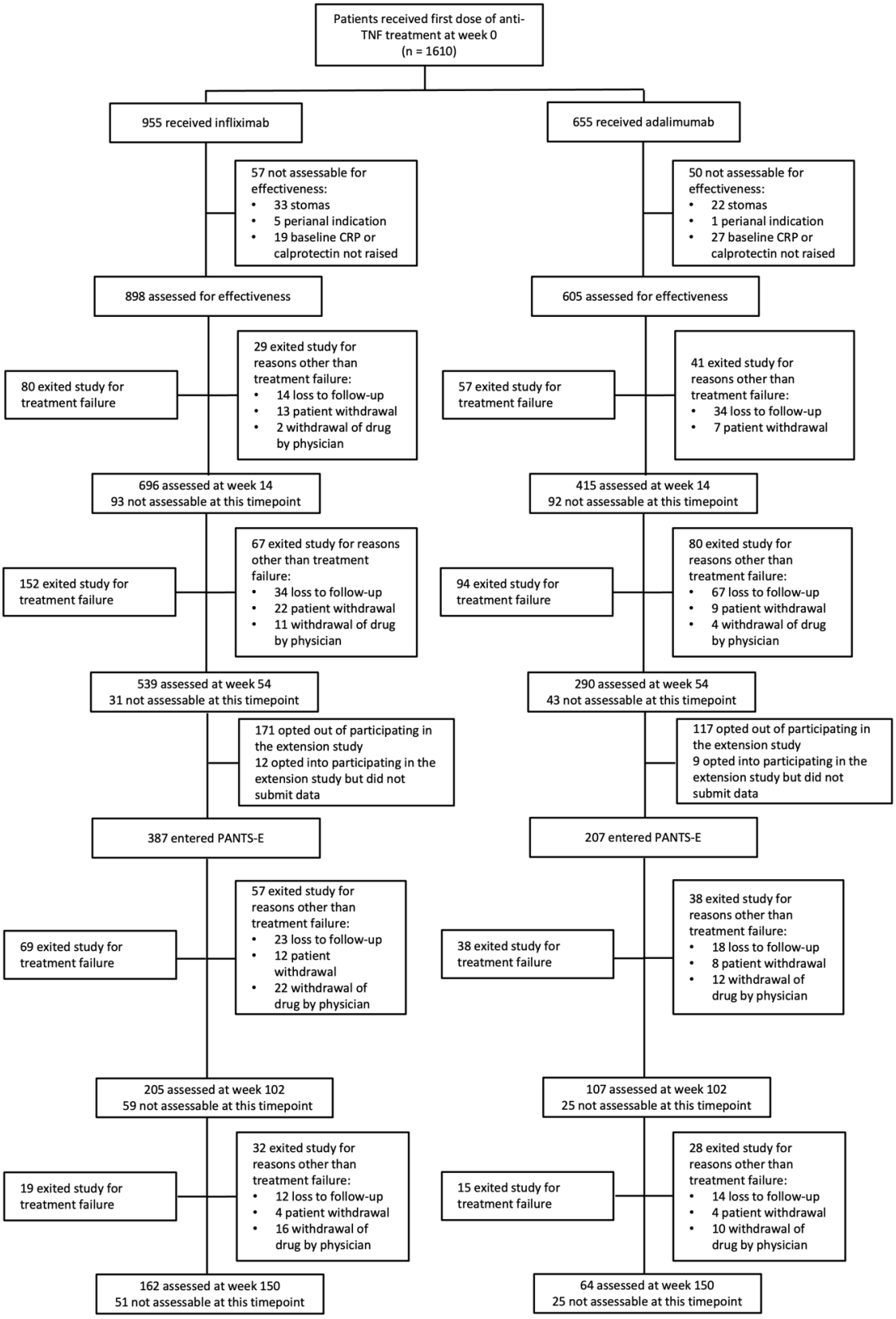
Findings

Participants

Between March 19th 2014 and September 21st 2017, 594 patients rolled over into the PANTS-E: 387 (65.1%) were treated with infliximab (263 [68%] originator infliximab, and 76 [19.6%] with biosimilar, and 48 [12.4%] having switched to biosimilar in the first year of study) and 207 (34.8%) were treated with adalimumab (Figure 1, Supplementary Table 2). By year 3, most participants had switched from infliximab originator (Remicade) to infliximab biosimilar (CT-P13) (Supplementary Figure 2).

Figure 1: Study profile

Patients were not assessable when one or more key data items were missing.



CRP=C-reactive protein.

At entry into PANTS-E, several baseline characteristics were significantly different between infliximab- and adalimumab-treated patients, including age, body mass index, disease duration, disease behaviour, and presence of perianal disease (Table 1). Compared to adalimumab-treated patients, those treated with infliximab had higher rates of immunogenicity (infliximab: 56% [218/387] adalimumab: 31% (65/207]), higher faecal calprotectin concentrations (infliximab: 444 µg/g [IQR 201 – 945] adalimumab: 293 (µg/g) [141 – 626), and increased rates of immunomodulator use (infliximab: 67% [258/387] adalimumab: 53% [109/207]) (all $p < 0.001$).

Table 1: Baseline demographic, clinical, and therapeutic drug monitoring characteristics of participants who entered extension study, by drug.

Data are number (%) or median (IQR). p values were calculated using Fisher's exact or Mann Whitney U tests. *BMI=Body Mass Index. PNR=Primary Non Response.*

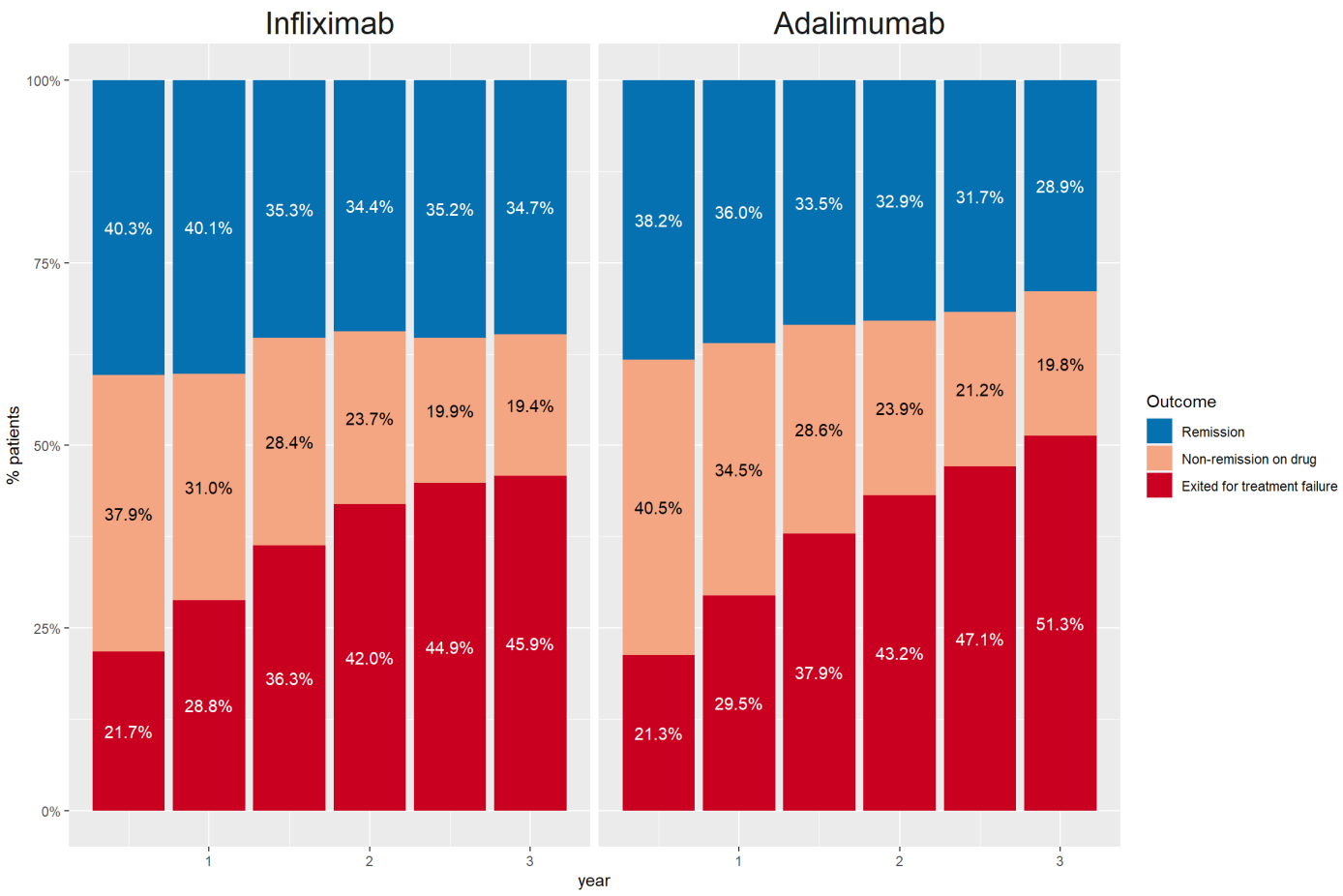
Variable	Level	Infliximab n = 387	Adalimumab n = 207
Sex (male)	Female	193/387 (50%)	112/207 (54%)
	Male	194/387 (50%)	95/207 (46%)
Age (years)		28.8 (18.4 - 43)	40.8 (29.7 - 51.7)
Disease duration (years)		2.2 (0.6 - 6.9)	3.7 (0.9 - 12.3)
Baseline immunomodulator		258/387 (67%)	109/207 (53%)
Montreal location	L1	103/383 (27%)	60/203 (30%)
	L2	93/383 (24%)	52/203 (26%)
	L3	184/383 (48%)	90/203 (44%)
	L4	3/383 (1%)	1/203 (0%)
Montreal behaviour	B1	259/385 (67%)	118/205 (58%)
	B2	92/385 (24%)	73/205 (36%)
	B3	34/385 (9%)	14/205 (7%)
Perianal		69/387 (18%)	18/207 (9%)
Previous resectional surgery		68/387 (18%)	48/207 (23%)
Charlson comorbidity score	0	359/387 (93%)	180/207 (87%)
	1	25/387 (6%)	21/207 (10%)

	>=2	3/387 (1%)	6/207 (3%)
Baseline BMI category	Normal	185/387 (48%)	102/207 (49%)
	Underweight	76/387 (20%)	11/207 (5%)
	Overweight	87/387 (22%)	66/207 (32%)
	Obese	39/387 (10%)	28/207 (14%)
Baseline current smoker		50/383 (13%)	33/206 (16%)
Baseline haemoglobin (g/L)		124 (113 - 135)	131 (120 - 140)
Baseline white cell count ($\times 10^9$/L)		8 (6.1 - 10.1)	7.5 (5.5 - 9.5)
Baseline platelet count ($\times 10^9$/L)		350 (288 - 422)	304 (255 - 384)
Baseline albumin (g/L)		39 (34 - 42)	40 (36 - 43)
Baseline faecal calprotectin (µg/g)		444 (201 - 945)	293 (141 - 626)
Week 14 drug level (mg/L)		4.1 (2.2 - 8.2)	11.3 (8.8 - 14.6)
Week 14 antibody level (AU/mL)		3 (2 - 5)	3 (2 - 4)
Week 14 status	Remission	194/349 (56%)	94/180 (52%)
	Response	61/349 (17%)	26/180 (14%)
	Grey zone	65/349 (19%)	35/180 (19%)
	PNR	29/349 (8%)	25/180 (14%)
Immunogenicity in first year	Antibody -ve	169/387 (44%)	142/207 (69%)
	Antibody +ve drug +ve	129/387 (33%)	49/207 (24%)
	Antibody +ve drug -ve	89/387 (23%)	16/207 (8%)

Remission

Across all patients recruited to the PANTS study, the estimated proportions of infliximab-treated patients in remission at the end of years one, two, and three were 40.1% (95%CI 36.7 – 43.7), 34.4% (95%CI 29.9 – 39), and 34.7% (95%CI 29.8 – 39.5), respectively (Figure 2). For adalimumab-treated patients, the estimated proportion in remission at years one, two, and three were 36% (95%CI 31.4 – 50.6), 32.9% (95%CI 26.8 – 39.2), and 28.9% (95%CI 21.9 – 36.3), respectively. Estimated proportions for CT-P13 and Remicade-treated patients were similar (Supplementary Figure 3).

Figure 2: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, by anti-TNF



Of infliximab-treated patients estimated to be in remission at week 14, the chance of being in remission at years 1, 2, and 3 were 63.3% (95%CI 57.7 – 68.9), 53.9% (95%CI 46.5 – 61.7), and 54.2% (95%CI 46.2 – 62.1%), respectively. Of adalimumab-treated patients estimated to be in remission at week 14, the chance of being in remission at years 1, 2, and 3 were 60% (95%CI 52 – 67.6), 46.8% (95%CI 36.4 – 57.2), and 48.7% (95%CI 36.1 – 61.3%), respectively.

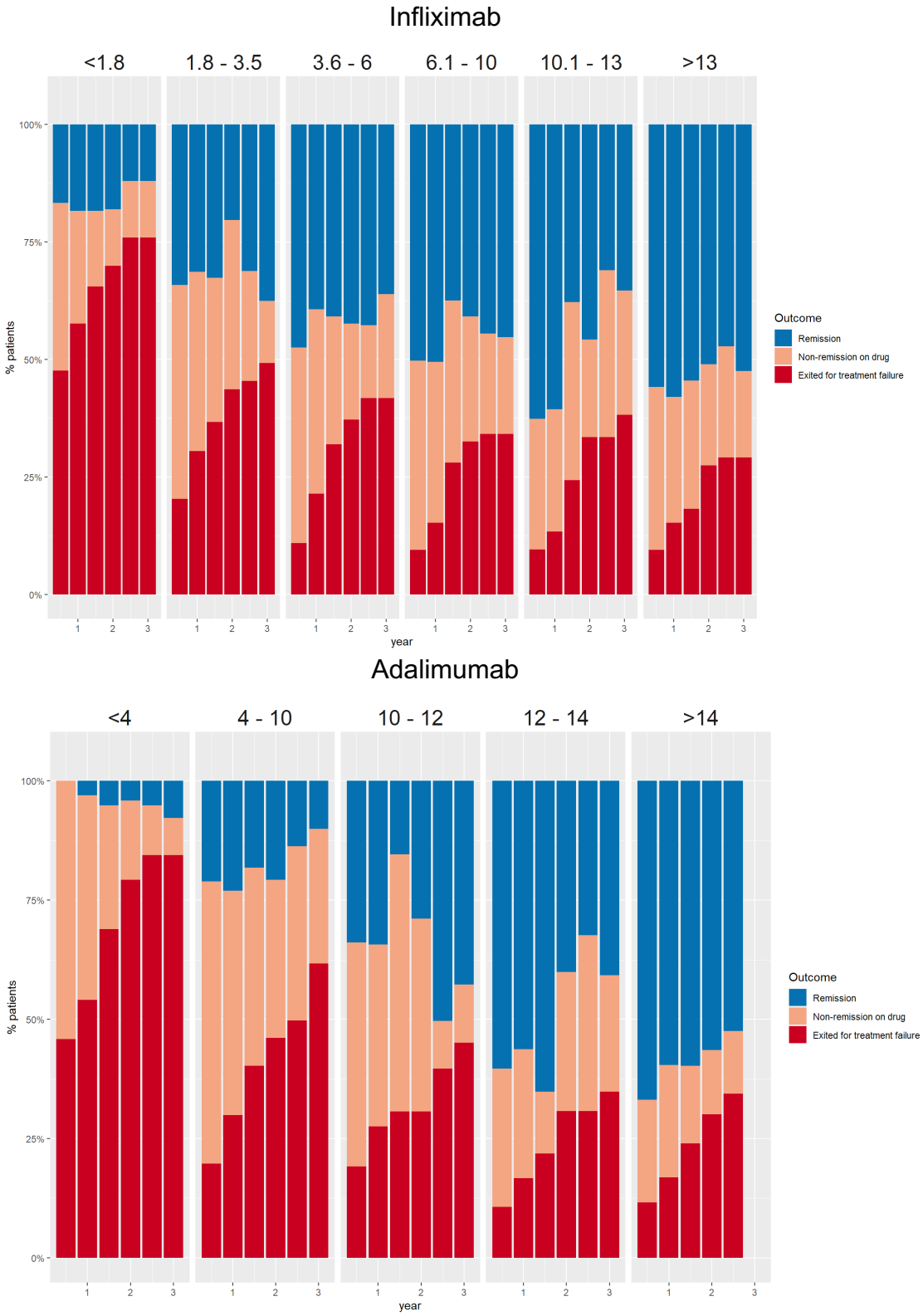
Of infliximab-treated patients estimated to be in remission at year 1, the chance of being in remission at years 2 and 3 were 70.3% (95%CI 63 – 77.3) and 62.7% (95%CI 54.4 – 70.5%), respectively. Of adalimumab-treated patients estimated to be in remission at year 1, the chance of being in remission at years 2 and 3 were 69.6% (95%CI 58.8 – 79.9) and 66.4% (95%CI 49.7 – 81.2%), respectively.

Female sex was associated with decreased rates of remission to infliximab, but not adalimumab, at years 2 and 3 (Supplementary Figure 4). A dose-response association was seen for week 14 drug concentration and remission rates at year 2 and 3 (Figure 3). Determined graphically, optimal drug concentration thresholds at week 14 to predict remission at years 1, 2 and 3 were about 6.1 - 10 and 10 - 12 mg/L for infliximab and adalimumab, respectively. For both infliximab and adalimumab, optimal week 14 drug concentrations were associated with increased remission rates at years 2 (infliximab: odds ratio [OR] 2.2 [95%CI 1.4 – 3.6] adalimumab: OR 3.6 [95%CI 1.8 – 8.7]) and 3 (infliximab: OR 1.9 [95%CI 1.2 – 3.1], adalimumab: OR 6.2 [95%CI 2.5 – 23.2]). In addition, presence of anti-drug antibodies at week 14 was associated with decreased remission rates at years 2 (infliximab: OR 0.44 [95%CI 0.21 – 0.81], adalimumab: OR 0.16 [0 – 0.46]) and 3

(infliximab: OR 3.72 [95%CI 0.15 – 0.72], adalimumab: OR 0.21 [0.08 – 0.71])

(Supplementary Figures 5 and 6).

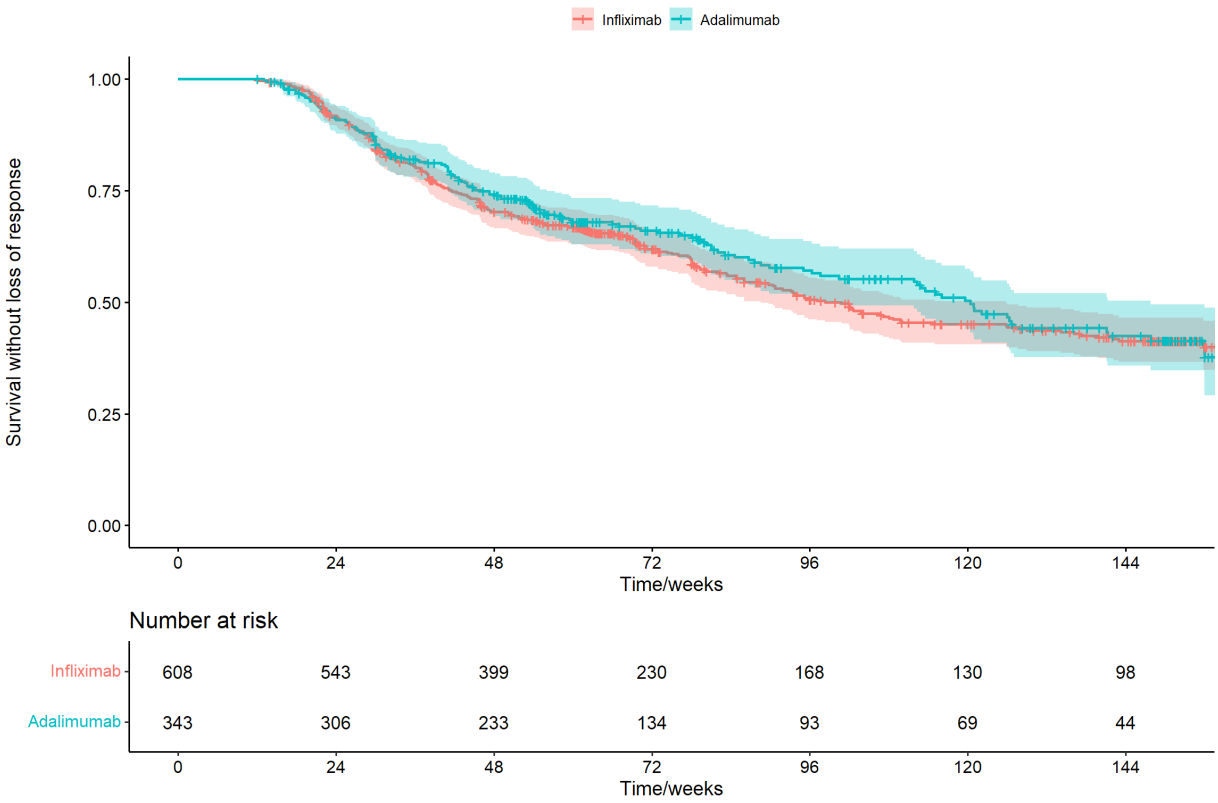
Figure 3: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, by week 14 drug level



Loss of response events

After excluding patients who experienced primary non-response, the estimated proportion of infliximab-treated patients who experienced loss of response events by years one, two, and three were 34.5% (30.5–38.2), 54.5% (49.5 – 59), and 60.1% (54.2 – 65.2), respectively (Figure 4). For adalimumab-treated patients, the rates of loss of response events by years one, two, and three were 32.5% (27 – 37.5), 47.6% (40.5 – 53.7), and 68.6% (51.2 – 79.8), respectively. Estimated rates for loss of response events for CT-P13 and Remicade-treated patients were similar (Supplementary Figure 7).

Figure 4: Time to loss of response, or exit for treatment failure using Kaplan-Meier and Cox proportional hazards methods, by anti-TNF



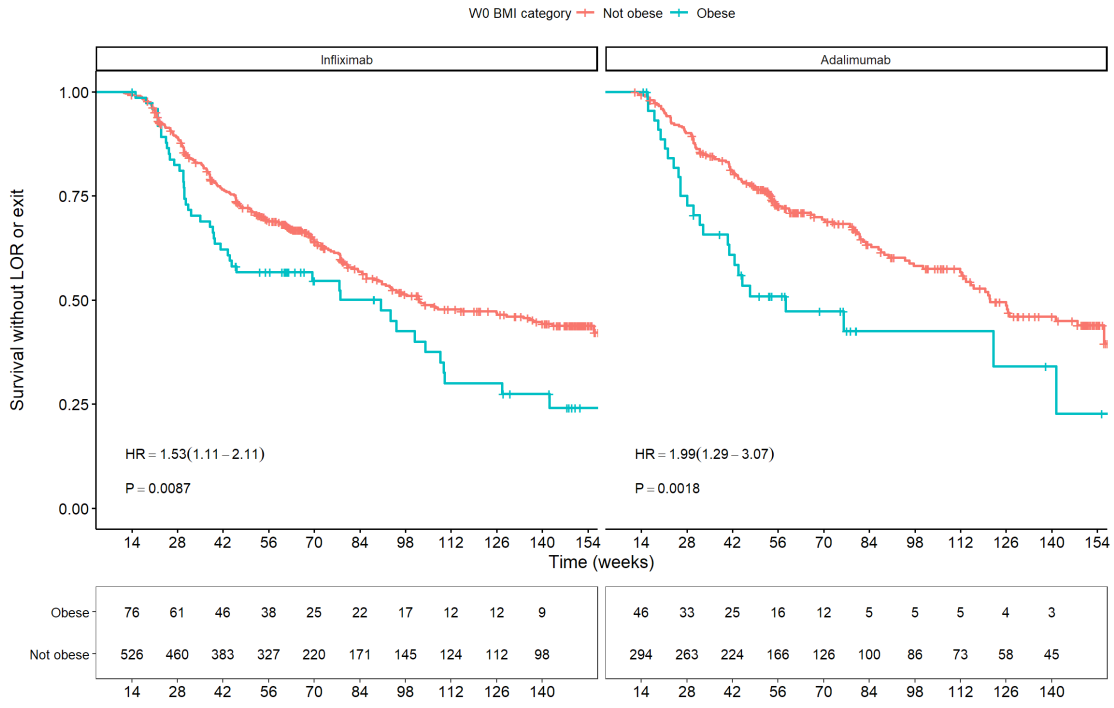
The univariable factors associated with time to loss of response or exit for treatment failure are shown in figure 5. For infliximab- and adalimumab-treated patients, these included BMI, drug quartile at week 14, immunogenicity at week 14, anti-TNF drug quartile at week 54, and immunogenicity at week 54. For infliximab-treated patients only, baseline thiopurine drug quartile and female sex were associated with loss of response. For neither drug, immunomodulator use nor smoking were associated with loss of response or exit.

Figure 5: Univariable associations of time to loss of response or exit using Kaplan-Meier and Cox proportional hazards methods

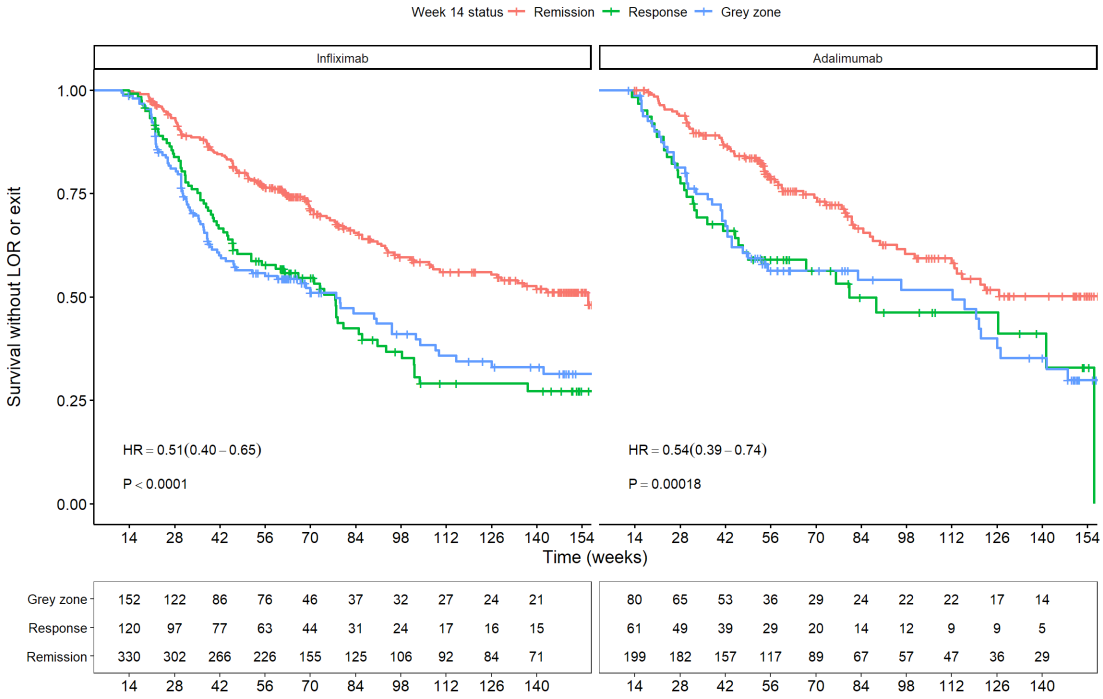
Kaplan-Meier graphs for survival without loss of response or exit from study according to body mass index (A), week 14 status (B), week 14 drug quartile (C), week 14 immunogenicitry (D), Week 54 drug quartile (E), Week 54 immunogenicity (F) for both drugs, and thiopurine drug quartile (G) and sex (H) for infliximab-treated patients. p values and HRs are derived from Cox proportional hazards models for each individual variable. The data for week 14 drug quartile excludes anyone who developed immunogenicity or exited the study before week 14, and is based on the log10 of the drug concentration. Therefore, the data show the HR for each ten-fold increase. HR=hazard ratio, LOR=loss of response.

Infliximab and adalimumab

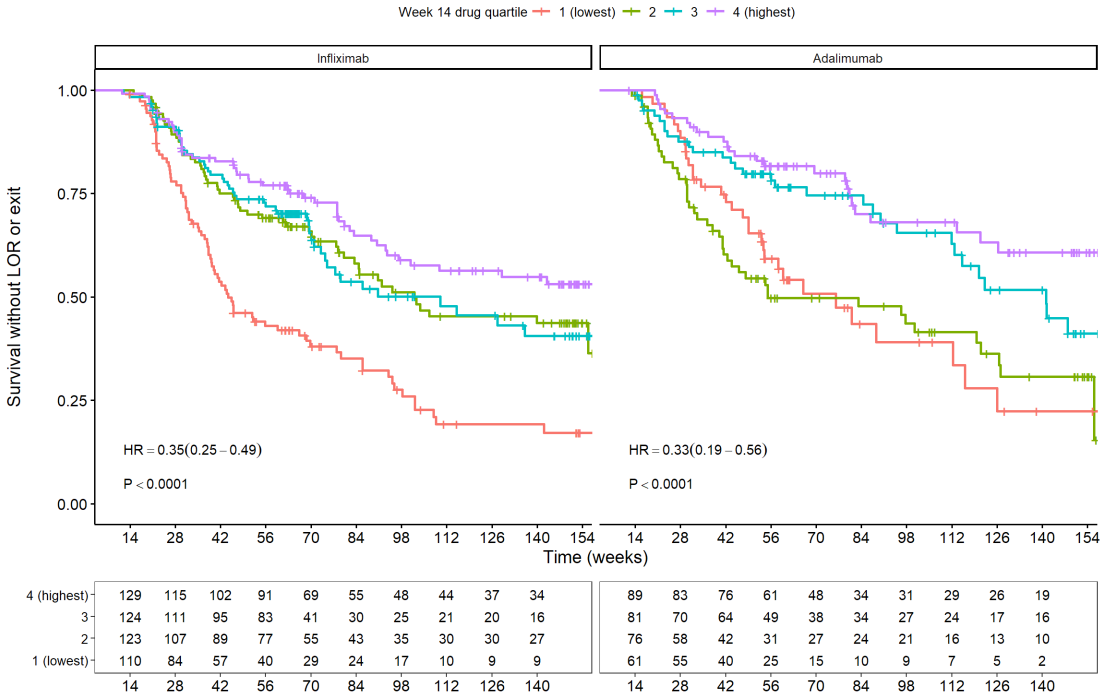
A) Body mass index



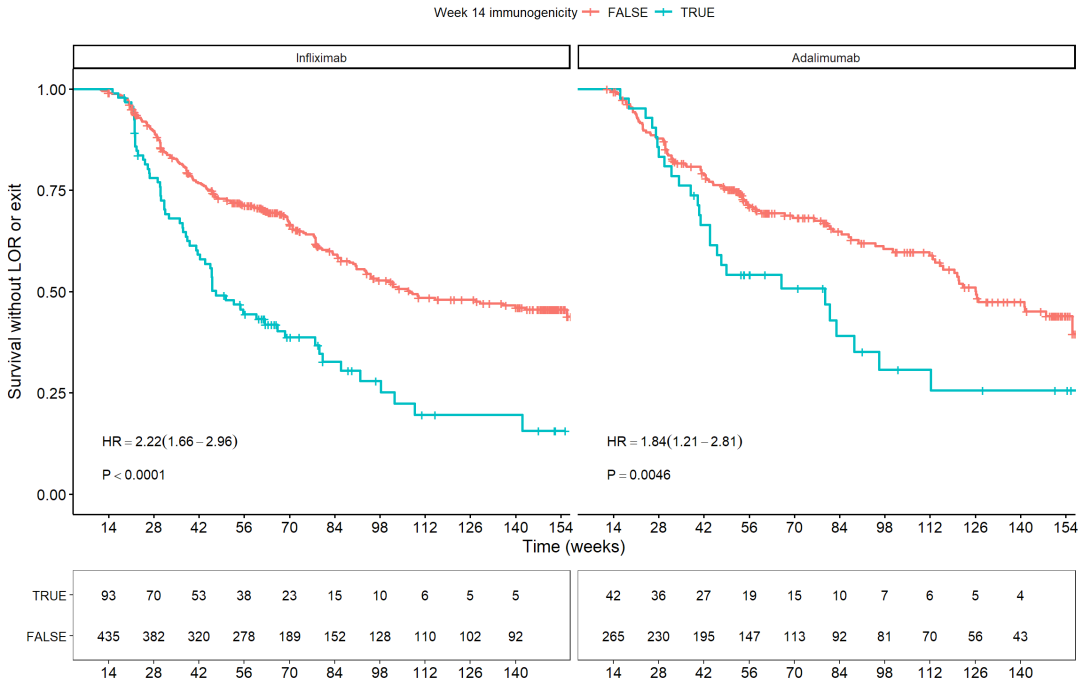
B) Week 14 status



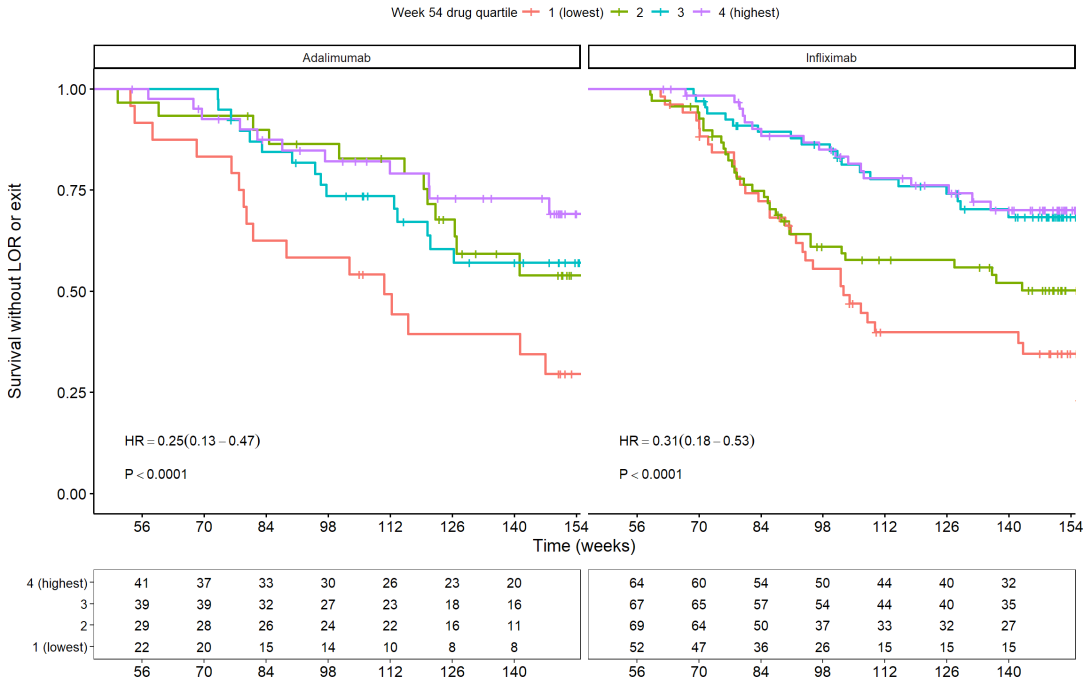
C) Week 14 drug quartile



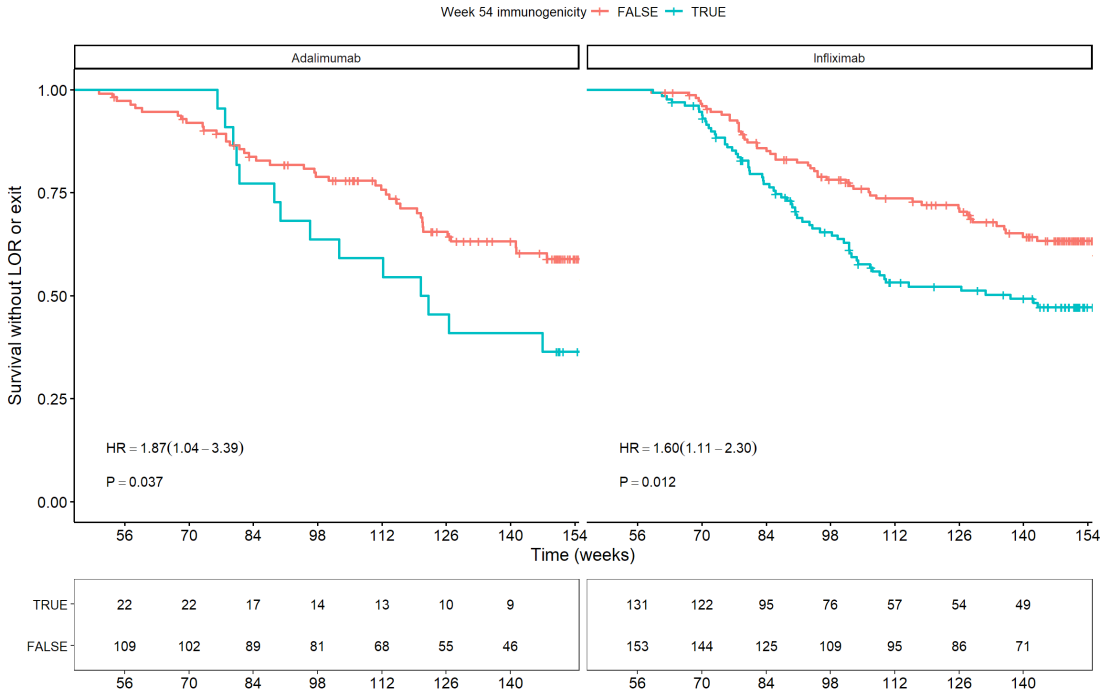
D) Week 14 immunogenicity



E) Week 54 drug quartile



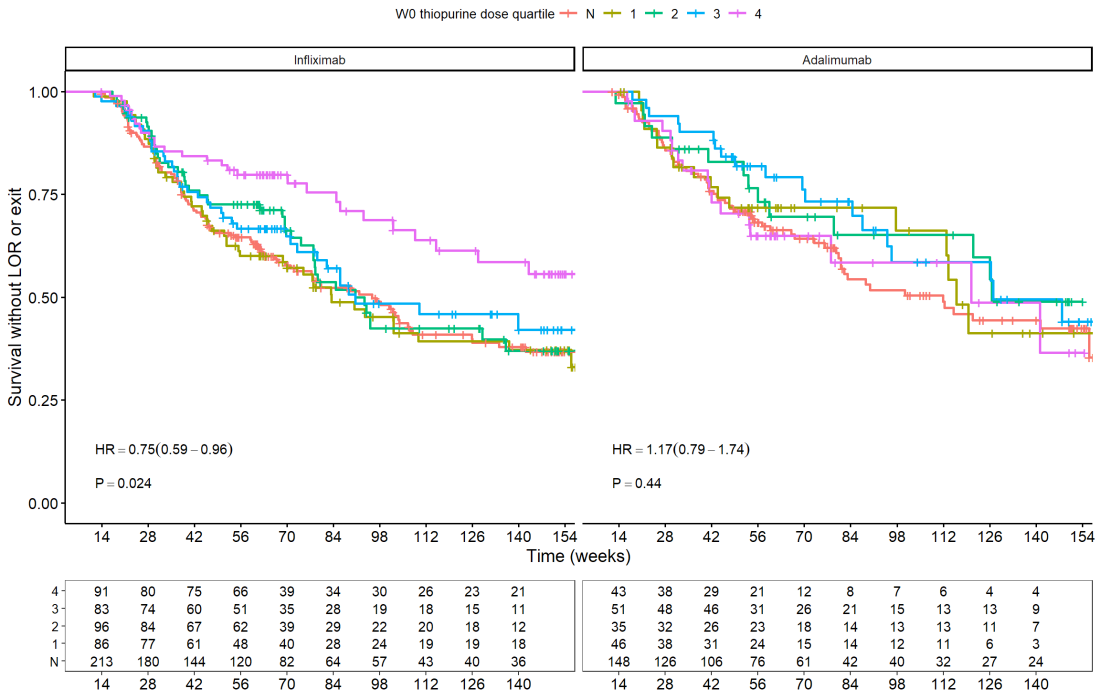
F) Week 54 immunogenicity



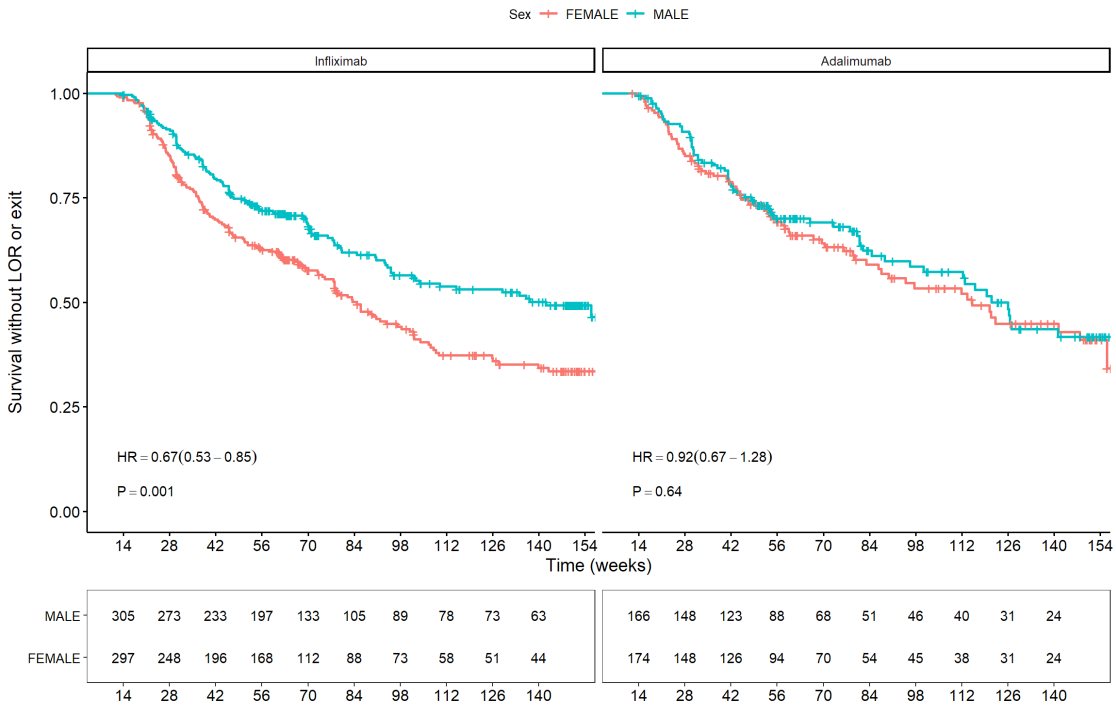
Infliximab only

G) Thiopurine dose quartiles (baseline)

1 (azathioprine: 0.18 – 1.40 mg/kg, mercaptopurine: 0.17 – 0.67 mg/kg)
2 (azathioprine: 1.41 – 1.85 mg/kg, mercaptopurine: 0.67 – 0.89 mg/kg)
3 (azathioprine: 1.86 – 2.198 mg/kg, mercaptopurine: 0.89 – 1.05 mg/kg)
4 (azathioprine: 2.199 – 4.15 mg/kg, mercaptopurine: 1.06 – 2.95 mg/kg)



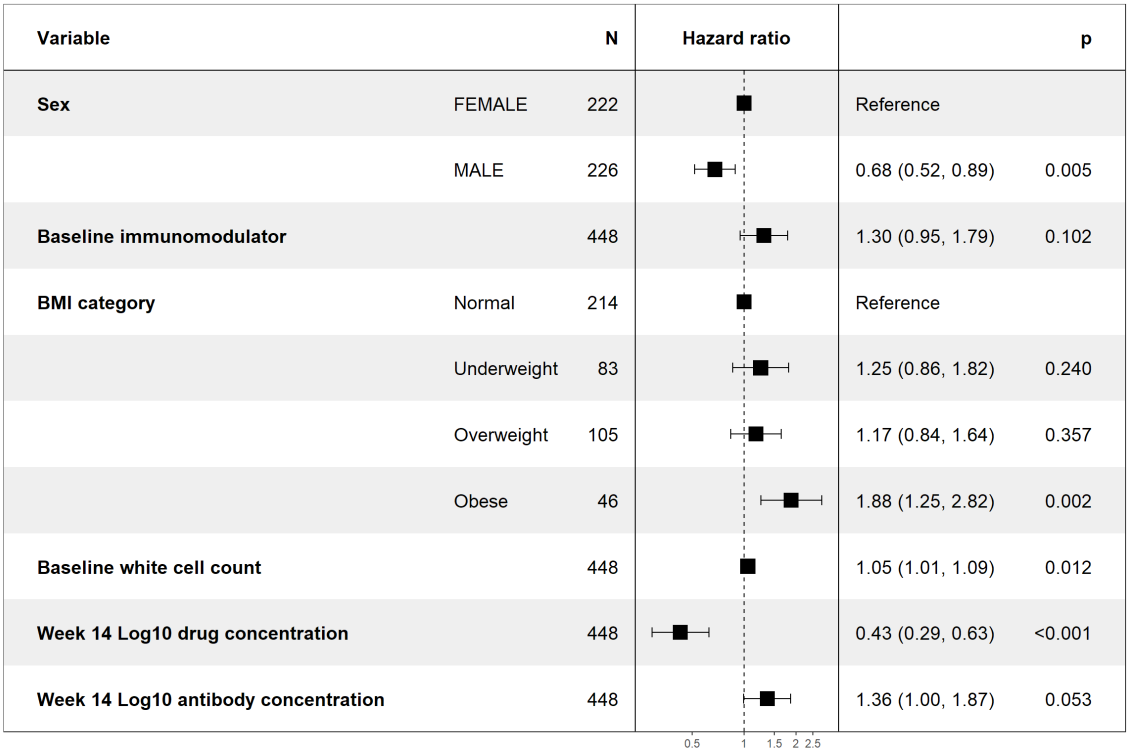
h)
Sex



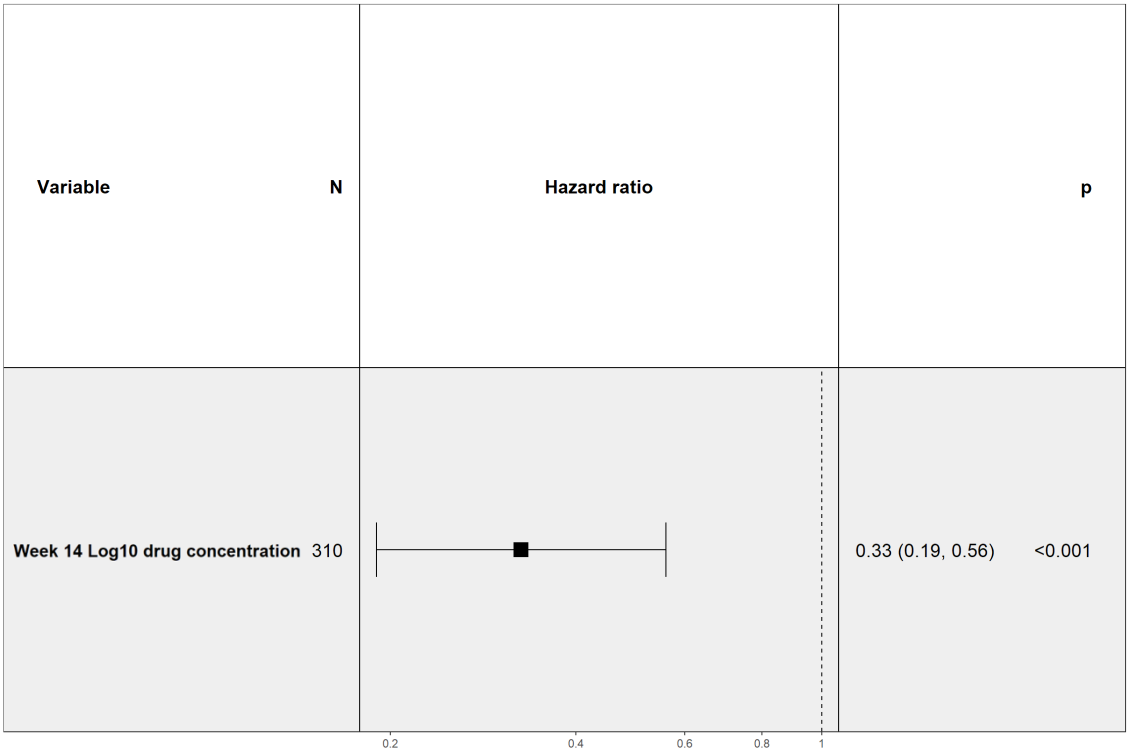
Multivariable analyses showed that drug concentration at week 14 was the major independent risk factor associated with loss of response for both drugs at year 2 and 3 (Figure 6). In addition, for infliximab-treated patients, female sex and obesity, but not baseline immunomodulator use or presence of immunogenicity at week 14, were associated with loss of response. For adalimumab-treated patients, only drug concentration at week 14 was significant.

Figure 6: Forest plot showing the coefficients from a multivariable logistic regression model of associations with loss of response, by drug

Infliximab



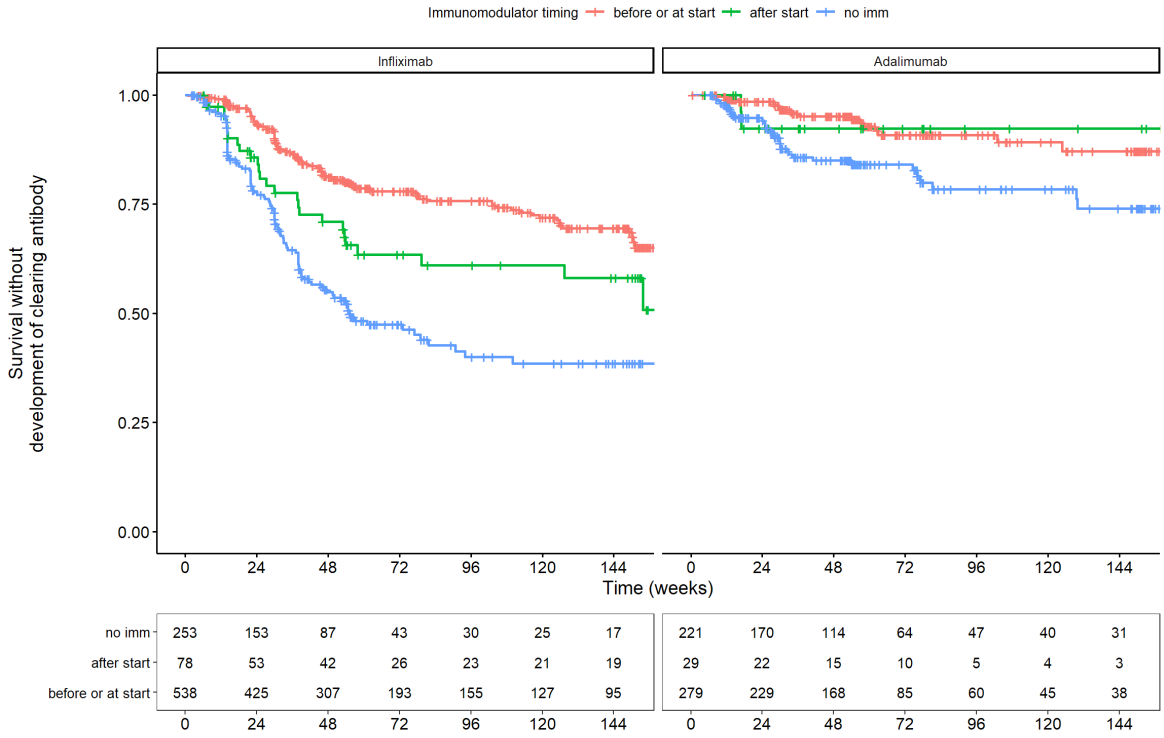
Adalimumab



Immunogenicity

For infliximab-treated individuals, rates of development of drug-clearing antibodies for years one, two, and three were 31.3% (95% CI 27.7 – 34.7), 37% (95% CI 32.8 – 40.8), and 44% (95% CI 38.1 – 49.4), respectively. For adalimumab-treated patients, rates for years one, two, and three were 12.5% (95% CI 9 – 15.8), 15.5% (95% CI 11.2 – 19.6), and 20.3% (95% CI 13.8 – 26.2), respectively. Estimated rates for immunogenicity for CT-P13 and Remicade-treated patients were similar (Supplementary Figure 8). Concomitant use of an immunomodulator prior to, or on the day of starting infliximab, was associated with increased survival without the development of drug-clearing antibodies when compared to use of infliximab alone, or introduction of an immunomodulator anti-TNF initiation (Figure 7).

Figure 7: Time to development of drug-clearing immunogenicity, using Kaplan-Meier and Cox proportional hazards methods, stratified by anti-TNF and timing of immunomodulator (days from starting anti-TNF therapy)



Using a Cox proportional hazards model, we demonstrated low anti-TNF concentration at week 14 was associated with a shorter time to development of drug-clearing antibodies (infliximab: hazards ratio [HR] 0.15 [95%CI 0.09 – 0.25], adalimumab: HR 0.02 [95%CI 0.01 – 0.04] for each 10-fold change in drug concentration). Using a time-varying approach to account for individual changes in antibody status throughout PANTS-E, the presence of a drug-clearing antibody, but not antibodies detected in the presence of drug ('double positive' status), was associated with an increased risk of exit for loss of response or treatment failure for infliximab (drug-clearing: HR 2.91 [95% CI 2.11 – 4], double positive: HR 1.29

[95%CI 0.94 – 1.76]), and adalimumab (drug-clearing: HR 4.03 [95%CI 1.96 – 8.30], double positive: HR 1.51 [95%CI 0.93 – 2.46]).

Of the 522 infliximab-treated patients with a positive anti-drug antibody test, 442 were re-tested at least four weeks later. 17.2% (76/442) patients' repeat antibody tests were negative and 82.8% (366/442) positive. The median anti-drug antibody concentration of the initial test was 11 AU/mL [IQR 9 – 17.3] for patients who subsequently tested negative and 18 AU/mL [IQR 12 – 34] for patients who subsequently tested positive. Of 191 adalimumab-treated patients with a positive anti-drug antibody test, 126 were re-tested at least four weeks later. 27% (34/126) patients' repeat antibody tests were negative and 73% (92/126) positive. The median anti-drug antibody concentration of the initial positive test was 8.4 AU/mL [IQR 6 – 15] for patients who subsequently tested negative and 15 AU/mL [IQR 7 – 54] for patients who subsequently tested positive. Estimated rates of drug clearance one year after the second positive antibody, inclusive of those remained positive for drug only, were 37% (95%CI 28 - 45) for infliximab- and 24% (95%CI 9 – 36) for adalimumab- treated patients.

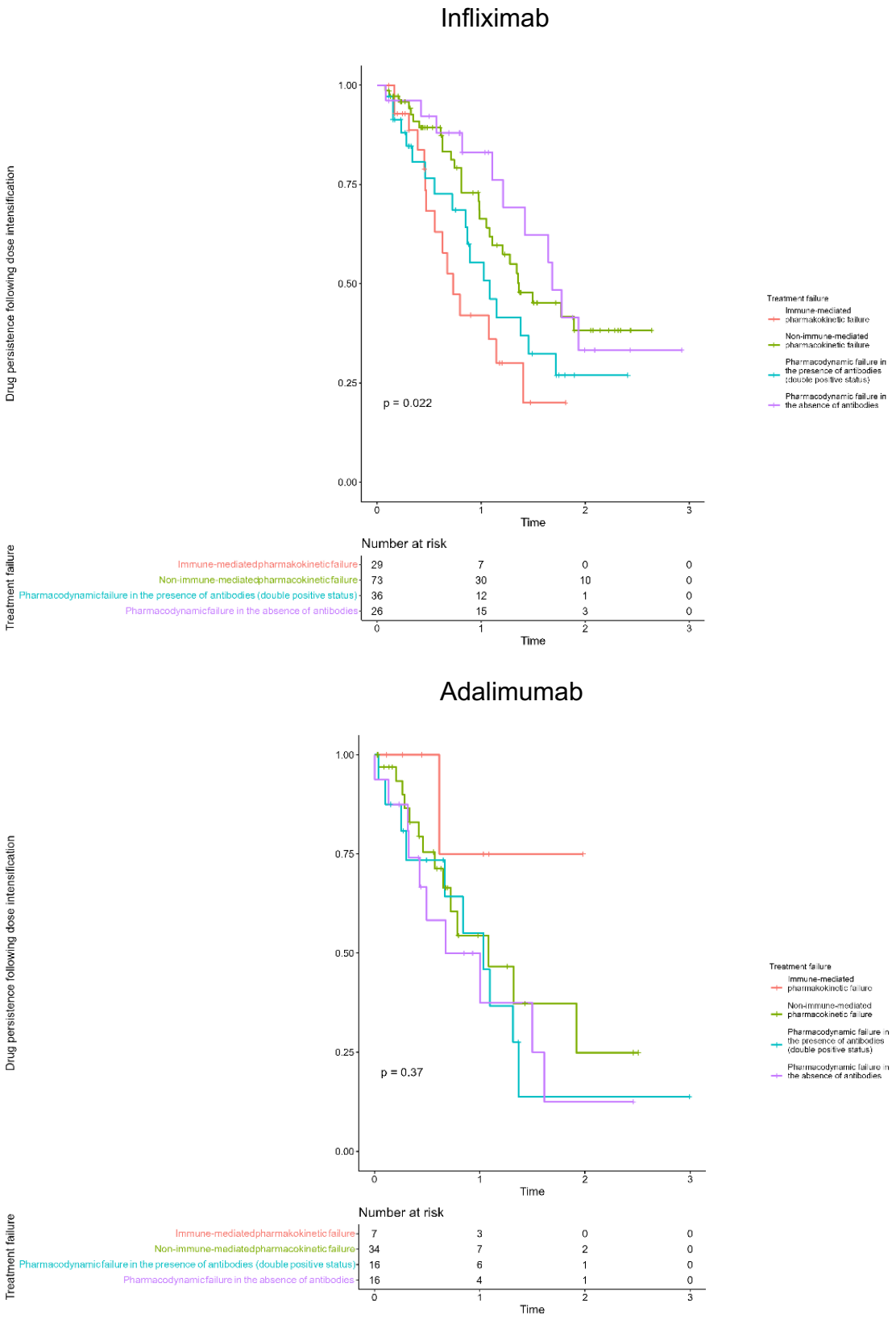
Management of loss of response

Over the study period, there were 686 episodes of loss of response. Anti-infliximab and -adalimumab antibodies were present in 43.8% (172/393) and 30.3% (70/231) episodes, respectively. In those who experienced loss of response, 48% (188/392) infliximab-treated patients and 30.3% (70/231) adalimumab-treated patients had antibodies at the time of loss of response.

Of the 188 infliximab-treated patients with a positive anti-drug antibody test, 70 were re-tested at least 4 weeks after the loss of response event. 18.6% (13/70) patients' repeat antibody tests were negative and 81.4% (57/70) positive. The median anti-drug antibody concentration of the initial positive test was 13 AU/mL [IQR 11 – 17] for patients who subsequently tested negative and 45 AU/mL [IQR 25 – 85] for patients who subsequently tested positive. Of the 70 adalimumab-treated patients with a positive anti-drug antibody test, 29 were re-tested at least 4 weeks after the loss of response event. 31% (9/29) patients' repeat antibody tests were negative and 69% (20/29) positive. The median anti-drug antibody concentration of the initial positive test was 7 AU/mL [IQR 6 – 8] for patients who subsequently tested negative and 113 AU/mL [IQR 22 – 173] for patients who subsequently tested positive.

Across 686 loss of response episodes, 732 clinician actions were taken to manage loss of response. 39.3% (288/732) resulted in anti-TNF dose intensification, 6.8% (50/732) commencement or increased dose of an immunomodulator, 15.6% (114/732) resulted in a course of steroids, 3.3% (24/732) in surgery, and 35% (256/732) stopped the drug (Supplementary Figure 9). Amongst infliximab-treated patients who received intensified anti-TNF therapy at the point of loss of response, those who experienced immune-mediated pharmacokinetic failure had the lowest rates of drug persistence compared to patients who had non-immune-mediated pharmacokinetic failure ($p = 0.04$) and pharmacodynamic failure in the absence of antibodies ($p = 0.03$) (Figure 8). This association was not seen for adalimumab-treated patients.

Figure 8: Drug persistence in dose-intensified patients, stratified by drug level and antibody status at time of loss of response, by anti-TNF



Adverse events

Risk of adverse events at any point during the three-year study were similar between adalimumab- and infliximab- treated patients (Supplementary Table 3).

In year 1 adverse events leading to treatment withdrawal were reported by 8.8% (84/955) infliximab-treated patients and 6.4% (42/655) adalimumab-treated patients. A further 4.3% (16/374; 95% CI 2.5 – 6.9) and 5.8% (11/189; 95% CI 2.9 – 10.1) infliximab- and adalimumab-treated patients, respectively, patients experienced adverse events leading to treatment withdrawal in years 2 and 3. (Supplementary Table 4).

Eight patients died during the course of the 3-year; five were treated with infliximab. The median age was 65.6 years (47.8 – 72.8). Of the five who died within the first year of the study, none responded to treatment by the time of death: two died of pneumonia, two died of intra-abdominal sepsis, and one of Crohn's disease-related malnutrition. Three died in the PANTS extension study: one of bowel perforation, one of suicide, and one of metastatic malignant melanoma. Five patients were taking concomitant corticosteroids at time of death and three were taking azathioprine.

In addition to the serious infections reported in the first year of study (infliximab: 38/955, adalimumab 21/655), a further 2.1% (8/374; 95% CI 0.9 - 4.2) of infliximab-treated patients and 1.1% (2/189; 95% CI 0.1 - 3.8) of adalimumab-treated patients reported serious infections during years 2 and 3 including active tuberculosis in one patient treated with adalimumab. In years 2 and 3 infusion reactions occurred in

four infliximab-treated patients and injection site reactions in two adalimumab-treated patients.

Discussion

Key results

Only about one-third of patients with active luminal Crohn's disease treated with an anti-TNF drug were estimated to be in remission at the end of two and three years. This was predicted by remission status at the end of treatment induction and year one. For both infliximab and adalimumab, low week 14 anti-TNF concentrations and presence of immunogenicity, were predictive of lower year two and year three remission rates. Two-thirds of initial responders experienced loss of response events by the end of year three. Loss of response events, for both infliximab and adalimumab-treated patients, were predicted by low anti-TNF concentrations at week 14, and for infliximab-treated patients, lower thiopurine dose quartiles. Drug-clearing anti-drug antibodies, detected in almost a half of infliximab-treated patients and one fifth of adalimumab-treated patients by three years, were associated with loss of response or treatment failure. Concomitant use of an immunomodulator, started prior to, or on the day of the first infliximab infusion, was associated with increased survival without the development of drug-clearing antibodies. Infliximab dose intensification in the setting of immune-mediated pharmacokinetic failure was associated with low rates of drug persistence.

Factors associated with long-term treatment failure

Most previous studies of anti-TNF therapy have been limited to estimating rates of treatment failure to one year only.[5–8] At the end of one year of treatment with infliximab, we previously demonstrated that female compared to male sex, was associated with lower remission rates.[3] Consistent with this, during PANTS-E, female sex was associated with both loss of response and non-remission through

years two and three. Similar findings were reported in a single-center retrospective cohort analysis of 210 patients with Crohn‘s disease treated with infliximab.[25] The biological basis for this association is not known. It might be explained by increased reporting of adverse events, increased rates of non-adherence and treatment discontinuation reported in female patients.[26]

We previously reported that obesity was associated with decreased remission rates at week 54 in adalimumab-treated patients only and we attributed this to the fixed dosing schedule. Herein, we report a similar association for infliximab-treated patients in years 2 and 3 of treatment, despite the weight-based dosing schedule. Similar findings have been reported in a single-center retrospective cohort of 124 patients initiating infliximab therapy [27], and a meta-analysis of anti-TNF treatment failure in several rheumatic diseases.[28] In contrast, no difference in clinical remission or response rates based on BMI was observed in a pooled data analysis of 1205 infliximab-treated IBD patients from four pivotal RCTs [29]. The association between obesity and anti-TNF loss of response events might be explained by larger volumes of body surface area, enhanced proteolysis, and TNF stored in adipose tissue.[30]

Drug concentrations and immunogenicity

Here we have demonstrated a clear dose-response relationship between low anti-TNF drug concentrations at weeks 14 and 54 and rates of treatment failure across three years of treatment. We report here the optimal drug concentrations at week 14 associated with remission at years one, two and three were about 7 mg/L and 12 mg/L for infliximab and adalimumab, respectively. This is considerably higher

than the target drug concentrations derived from previous observational studies.[31] Arguably, based on our data, most patients were under-dosed, in routine clinical care between 2013 to 2016, suggesting that true pharmacodynamic treatment failure may be more uncommon than observed in this study.

Whilst inter-individual differences in the pharmacokinetics of the anti-TNF drugs clearly influence the heterogeneity in response to treatment [32–34], a role for proactive TDM driven dosing, particularly during induction remains controversial. Most, but not all, prospective studies [35] have failed to show improved clinical outcomes when compared to conventional care. This may reflect facets of study design including the timing of dose optimization and the target drug concentration employed. Critically, these studies used significantly lower target drug concentrations (0.5 – 7 mg/L) than the optimal cut-offs observed in the current study.[36–38] Because we found no difference in clinical and pharmacokinetic outcomes between biosimilar and originator infliximab, any additional drug costs associated with dose intensification should be offset by use of increasingly inexpensive biosimilar preparations.

In the PANTS-E study, we employed a drug tolerant anti-drug antibody assay and demonstrated that only drug-clearing antibody development was associated with loss of response or treatment failure. Antibodies to the anti-TNF drugs were most likely to be detected in the first year of treatment; only 13% of infliximab- and 8% of adalimumab-treated patients developed drug-clearing antibodies after year one. Whilst this may suggest little benefit of using a drug-tolerant over a drug-sensitive assay, the former does allow for earlier detection of immunogenicity and a window

of opportunity to add an immunomodulator to reduce the risk of subsequent drug clearance and treatment failure.[39,40]

Implications for clinical practice

Loss of response events and non-remission in years two and three of anti-TNF treatment are predicted by low drug concentrations at week 14 and week 52. Whilst the direction of this dose-response association is uncertain it is plausible that achieving higher drug concentrations during year one, particularly during induction may lead to better long-term outcomes. Because most loss of response events occurred in the first year of treatment, the benefit of proactive TDM is likely to be limited after year one [36] and reactive TDM in the setting of treatment failure is then likely to be more cost-effective.[37] Further prospective studies of early dose optimisation using proactive TDM are underway. [41,42]

There are limited data regarding the optimal dose of thiopurines when used in combination with anti-TNF therapy. Several studies have suggested that the thioguanine nucleotide concentrations required to mitigate immunogenicity to anti-TNF therapy are lower that the therapeutic concentration targeted when thiopurines are used as monotherapy. However, these studies have been limited by retrospective design, small sample size, and short-term follow-up.[43–45] Contrary to these reports, we found that patients in the highest weight-based quartile of thiopurine dosing were least likely to experience loss of response. Our data suggest a target dose of at least 2.2 mg/kg of azathioprine and 1.1 mg/kg of mercaptopurine, when used alongside anti-TNF therapy.

We have demonstrated that use of a concomitant immunomodulator reduces the risk of developing drug clearing immunogenicity to both infliximab and adalimumab. We and others have shown that for infliximab, concomitant treatment with an immunomodulator translates to better outcomes.[3,7] With the increasingly early introduction of infliximab, commencement of a concomitant thiopurine may be delayed whilst waiting on a thiopurine methyltransferase laboratory result, or to allow steroid taper to minimise the risks of triple immunosuppression. Our data suggests this delay may increase the risk of immunogenicity to infliximab and should be avoided.

In the setting of loss of response, anti-TNF dose intensification was carried out in only 40% of episodes, and more than one-third of patients had their anti-TNF treatment withdrawn. This low rate of dose intensification is likely reflective of clinical practice at the time the study was conducted. We looked at the outcome of dose intensification, stratified by drug and antibody concentrations at the time of loss of response. In the setting of immune-mediated pharmacokinetic failure (undetectable drug concentration with antibodies), dose intensification resulted in shorter drug persistence compared to patients with non-immune mediated pharmacokinetic failure (undetectable or subtherapeutic drug concentration without antibodies). These observations support the current practice of dose intensification in the setting of low drug concentrations without immunogenicity and switching out of class in the setting of unrecordable drug concentrations and presence of antibodies.[13,14]. Our observation that dose intensification was associated with increased drug persistence in patients who had treatment failure despite adequate infliximab concentrations may imply that for some individuals, even higher drug

concentrations are required to achieve remission.[31,46] Alternatively, the practice of continuing anti-TNF treatment even in the setting of treatment failure may reflect the limited treatment options available at the time study.

Limitations and generalisability

We acknowledge the following limitations. Firstly, consistent with registration trials and other real-world prospective cohort studies, about one-third of patients completing the first year of PANTS did not enter the extension phase. To mitigate possible observation bias, we used a modified survival technique and permutation testing to estimate the number of patients in remission throughout the entire study. Secondly, in the absence of standardised definitions of treatment response and loss of response, we used pragmatic definitions combining corticosteroid use, clinical and biochemical markers of disease activity, and clinician action. We did not use endoscopic outcomes which we acknowledge would have strengthened our data. Finally, unlike in year one of the study, when visits were scheduled eight-weekly, during the extension phase visits were scheduled every six months. This reduced the granularity of our data. In particular, we were not able to assess in detail the immediate impact of anti-TNF dose intensification on drug concentrations and clinical outcomes.

We collected data from >120 sites from across the UK. Our findings are likely to be generalisable to patients with Crohn's disease, and to similar patient cohorts from other high-income countries. Whether our results are generalisable to other anti-TNFs, including certolizumab and golimumab, or across ulcerative colitis, remains unknown.

Conclusions

Only about one-third of patients with active luminal Crohn's disease treated with an anti-TNF drug are in remission at the end of three years of treatment. Low drug concentrations at the end of induction predict loss of response and non-remission to year 3 of treatment, suggesting higher drug concentrations during the first year of treatment, particularly during induction, may lead to better long-term outcomes. Drug-clearing anti-drug antibodies are associated with long-term loss of response and treatment failure, and can be mitigated by an immunomodulator, started prior to, or on the day of the first infliximab infusion.

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Supplementary Table 1

PANTS consortium

All UK gastroenterologists were invited to participate in the PANTS study which was promoted through the UK National Institute for Health Service Research (NIHR) and the British Society of Gastroenterology (BSG).

Hospital or Trust name	**City**	**Name**	**Job Title**
Tameside Hospital NHS Foundation Trust	Ashton U Lyne	Dr Vinod Patel	Consultant Gastroenterologist
Basildon and Thurrock University Hospitals NHS Foundation Trust	Basildon	Dr Zia Mazhar	Consultant Gastroenterologist
Hampshire Hospitals NHS Foundation Trust	Basingstoke	Dr Rebecca Saich	Consultant Gastroenterologist
Royal United Hospital	Bath	Dr Ben Colleypriest	Consultant Gastroenterologist
Ulster Hospital	Belfast	Dr Tony C Tham	Consultant Gastroenterologist

University Hospital's Birmingham NHS Foundation Trust	Birmingham	Dr Tariq H Iqbal	Consultant Gastroenterologist
East Lancashire NHS Teaching Trust	Blackburn	Dr Vishal Kaushik	Consultant Gastroenterologist
Blackpool Teaching Hospitals NHS Foundation Trust	Blackpool	Dr Senthil Murugesan	Consultant Gastroenterologist
Bolton NHS Trust	Bolton	Dr Salil Singh	Consultant Gastroenterologist
Royal Bournemouth Hospital	Bournemouth	Dr Sean Weaver	Consultant Gastroenterologist
Bradford Teaching Hospitals Foundation Trust - (St Lukes Hospital &Bradford Royal Infirmary)	Bradford	Dr Cathryn Preston	Consultant Gastroenterologist
Brighton and Sussex University Hospitals NHS Trust	Brighton	Dr Assad Butt	Paediatric Consultant Gastroenterologist

Brighton and Sussex University Hospitals NHS Trust	Brighton	Dr Melissa Smith	Consultant Gastroenterologist
University Hospitals Bristol NHS Foundation Trust	Bristol	Dr Dharamveer Basude	Consultant Paediatric Gastroenterologist
University Hospitals Bristol NHS Foundation Trust	Bristol	Dr Amanda Beale	Consultant Gastroenterologist
Frimley Park Hospital NHS Foundation Trust	Camberley	Dr Sarah Langlands	Consultant Gastroenterologist
Frimley Park Hospital NHS Foundation Trust	Camberley	Dr Natalie Direkze	Consultant gastroenterologist
Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Miles Parkes	Consultant Gastroenterologist
Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Franco Torrente	Consultant Paediatric Gastroenterologist

Cambridge University Hospitals NHS Foundation Trust	Cambridge	Dr Juan De La Revella Negro	Research fellow
North Cumbria University Hospitals NHS Trust	Carlisle	Dr Chris Ewen MacDonald	Consultant Gastroenterologist
Ashford & St Peter's Hospitals NHS Foundation Trust	Chertsey	Dr Stephen M Evans	Consultant Gastroenterologist
St Peter's Hospital	Chertsey	Dr Anton V J Gunasekera	Consultant Gastroenterologist
Ashford & St Peter's Hospitals NHS Foundation Trust	Chertsey	Dr Alka Thakur	Paediatric Consultant
Chesterfield Royal NHS Foundation Trust	Chesterfield	Dr David Elphick	Consultant Gastroenterologist
Colchester Hospital University NHS Foundation Trust	Colchester	Dr Achuth Shenoy	Consultant Gastroenterologist
University Hospitals Coventry and	Coventry	Prof Chuka U Nwokolo	Consultant Gastroenterologist

Warwickshire NHS Trust			
County Durham and Darlington NHS Foundation Trust	Darlington	Dr Anjan Dhar	Consultant Gastroenterologist & Hon. Clinical Lecturer
Derby Hospital NHS Foundation NHS Trust	Derby	Dr Andrew T Cole	Consultant Gastroenterologist
Doncaster and Bassetlaw Hospitals NHS Foundation Trust	Doncaster	Dr Anurag Agrawal	Consultant Gastroenterologist
Dorset County Hospital NHS Foundation Trust	Dorchester	Dr Stephen Bridger	Consultant Gastroenterologist
Dorset County Hospitals Foundation Trust	Dorchester	Dr Julie Doherty	Paediatric Consultant
Dudley Group NHS Foundation Trust	Dudley	Dr Sheldon C Cooper	Consultant Gastroenterologist
Russells Hall Hospital, The	Dudley	Dr Shanika de Silva	Consultant Gastroenterologist

Dudley Group NHS Foundation Trust			
Ninewells Hospital & Medical School	Dundee	Dr Craig Mowat	Consultant Gastroenterologist
East Sussex Healthcare Trust	Eastborne	Dr Phillip Mayhead	Consultant Gastroenterologist
NHS Lothian	Edinburgh	Dr Charlie Lees	Consultant Gastroenterologist and Honorary Senior Lecturer
NHS Lothian	Edinburgh	Dr Gareth Jones	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Tariq Ahmad	Consultant Gastroenterologist
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr James W Hart	Consultant Paediatrician
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Nicholas A Kennedy	Consultant Gastroenterologist

Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr James R Goodhand	Consultant Gastroenterologist
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Simeng Lin	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Neil Chanchlani	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Rachel Nice	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Timothy J McDonald	Consultant clinical scientist
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Claire Bewshea	Group Manager

Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Yusur Al-Nuaimi	Consultant dermatologist
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Ellen Richards	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Richard Haigh	Consultant rheumatologist
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Huw Greenish	Research fellow
Royal Devon University Healthcare NHS Foundation Trust	Exeter	Dr Harry Heath	Research fellow
Glasgow Royal Infirmary	Glasgow	Dr Daniel R Gaya	Consultant Gastroenterologist

Royal Hospital for Children	Glasgow	Prof Richard K Russell	Consultant Paediatric Gastroenterologist
Royal Hospital for Children	Glasgow	Dr Lisa Gervais	Research fellow
Gloucestershire Hospitals NHS Trust	Gloucester	Dr Paul Dunckley	Consultant Gastroneterologist
United Lincolnshire Hospitals NHS Trust	Grantham	Dr Tariq Mahmood	Consultant Gastroenterologist
James Paget University Hospitals NHS Foundation Trust	Great Yarmouth	Dr Paul J R Banim	Consultant Gastroneterologist
Calderdale and Huddersfield NHS Trust	Halifax	Dr Sunil Sonwalkar	Consultant Gastroenterologist
Princess Alexandra Hospital NHS Trust	Harlow	Dr Deb Ghosh	Consultant Gastroenterologist
Princess Alexandra Hospital NHS Trust	Harlow	Dr Rosemary H Phillips	Consultant Gastroenterologist

Hull and East Yorkshire NHS Trust	Hull	Dr Amer Azaz	Paediatric Consultant Gastroenterologist
Hull and East Yorkshire NHS Trust	Hull	Dr Shaji Sebastian	Consultant Gastroenterologist
Airedale NHS Foundation Trust	Keighley	Dr Richard Shenderey	Consultant Gastroenterologist
Crosshouse Hospital	Kilmarnock	Dr Lawrence Armstrong	Consultant Paediatrician
Crosshouse Hospital	Kilmarnock	Dr Claire Bell	Research fellow
The Queen Elizabeth Hospital NHS Foundation Trust	Kings Lynn	Dr Radhakrishnan Hariraj	Consultant Gastroenterologist
Kingston Hospital NHS Trust	Kingston upon Thames	Dr Helen Matthews	Consultant Gastroenterologist
NHS Fife	Kirkcaldy	Dr Hasnain Jafferbhoy	Consultant Gastroenterologist
Leeds Teaching Hospitals NHS Trust	Leeds	Dr Christian P Selinger	Consultant Gastroenterologist

Leeds Teaching Hospitals NHS Trust	Leeds	Dr Veena Zamvar	Paediatric Consultant Gastroenteorlogist
University Hospitals of Leicester NHS Trust	Leicester	Prof John S De Caestecker	Consultant Gastroenterologist
University Hospitals of Leicester NHS Trust	Leicester	Dr Anne Willmott	Paediatric Consultant Gastroenterologist
Mid Cheshire Hospitals NHS Foundation Trust	Leighton	Mr Richard Miller	Research Nurse
United Lincolnshire Hospitals NHS Trust	Lincoln	Dr Palani Sathish Babu	Consultant Gastroenterologist
Alder Hey Childrens Hospital	Liverpool	Dr Christos Tzivinikos	Consultant Paediatric Gastroenterologist
University College London Hospitals NHS Foundation Trust	London	Dr Stuart L Bloom	Consultant Gastroenterologist

Kings College Hospital NHS Foundation Trust	London	Dr Guy Chung-Faye	Consultant Gastroenterologist
Royal London Childrens Hospital, Barts Health NHS Trust	London	Prof Nicholas M Croft	Paediatric Consultant Gastroenterologist
Chelsea & Westminster Hospital	London	Dr John ME Fell	Consultant Paediatric Gastroenterologist
Chelsea and Westminster Hospital NHS Foundation	London	Dr Marcus Harbord	Consultant Gastroenterologist
North West London Hospitals NHS Trust	London	Dr Ailsa Hart	Consultant Gastroenterologist
Kings College Hospital NHS Foundation Trust	London	Dr Ben Hope	Consultant Paediatrician
Guys & St Thomas' NHS Foundation Trust	London	Dr Peter M Irving	Consultant Gastroenterologist

Barts and The London NHS Trust	London	Prof James O Lindsay	Consultant Gastroenterologist
Guy's and St Thomas' NHS trust	London	Dr Joel E Mawdsley	Gastroenterology Consultant
Lewisham and Greenwich Healthcare NHS Trust	London	Dr Alistair McNair	Consultant Gastroenterologist
Chelsea and Westminster Hospital NHS Foundation	London	Dr Kevin J Monahan	Consultant Gastroenterologist
Royal Free London NHS Foundation Trust	London	Dr Charles D Murray	Consultant Gastroenterologist
Imperial College Healthcare NHS Trust	London	Prof Timothy Orchard	Consultant Gastroenterologist
St George's Healthcare NHS Trust	London	Dr Thankam Paul	Paediatric Consultant Gastroenterologist
St George's Healthcare NHS Trust	London	Dr Richard Pollok	Reader and Consultant Gastroenterologist

Great Ormond Street Hospital for Children NHS Foundation Trust	London	Dr Neil Shah	Consultant Gastroenterologist
North West London Hospitals NHS Trust	London	Dr Sonia Bouri	Research fellow
The Luton & Dunstable University Hospital	Luton	Dr Matt W Johnson	Consultant Gastroenterologist
Luton and Dunstable Hospital Foundation Trust	Luton	Dr Anita Modi	Paediatric Consultant with Allergy and Gastroenterology interest
The Luton & Dunstable University Hospital	Luton	Dr Kasamu Dawa Kabiru	Research fellow
Maidstone and Tunbridge Wells NHS Trust	Maidstone	Dr B K Baburajan	Consultant Gastroenterologist
Maidstone and Tunbridge Wells NHS Trust	Maidstone	Prof Bim Bhaduri	Paediatric Consultant Gastroenterologist

Manchester University Hospitals NHS Foundation Trust	Manchester	Dr Andrew Adebayo Fagbemi	Consultant Gastroenterologist
Central Manchester University Hospitals NHS Foundation Trust	Manchester	Dr Scott Levison	Consultant Gastroenterologist
The Pennine Acute Hospitals NHS Trust	Manchester	Dr Jimmy K Limdi	Consultant Gastroenterologist
Manchester University NHS Foundation Trust, Wythenshawe Hospital	Manchester	Dr Gill Watts	Consultant Gastroenterologist
Sherwood Forest Hospitals NHS Foundation Trust	Mansfield	Dr Stephen Foley	Consultant Gastroenterologist
South Tees Hospital NHS Foundation Trust	Middlesbrough	Dr Arvind Ramadas	Consultant Gastroenterologist

Milton Keynes Hospital NHS Foundation Trust	Milton Keynes	Dr George MacFaul	Consultant Gastroenterologist
Newcastle Upon Tyne Hospital Trust	Newcastle	Dr John Mansfield	Consultant Gastroenterologist
Isle of Wight NHS Foundation Trust	Newport	Dr Leonie Grellier	Consultant Gastroenterologist
Norfolk & Norwich University Hospital NHS Foundation Trust	Norwich	Dr Mary-Anne Morris	Consultant Paediatric Gastroenterologist
Norfolk & Norwich University Hospital NHS Foundation Trust	Norwich	Dr Mark Tremelling	Consultant Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Prof Chris Hawkey	Consultant Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Dr Sian Kirkham	Consultant Paediatric Gastroenterologist
Nottingham University Hospitals NHS Trust	Nottingham	Dr Charles PJ Charlton	Consultant gastroenterologist

Oxford University Hospitals NHS Foundation Trust	Oxford	Dr Astor Rodrigues	Paediatric Consultant Gastroenterologist
Oxford University Hospitals NHS Trust	Oxford	Prof Alison Simmons	Consultant Gastroenterologist
Plymouth Hospitals NHS Trust	Plymouth	Dr Stephen J Lewis	Consultant Gastroenterologist
Poole Hospital NHS Foundation Trust	Poole	Dr Jonathon Snook	Consultant Gastroenterologist
Poole Hospital NHS Foundation Trust	Poole	Dr Mark Tighe	Paediatric Consultant with interest in Oncology and Gastroenterology
Portsmouth Hospitals NHS Trust	Portsmouth	Dr Patrick M Goggin	Consultant Gastroenterologist
Royal Berkshire NHS Foundation Trust	Reading	Dr Aminda N De Silva	Consultant Gastroenterologist
Salford Royal NHS Foundation Trust	Salford	Prof Simon Lal	Consultant Gastroenterologist

Shrewsbury and Telford Hospital NHS Trust	Shrewsbury	Dr Mark S Smith	Consultant Gastroenterologist
South Tyneside NHS Foundation Trust	South Shields	Dr Simon Panter	Consultant Gastroenterologist
Southampton University Hospitals NHS Trust	Southampton	Dr JR Fraser Cummings	Consultant Gastroenterologist
Southampton University Hospitals NHS Trust	Southampton	Dr Suranga Dharmisari	Research fellow
East and North Herts NHS Trust	Stevenage	Dr Martyn Carter	Consultant Gastroenterologist
NHS Forth Valley	Stirling	Dr David Watts	Consultant Gastroenterologist
Stockport NHS foundation Trust	Stockport	Dr Zahid Mahmood	Consultant Gastroenterologist
North Tees and Hartlepool NHS Foundation Trust	Stockton	Dr Bruce McLain	Paediatric Consultant Gastroenterologist
University Hospitals of North Staffordshire	Stoke-on Trent	Dr Sandip Sen	Consultant Gastroenterologist

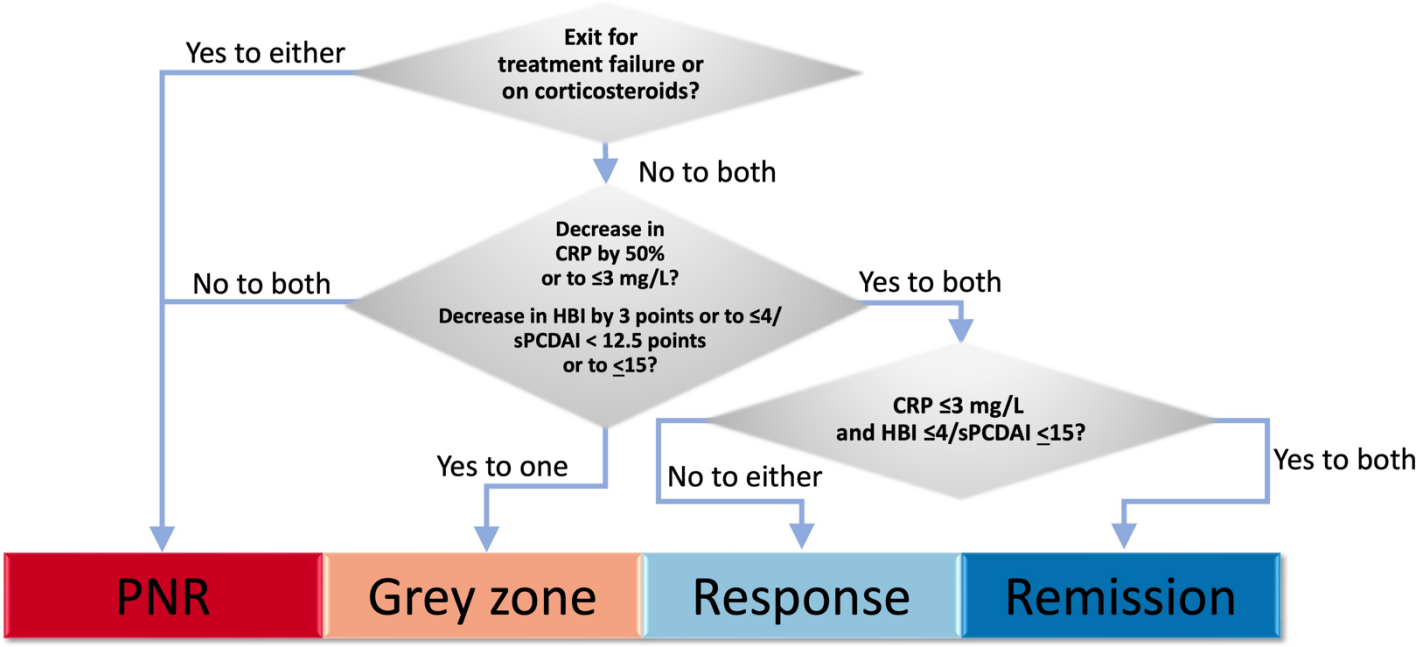
University Hospitals of North Midlands NHS Trust	Stoke-on-Trent	Dr Anna J Pigott	Consultant Paediatric Gastroenterologist
City Hospitals Sunderland NHS Foundation Trust	Sunderland	Dr David Hobday	Consultant Gastroenterologist
Taunton and Somerset NHS Foundation Trust	Taunton	Dr Emma Wesley	Consultant Gastroenterologist
South Devon Healthcare NHS Foundation Trust	Torquay	Dr Richard Johnston	Consultant Gastroenterologist
South Devon Healthcare NHS Foundation Trust	Torquay	Dr Cathryn Edwards	Consultant gastroenterologist
Royal Cornwall Hospitals NHS Trust	Truro	Dr John Beckly	Consultant Gastroenterologist
Mid Yorkshire Hospitals NHS Trust	Wakefield	Dr Deven Vani	Consultant Physician & Gastroenterologist
Warrington& Halton NHS Foundation	Warrington	Dr Subramaniam Ramakrishnan	Consultant Gastroenterologist

West Hertfordshire Hospitals NHS Trust	Watford	Dr Rakesh Chaudhary	Consultant Gastroenterologist
Sandwell and West Birmingham Hospitals NHS Trust	West Bromwich	Dr Nigel J Trudgill	Consultant Gastroenterologist
Sandwell and West Birmingham Hospitals NHS Trust	West Bromwich	Dr Rachel Cooney	Consultant gastroenterologist
Weston Area Health NHS Trust	Weston-Super-Mare	Dr Andy Bell	Consultant Gastroenterologist
Royal Albert Edward Infirmary, Wrightington, Wigan & Leigh NHS Foundation Trust	Wigan	Dr Neeraj Prasad	Consultant Gastroenterologist
Hampshire Hospitals NHS Foundation Trust	Winchester	Dr John N Gordon	Consultant Gastroenterologist
Royal Wolverhampton	Wolverhampton	Prof Matthew J Brookes	Consultant Gastroenterologist

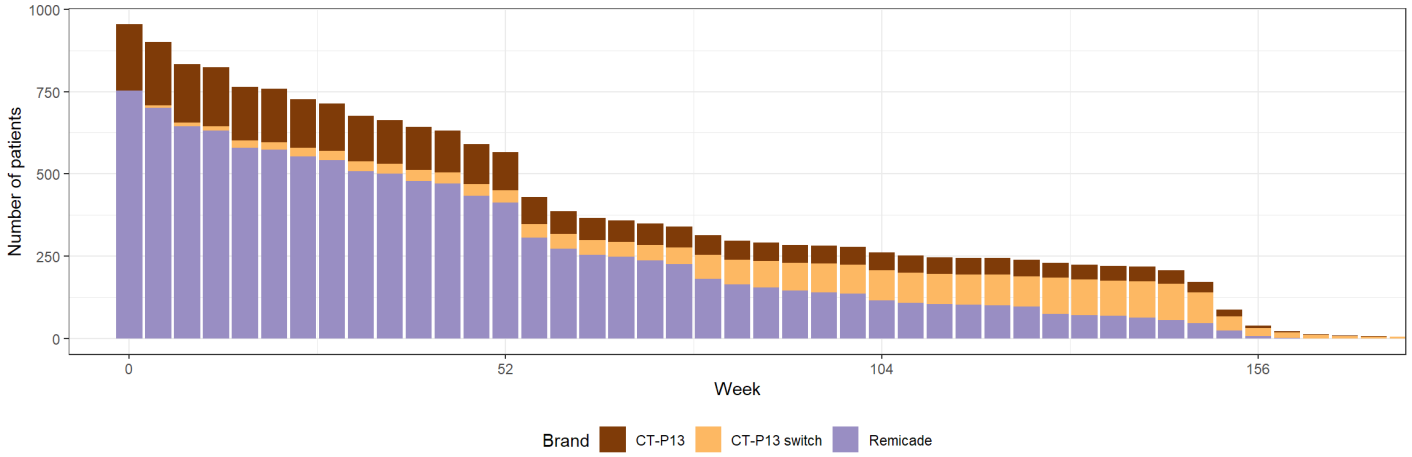
Hospitals NHS Trust			
Western Sussex Hospitals NHS Trust	Worthing	Dr Andy Li	Consultant Gastroenterologist
Yeovil District Hospital NHS Foundation Trust	Yeovil	Dr Stephen Gore	Consultant Gastroenterologist

Supplementary Figures

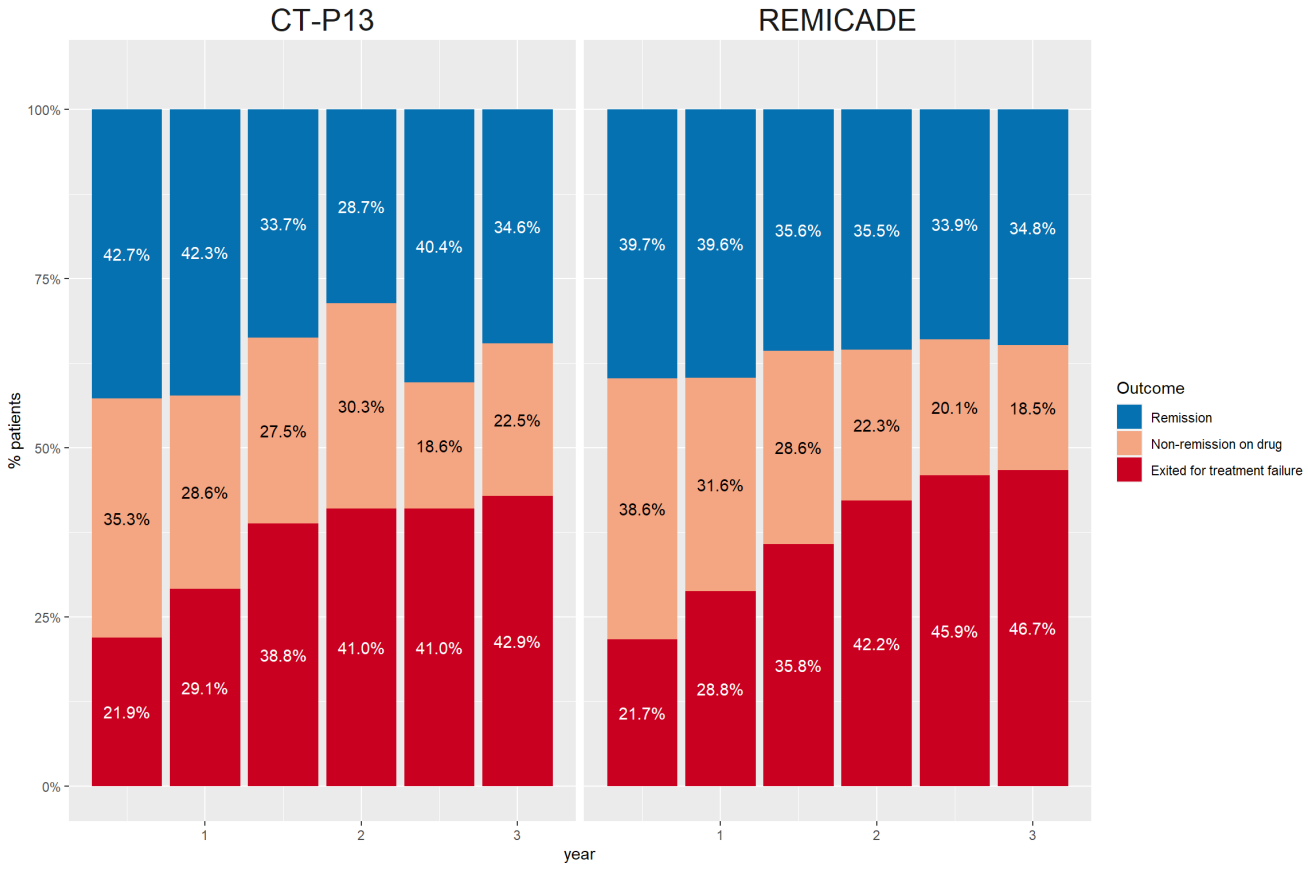
Supplementary Figure 1: Definition of outcomes at week 14. *CRP=C-reactive protein; HBI=Harvey-Bradshaw index; sPCDAI=short Paediatric Crohns Disease Activity Index; PNR=primary non-response.*



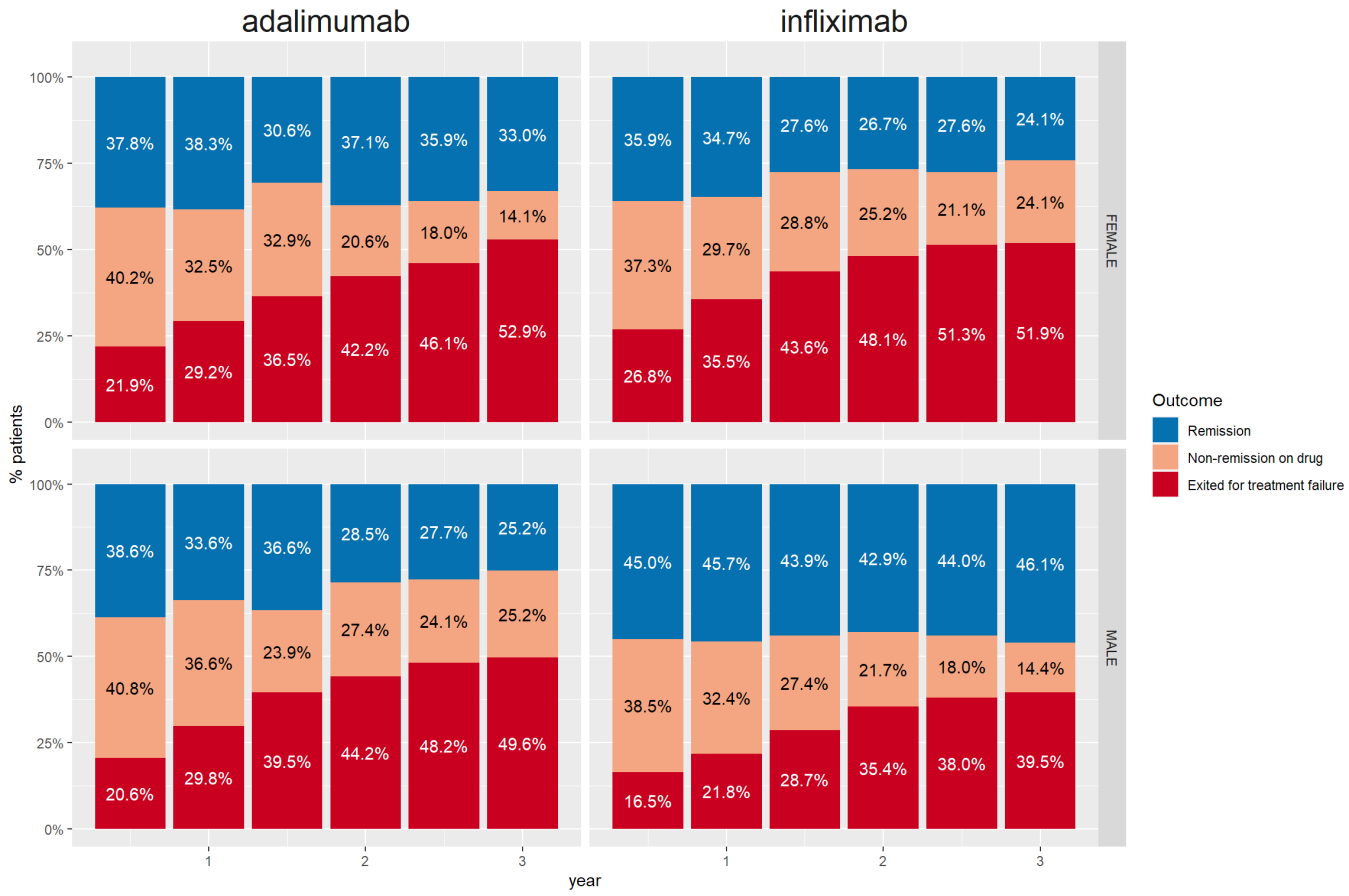
Supplementary Figure 2: Brand of infliximab treatment, over study period



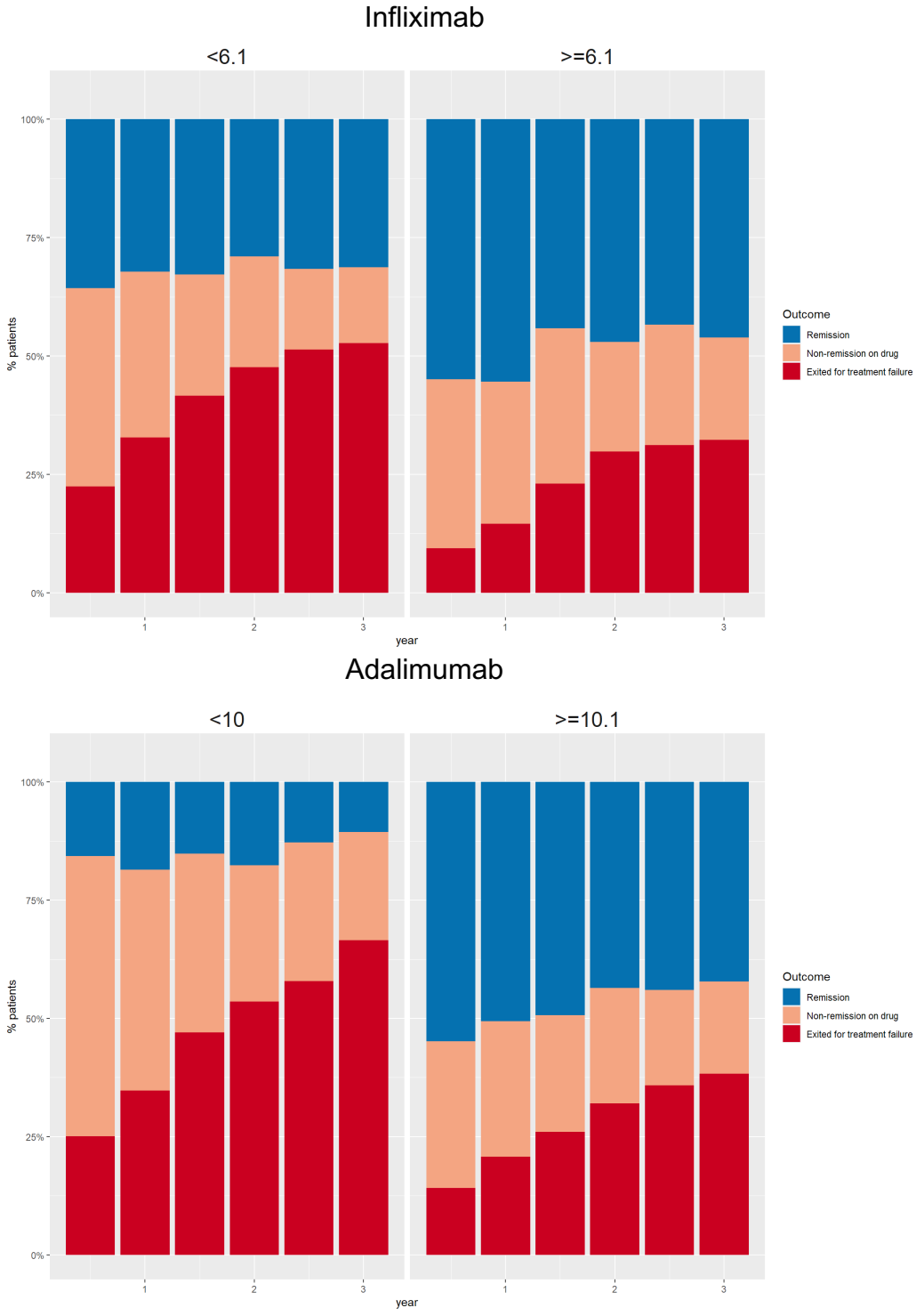
Supplementary Figure 3: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, by infliximab type



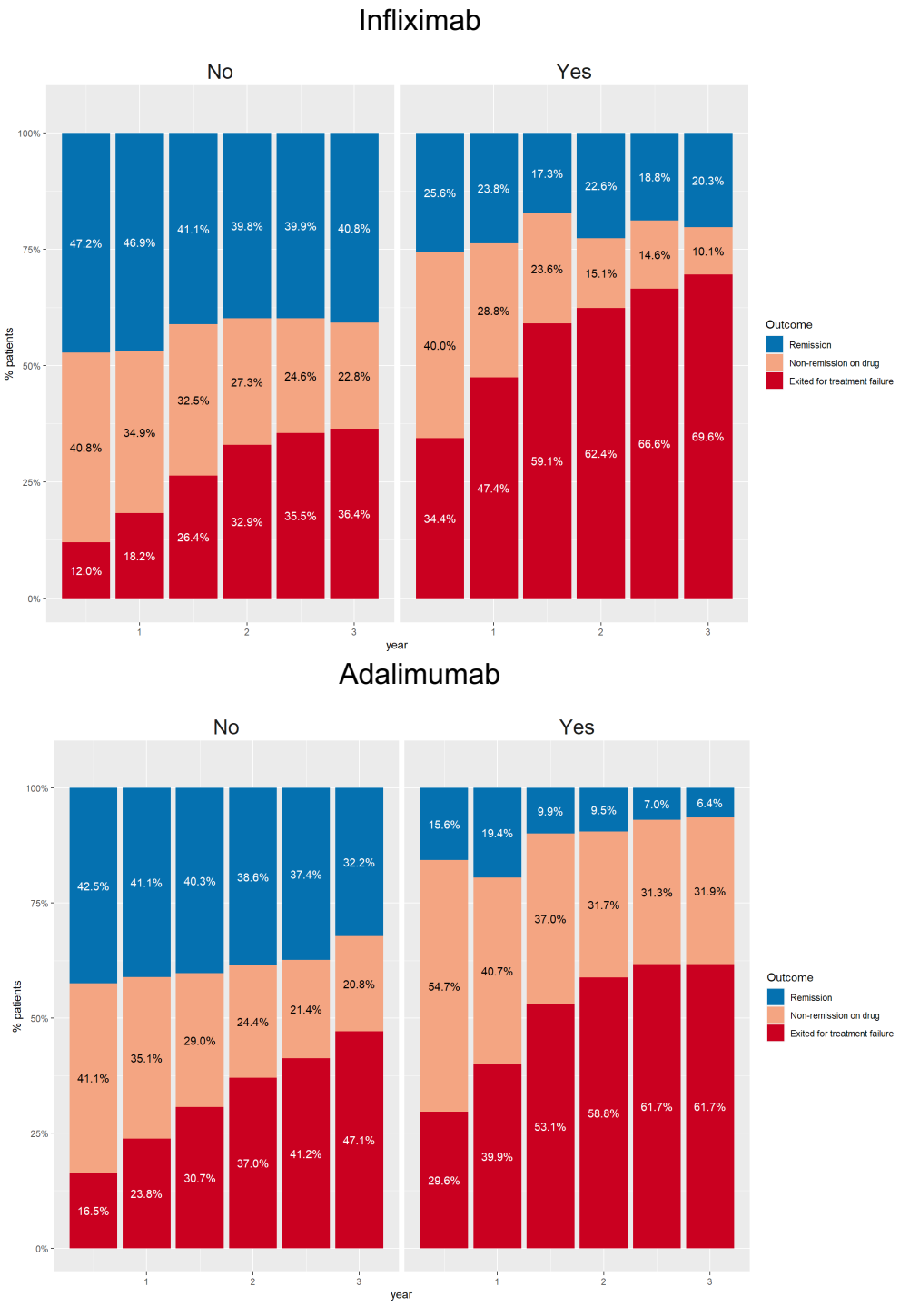
Supplementary Figure 4: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, by anti-TNF and sex



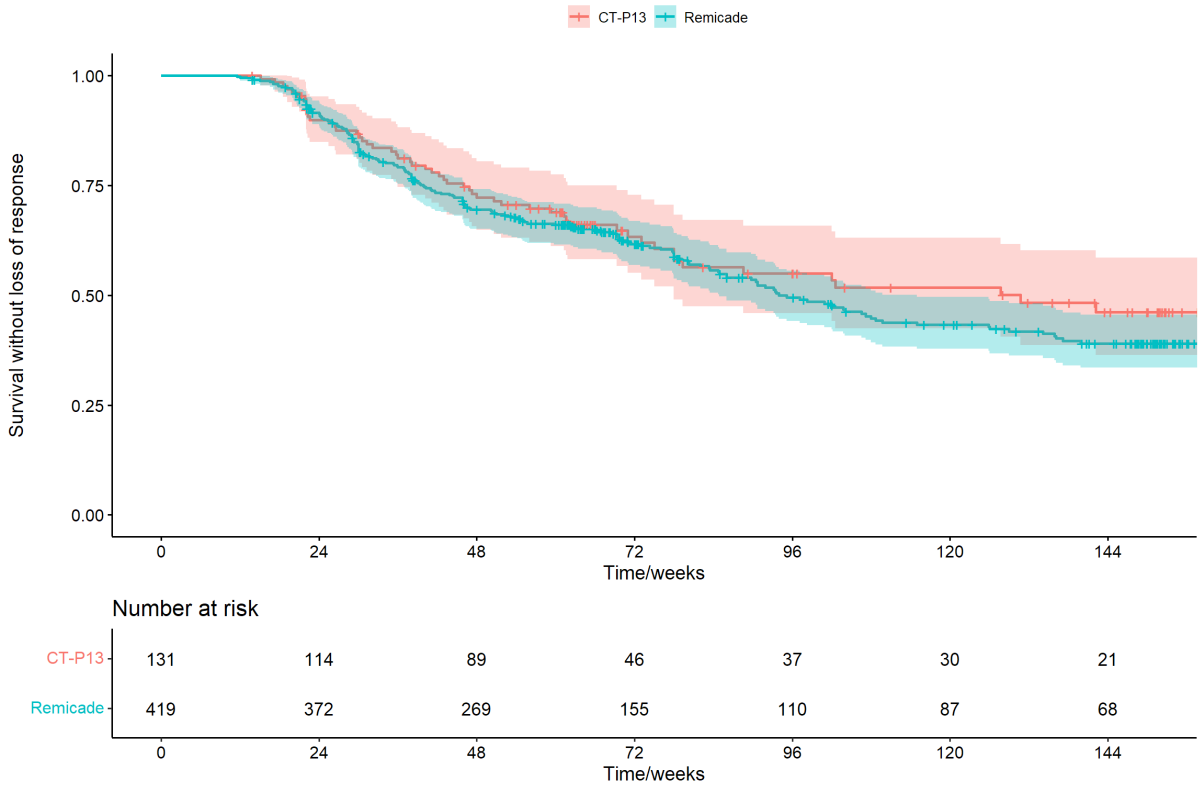
Supplementary Figure 5: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, permutation testing of week 14 drug level (with respect to optimal drug concentration thresholds)



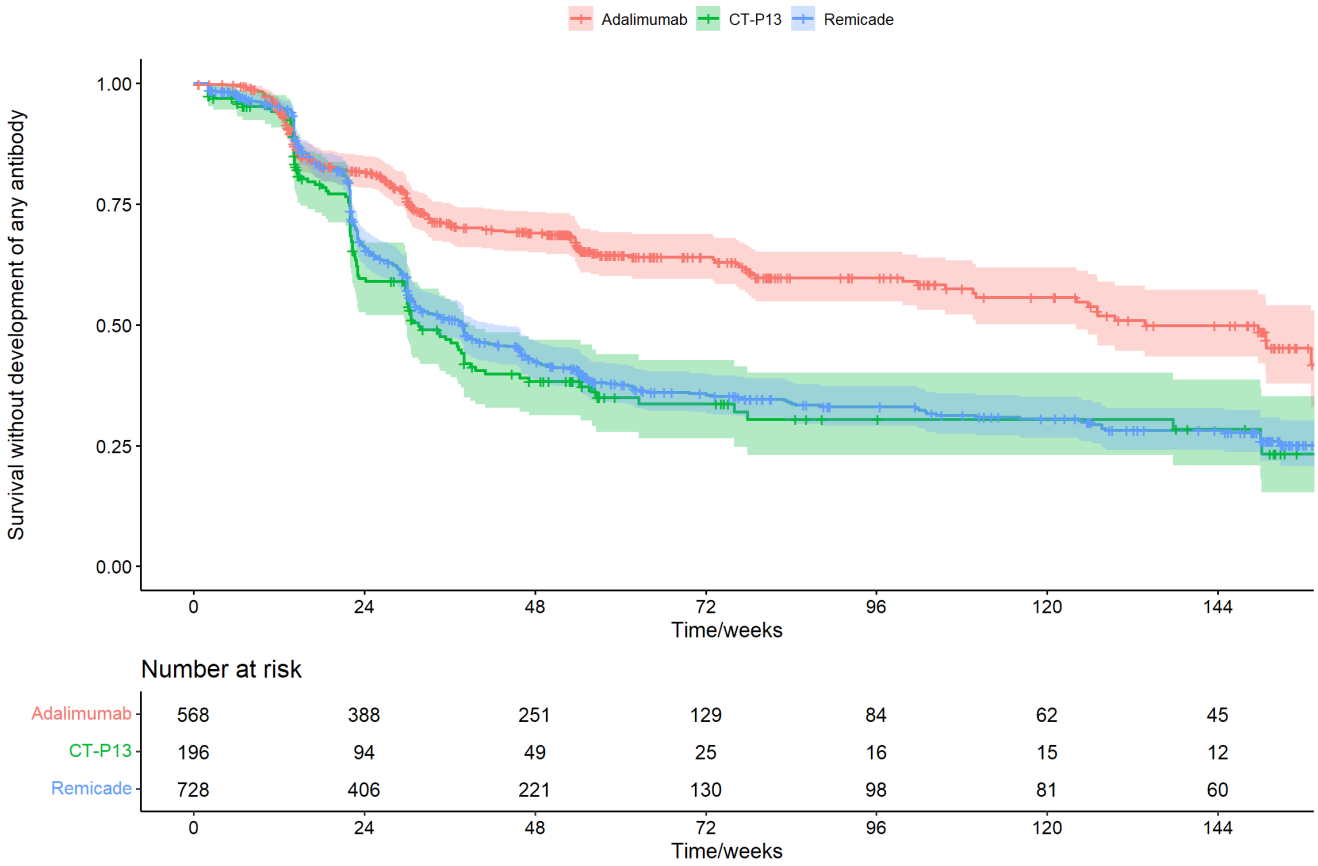
Supplementary Figure 6: Estimated proportions of patients in remission, non-remission, or exit for treatment failure at end of years one, two, and three of study, permutation testing of immunogenicity



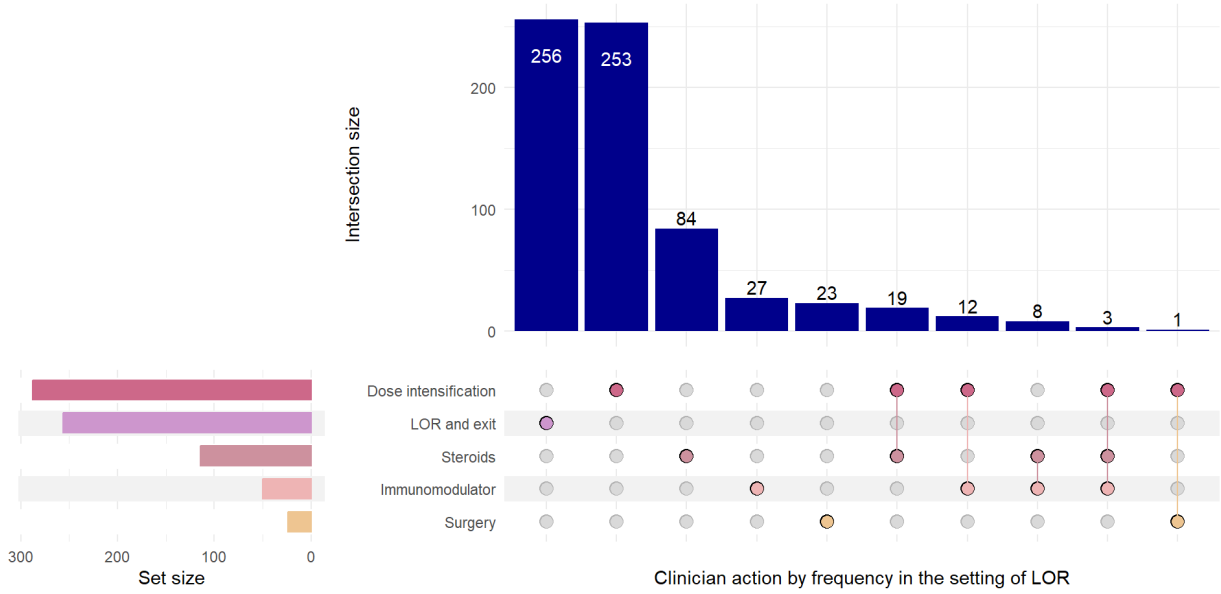
Supplementary Figure 7: Time to loss of response, or exit for treatment failure using Kaplan-Meier and Cox proportional hazards methods, by infliximab type



Supplementary Figure 8: Time to immunogenicity, using Kaplan-Meier and Cox proportional hazards methods, by anti-TNF and infliximab type



Supplementary Figure 9: Upset plot describing clinician action in the setting of loss of response. *LOR=loss of response*.



Supplementary Tables 2 – 4

Table 2: Baseline demographic, clinical, and TDM characteristics, participants who entered extension study compared to those who did not enter extension study. Data are number (%) or median (IQR). p values were calculated using Fisher's exact or Mann Whitney U tests. BMI = Body Mass Index.

Variable	**Level**	**Did not enter extension n = 909**	**Entered extension n = 594**
Drug	Infliximab	511/909 (56%)	387/594 (65%)
	Adalimumab	398/909 (44%)	207/594 (35%)
Sex (male)	Female	462/909 (51%)	305/594 (51%)
	Male	447/909 (49%)	289/594 (49%)
Age (years)		32.9 (22.8 - 46.2)	32.6 (22.2 - 47)
Disease duration (years)		2.4 (0.7 - 8.9)	2.6 (0.7 - 8.8)
Baseline immunomodulator		503/909 (55%)	367/594 (62%)
Montreal location	L1	276/901 (31%)	163/586 (28%)
	L2	206/901 (23%)	145/586 (25%)
	L3	409/901 (45%)	274/586 (47%)
	L4	10/901 (1%)	4/586 (1%)
Montreal behaviour	B1	540/897 (60%)	377/590 (64%)
	B2	277/897 (31%)	165/590 (28%)
	B3	80/897 (9%)	48/590 (8%)
Perianal		95/909 (10%)	87/594 (15%)
Previous resectional surgery		184/909 (20%)	116/594 (20%)
	0	828/908 (91%)	539/594 (91%)

Charlson comorbidity score	1	55/908 (6%)	46/594 (8%)
	>=2	25/908 (3%)	9/594 (2%)
Baseline BMI category	Normal	418/898 (47%)	287/594 (48%)
	Underweight	109/898 (12%)	87/594 (15%)
	Overweight	214/898 (24%)	153/594 (26%)
	Obese	157/898 (17%)	67/594 (11%)
Baseline current smoker		176/898 (20%)	83/589 (14%)
Baseline haemoglobin (g/L)		127 (116 - 138)	126 (116 - 138)
Baseline white cell count ($\times 10^9$/L)		8 (6.3 - 10.3)	7.8 (5.9 - 9.9)
Baseline platelet count ($\times 10^9$/L)		332 (269 - 406)	335 (277 - 410)
Baseline albumin (g/L)		39 (34 - 42)	39 (35 - 42)
Baseline faecal calprotectin (µg/g)		366 (162 - 763)	390 (165 - 825)
Week 14 drug level (mg/L)		6 (2.3 - 10.5)	7 (3 - 11.5)
Week 14 antibody level (AU/mL)		3 (2 - 6)	3 (2 - 5)
Week 14 status	Remission	242/711 (34%)	288/529 (54%)
	Response	97/711 (14%)	87/529 (16%)
	Grey zone	138/711 (19%)	100/529 (19%)
	Primary non-response	234/711 (33%)	54/529 (10%)
Immunogenicity in first year	Antibody -ve	507/804 (63%)	311/594 (52%)
	Antibody +ve drug +ve	162/804 (20%)	178/594 (30%)
	Antibody +ve drug -ve	135/804 (17%)	105/594 (18%)
Week 14 antibody positive		129/569 (23%)	72/509 (14%)

Supplementary Tables 3a: Adverse events at any point during the study.

Numbers in brackets are per 100 patient years.

	Adalimumab	**Infliximab**
Adverse event	311 (35.9)	543 (36.4)
Hospitalization	131 (15.1)	203 (13.6)
Hospitalization, not Crohn's related	106 (12.2)	170 (11.4)
Infection, not Crohn's related	83 (9.6)	168 (11.3)
Adverse event, not Crohn's related	289 (33.4)	509 (34.1)
Serious	145 (16.7)	236 (15.8)
Serious infection	27 (3.1)	48 (3.2)
Serious, not Crohn's related	119 (13.7)	205 (13.7)
Death	3 (0.3)	5 (0.3)
Exit for adverse event	53 (6.1)	111 (7.4)
Exit for adverse event, not Crohn's related	53 (6.1)	111 (7.4)

Supplementary Tables 3b: Adverse events of special interest at any point during the study

Adverse event type	Adalimumab	Infliximab
All infections	83 (9.6)	168 (11.3)
Adverse skin reaction	43 (5)	92 (6.2)
Infusion/Injection reaction	38 (4.4)	45 (3)
Headache	30 (3.5)	37 (2.5)
Lower respiratory tract infections	15 (1.7)	36 (2.4)
Nausea/vomiting	12 (1.4)	34 (2.3)
Upper respiratory tract infections	14 (1.6)	21 (1.4)
Fatigue/lethargy/malaise	6 (0.7)	28 (1.9)
Paraesthesias and dysaesthesias	9 (1)	12 (0.8)
Infectious gastroenteritis	7 (0.8)	13 (0.9)
Urinary tract infections	4 (0.5)	14 (0.9)
Lupus-like syndrome	2 (0.2)	7 (0.5)
Cancer	2 (0.2)	3 (0.2)
Tuberculosis	1 (0.1)	3 (0.2)

Supplementary Tables 4a: Adverse events at any point in extension study (after year one). Numbers in brackets are percentages of cohort.

	Adalimumab	**Infliximab**
Adverse event	59 (31.2%)	108 (28.9)
Hospitalization	20 (10.6%)	39 (10.4)
Hospitalization, not Crohn's related	13 (6.9%)	31 (8.3)
Infection, not Crohn's related	21 (11.1%)	27 (7.2)
Adverse event, not Crohn's related	52 (27.5%)	97 (25.9)
Serious	23 (12.2%)	44 (11.8)
Serious infection	2 (1.1%)	8 (2.1)
Serious, not Crohn's related	16 (8.5%)	36 (9.6)
Death	1 (0.5%)	2 (0.5)
Exit for adverse event	11 (5.8%)	16 (4.3)
Exit for adverse event, not Crohn's related	11 (5.8%)	16 (4.3)

Supplementary Table 4b: Adverse events of special interest at any point in extension study (after year one). Numbers in brackets are percentages of cohort.

Adverse event type	**Adalimumab**	**Infliximab**
All infections	21 (11.1)	27 (7.2)
Adverse skin reactions	7 (3.7)	8 (2.1)
Headache	4 (2.1)	4 (1.1)
Infectious gastroenteritis	2 (1.1)	5 (1.3)
Upper respiratory tract infections	3 (1.6)	4 (1.1)
Nausea/vomiting	1 (0.5)	5 (1.3)
Infusion/injection reaction	2 (1.1)	4 (1.1)
Lower respiratory tract infections	3 (1.6)	0
Lupus-like syndrome	0	3 (0.8)
Fatigue/lethargy/malaise	0	3 (0.8)
Lupus-like syndrome	0	3 (0.8)
Tuberculosis	1 (0.5)	0
Urinary tract infections	0	1 (0.3)
Fatigue/lethargy/malaise	0	1 (0.3)
Cancer	1 (1.5%)	0

Chapter 7: Implications for sequencing of biologic therapy and choice of second anti-TNF in patients with inflammatory bowel disease: results from the Immunogenicity to Second Anti-TNF Therapy (IMSAT) therapeutic drug monitoring study

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Borg-Bartolo, Paul Knight, Michael B Sprakes, Julie Burton, Patricia Kane, Stephanie Lupton, Aimee Fletcher, Daniel R Gaya, Roghan Colbert, John Paul Seenan, Jonathan MacDonald, Iain McLachlan, Stephanie Shields, Richard Hansen, Lisa Gervais, Mwansa Jere, Muhammad Akhtar, Karen Black, Paul Henderson, Richard K Russell, Charlie W Lees, Lauranne AAP Derikx, Melanie Lockett, Frederica Betteridge, Aminda De Silva, Arif Hussenbux, John Beckly, Oliver Bendall, James W Hart, Amanda Thomas, Ben Hamilton, Claire Gordon, Desmond Chee, Timothy J McDonald, Rachel Nice, Marian Parkinson, Helen Gardner-Thorpe, Jeff R Butterworth, Asima Javed, Sarah Al-Shakhshir, Rekha Yadagiri, Sebrene Maher, Richard CG Pollok, Tze Ng, Priscilla Appiahene, Fiona Donovan, James Lok, Rajiv Chandy, Reema Jagdish, Daniyal Baig, Zahid Mahmood, Liane Marsh, Allison Moss, Amin Abdulgader, Angus Kitchin, Gareth J Walker, Becky George, Yuen-Hui Lim, James Gulliver, Stuart Bloom, Holly Theaker, Sean Carlson, JR Fraser Cummings, Robert Livingstone, Amanda Beale, Josiah O Carter, Andrew Bell, Archibald Coulter, Jonathon Snook, Helen Stone, Nicholas A Kennedy, James R Goodhand,Tariq Ahmad

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doi:10.1111/apt.17170.

Study aim

To assess whether immunogenicity to a patient's first anti-TNF would be associated with immunogenicity to their second, irrespective of drug sequence.

My role in the study

I was responsible for determining the study question, seeking ethical approval for the study protocol and design, conducting the study in full, and writing up the results. As part of designing the study protocol, I sought patient and public involvement by by working closely with Crohn's and Colitis UK (CCUK), the national patient group on this project. On 7th September 2019, I participated in the NIHR CCUK IBD Patient involvement in research Day in Manchester. I presented my findings from the PANTS study and explained how these data had raised further questions that the IMSAT study sought to address. I liaised directly with our local CCUK patient panel to help improve the lay summary and patient information sheet. I performed all analyses independently on this project, and wrote the abstracts for the British Society of Gastroenterology and European Crohn's and Colitis Organisation annual conferences, where it was accepted as an oral presentation, which I delivered. I wrote up the study, and submitted it to multiple journals, revising it at each stage to align with multiple editors' and peer reviewers' comments.

Findings

Irrespective of drug sequence, immunogenicity to the first anti-TNF was associated with immunogenicity to the second anti-TNF, which was mitigated by the

introduction of an immunomodulator in patients with immunogenic, but not pharmacodynamic treatment failure.

Relevance and impact on my learning

I was the study lead of this retrospective cohort study. I was responsible for authoring the study protocol, authoring the submission to the Integrated Research Application System, and navigating the processes by which a research study is approved in the UK – a skill that has led me to becoming an independent researcher.

As this was a national therapeutic drug monitoring study, I had to work collaboratively with the departments of biochemistry locally and nationally to recruit UK sites to join the study. I held 36 site initiation visits independently; a strategy that I believe led to higher quality data being entered into the database, compared to if I held the visits via video recording only. Therefore, I fostered a working relationship with personnel at each study site, meaning they felt open to communicating with me via email during the conduct of their data entry.

Learning how to code and analyse this breadth and complexity of data was a new skill that took many months to perform competently, but one that I will use increasingly during my research career.

Acknowledgements of co-authors and contributions to paper

Myself and Tariq Ahmad participated in the conception and design of this study. Rachel Nice and Timothy J Macdonald coordinated all biochemical analyses and central laboratory aspects of the project. All authors and collaborators were involved in the acquisition, analysis, or interpretation of data. Data analysis was done by myself, Simeng Lin, and Nicholas A Kennedy. Drafting of the manuscript was done by myself, James R Goodhand and Tariq Ahmad. Myself, James R Goodhand, Tariq Ahmad obtained the funding for the study. Myself, Simeng Lin, and Nicholas A Kennedy have verified the underlying data.

Abstract

Background and Aims

Anti-drug antibodies are associated with anti-TNF treatment failure in patients with inflammatory bowel disease (IBD). We hypothesized that immunogenicity to a patient's first anti-TNF would be associated with immunogenicity to their second, irrespective of drug sequence.

Methods

We conducted a UK-wide, multicentre, retrospective cohort study to report rates of immunogenicity and treatment failure of second anti-TNF therapies: 1058 patients with IBD who underwent therapeutic drug monitoring for both infliximab and adalimumab were included. The primary outcome was immunogenicity to the second anti-TNF drug, defined at any timepoint as an anti-TNF antibody concentration $\geq$ 9 AU/mL for infliximab and $\geq$ 6 AU/mL for adalimumab.

Results

In patients treated with infliximab then adalimumab, those who developed antibodies to infliximab were more likely to develop antibodies to adalimumab, compared to patients who did not develop antibodies to infliximab (OR 1.99, 95%CI 1.27 - 3.20, p = 0.002). Similarly, in patients treated with adalimumab then infliximab, immunogenicity to adalimumab was associated with subsequent immunogenicity to infliximab (OR 2.63, 95%CI 1.46 - 4.80, p < 0.001). For each 10-fold increase in anti-infliximab and anti-adalimumab antibody concentration, the odds of subsequently developing antibodies to adalimumab and infliximab increased by 1.73 (95% CI 1.38 – 2.17, p<0.001) and 1.99 (95%CI 1.34 – 2.99, p

<0.001), respectively. Patients who developed immunogenicity with undetectable drug levels to infliximab were more than twice as likely to then develop immunogenicity with undetectable drug levels to adalimumab (OR 2.37, 95% CI 1.39 – 4.19, p <0.001). Commencing an immunomodulator at the time of switching to the second anti-TNF was associated with improved drug persistence in patients with immunogenic, but not pharmacodynamic failure.

Conclusion

Irrespective of drug sequence, immunogenicity to the first anti-TNF was associated with immunogenicity to the second anti-TNF, which was mitigated by the introduction of an immunomodulator in patients with immunogenic, but not pharmacodynamic treatment failure.

Introduction

The anti-TNF monoclonal antibodies infliximab and adalimumab, have transformed the management of immune-mediated inflammatory diseases (IMIDs), including inflammatory bowel disease (IBD)[1].

Regrettably, however, anti-TNF treatment failure is common. Obesity, cigarette smoking, higher baseline markers of disease activity, anti-TNF monotherapy and the development of anti-drug antibodies are associated with low drug levels and anti-TNF treatment failure.[2] Loss of response is frequently associated with low anti-TNF drug levels and the formation of anti-drug antibodies, which can be predicted by the carriage of the HLA-DQA1*05 haplotype[3,4], and mitigated by concomitant immunomodulator use[2].

Whilst it is generally accepted that there is a diminishing return from second- and subsequent anti-TNF therapies[5,6], well-designed and adequately powered sequencing studies are scarce[7,8]. Most have been small and limited to the immunogenicity of second-line adalimumab, because historically infliximab has been used first. Estimates range from 28 - 40%[7,9–12] and 39 – 70%[7,12,13] for the risk of immunogenicity to second-line adalimumab and infliximab, respectively. Few studies have addressed whether the development of antibodies to the first anti-TNF drug is associated with immunogenicity[8,10–15] and treatment failure to a second.

The aim of the IMplications for Sequencing of biologic therapy and choice of second Anti-TNF in patients with IBD (IMSAT) study was to evaluate the

relationship between immunogenicity to the first anti-TNF therapy and immunogenicity and drug persistence to second anti-TNF therapy. We hypothesized that immunogenicity to the first anti-TNF would be associated with immunogenicity to the second anti-TNF, irrespective of drug sequence.

Materials and Methods

Study design, clinical setting and participants

We sought to define the:

1. Risk of immunogenicity to a second anti-TNF drug, stratified by immunogenicity to the first anti-TNF drug
2. Rates of drug persistence to a second anti-TNF, following treatment failure to the first anti-TNF, stratified by immunogenicity to the first anti-TNF drug
3. Strategies to mitigate development of immunogenicity to a second anti-TNF drug

We conducted a UK-wide, multicentre, retrospective cohort study to report rates of immunogenicity to second anti-TNF therapies in patients with IBD.

The Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust provides a therapeutic drug monitoring (TDM) service to hospitals throughout the United Kingdom (UK)[16]. All patients who had drug and anti-drug antibody levels undertaken for both infliximab and adalimumab, originator or biosimilar preparations, between 1st May 2013 and 31st November 2020 were eligible for inclusion. Sites who had sent samples for TDM measurement for > 2 patients were invited to take part in our study.

Patient eligibility was confirmed by local research sites. We included patients with a diagnosis of Crohn's disease, ulcerative colitis (UC), and IBD-unclassified (IBD-U) as determined by local sites. Using case note review of secondary care records, their disease courses were followed to the point of data entry or drug withdrawal.

Patients who had historically been treated with an anti-TNF drug prior to the index course with TDM measurement, those who had not been exposed to two anti-TNF drugs, and where the clinical data was incomplete were excluded.

Outcome measures

The primary outcome was immunogenicity to the second anti-TNF drug, defined at any timepoint as an anti-TNF antibody concentration $\geq$ 9 AU/mL for infliximab and $\geq$ 6 AU/mL for adalimumab, using the Immundiagnostik anti-drug antibody ELISA[16]. The secondary outcome was second anti-TNF drug persistence, defined as the length of time from initiation of second anti-TNF to discontinuation of therapy[17].

Treatment failure endpoints were primary non-response at week 20, loss of response after week 20, and adverse events leading to drug withdrawal:

Primary non-response: was defined as exit before week 20 because of treatment failure (including resectional inflammatory bowel disease surgery), corticosteroid use at week 20 (new prescriptions or if previous dose had not been stopped), or physician global assessment of no meaningful response at any time prior to drug withdrawal, even if drug continues beyond standard induction period.

Loss of response: in patients who did not have primary non-response was defined as symptomatic inflammatory bowel disease activity that warranted an escalation of steroid, immunomodulator or anti-TNF therapy, resectional surgery, or exit from

study due to treatment failure[2]. Timing of loss of response was defined as the time of treatment escalation, drug withdrawal, or surgery.

Adverse events were coded centrally according to the Medical Dictionary for Regulatory Activities (MedDRA) version 23.1. Serious adverse events included those that required hospitalization, were life-threatening, or resulted in persistent, permanent, or substantial disability or incapacity. Causality was graded according to the Good Clinical Practice framework guidelines as not related, unlikely, possibly, probably, or definitely related to treatment by local research sites[18].

We subsequently incorporated use of TDM-based decision making in the setting of primary non-response or loss of response, according to the results of their most recent drug level and anti-drug antibodies to the first anti-TNF [2,19–21].

1. Immunogenic - pharmacokinetic failure was defined as treatment failure with low anti-TNF drug levels (infliximab <3 mg/L, adalimumab <5 mg/L), and presence of anti-TNF antibodies (infliximab $\geq$ 9 AU/mL, adalimumab $\geq$ 6 AU/mL).
2. Immunogenic - pharmacodynamic failure was defined as treatment failure despite adequate anti-TNF drug levels (infliximab $\geq$ 3 mg/L, adalimumab $\geq$ 5 mg/L), and presence of anti-TNF antibodies (infliximab $\geq$ 9 AU/mL, adalimumab $\geq$ 6 AU/mL).
3. Non-immunogenic - pharmacokinetic failure was defined as treatment failure with low anti-TNF drug levels (infliximab < 3 mg/L, adalimumab < 5 mg/L), and without presence of anti-TNF antibodies (infliximab < 9 AU/mL, adalimumab < 6 AU/mL).

4. Non-immunogenic - pharmacodynamic failure was defined as treatment failure despite adequate anti-TNF drug levels (infliximab ≥ 3 mg/L, adalimumab ≥ 5 mg/L), and without presence of anti-TNF antibodies (infliximab < 9 AU/mL, adalimumab < 6 AU/mL).

Time to loss of response was defined as the duration of time from initiation of anti-TNF therapy to treatment failure. Non-treatment failure endpoints were withdrawal of anti-TNF therapy in patients with quiescent disease, by treating physician or patient choice.

Variables

We recorded demographic (sex, age, ethnicity, weight, smoking history), IBD-related data (date of diagnosis, phenotype according to Montreal Classification, and immunomodulator status (type, dosing and frequency at time of start and end of anti-TNF treatment, with no minimum duration required and anti-TNF treatment data (indication, dosing frequency, interval, reason for withdrawal, treatment plan after cessation, and any breaks in treatment ≥ 16 weeks).

Laboratory methods

All laboratory analyses were performed at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust. Anti-TNF drug and anti-drug antibodies were measured on the Dynex Technologies (Chantilly, Virginia, USA) DS2 automated ELISA platform.

The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor infliximab (K9654) and adalimumab (K9651) total anti-drug antibody assays allow semi-quantitative measurement of both free and bound anti-drug antibodies[22,23]. A pre-treatment acid dissociation step is used to separate anti-drug antibodies from the therapeutic antibody. The assay then follows a standard ELISA format using recombinant therapeutic antibody as a capture and detection antibody. The positivity threshold for anti-infliximab antibodies is 9 AU/ml and for anti-adalimumab antibodies is 6 AU/mL[16].

The IDKmonitor free infliximab (K9655) and adalimumab (K9657) drug level assays permit quantitative measurement of free therapeutic drug in serum[22,23]. The assays follow a standard ELISA format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody. The measuring range for both assays is 0.8 - 45 mg/L, with absence of drug being defined using a cutoff of <0.8 mg/L.

Statistical analysis

At the time of study design, we identified approximately 1000 patients who had TDM results for both anti-TNF drugs: 78% were treated with infliximab first, and 22% with adalimumab first. We assumed that the crude rates of immunogenicity according to biologic type were generalizable across the cohort and allowed for a 30% attrition rate. We calculated that our sample size provided 93% and 79% power at the 0.025 significance threshold level to detect a significant association between immunogenicity to the first and second anti-TNF, in the infliximab- and adalimumab- treated first cohorts, respectively.

Data were pseudonymized and entered into a purpose designed electronic database in REDCap (Vanderbilt University Medical Centre, Tennessee, US)[24]. Statistical analyses were undertaken in R 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two tailed and p-values <0.05 were considered significant. We included patients with missing clinical data in analyses for which they had data and have specified the denominator for each variable.

We performed univariable analyses using Fisher's exact and Mann-Whitney U tests to identify categorical and continuous variables associated with immunogenicity and treatment failure outcomes. Logistic regression analyses were used to assess whether the magnitude of anti-drug antibodies to the first anti-TNF were independently associated with antibody formation to the second anti-TNF. We performed sensitivity analyses according to drug clearance, which was defined as undetectable anti-TNF drug levels (infliximab <0.8 mg/L, adalimumab <0.8 mg/L), and presence of anti-TNF antibodies (infliximab $\geq$ 9 AU/mL, adalimumab $\geq$ 6 AU/mL).

Rates of drug persistence were estimated using the Kaplan-Meier method, and comparative analyses were performed using Cox proportional hazards regression. Patients were censored at the time of treatment failure to their second anti-TNF.

Youden's formula[25] was used to determine the optimal anti-drug antibody titre during first anti-TNF therapy to predict immunogenicity with undetectable drug level to second anti-TNF, and receiver operator characteristic curves and area under the

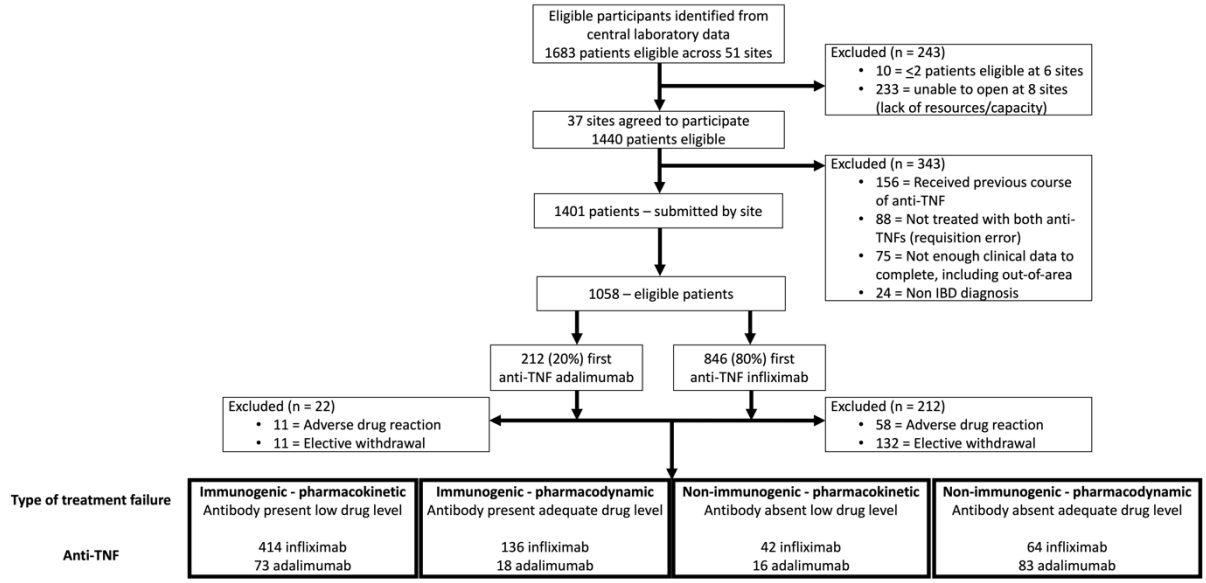
curve analyses with bootstrapping were used to estimate the diagnostic accuracy of the model.

Results

Patient identification and eligibility

Between 1st May 2013 and 31st November 2020, we identified 38940 and 14847 TDM results, from 13708 and 8662 patients, treated with infliximab (median 2, range [1-48]) and adalimumab (1, [1-17]), respectively. 1683 patients from 51 sites had both infliximab and adalimumab TDM results (Figure 1, Supplemental Table 1). Six sites submitted ≤ 2 patients (n=10 patients) so were not approached and eight sites (n=233 patients) opted not to take part.

Figure 1: Patient disposition



1440 patients were screened by research sites for eligibility, and data for 97.3% (1401/1440) patients were submitted. We excluded 11.1% (156/1401) patients who had received a previous course of anti-TNF therapy; 6.3% (88/1401) patients where a requisition error had occurred and who had never received a second anti-TNF; 5.4% (75/1401) patients with incomplete clinical data; and 1.7% (24/1401)

patients who did not have IBD.

Patient characteristics

Of the 1058 (50.3% [532] male) patients in the final analysis: 71.4% (755), 24.4% (258), and 4.3% (45) patients were diagnosed with Crohn's disease, UC, and IBD-U, respectively. Median time of follow-up from starting first anti-TNF to the point of data entry or drug withdrawal was 3.84 years (IQR 2.34 – 5.68). 80% (846) patients were treated with infliximab and then adalimumab, and 20% (212) patients were treated with adalimumab and then infliximab. There was no difference in the duration of treatment with the first anti-TNF drug (infliximab: 1.4 years [IQR 0.7 - 2.9], adalimumab: 1.3 [IQR 0.6 -2.5], p = 0.18). The first anti-TNF was discontinued in 80% (846/1058) patients because they did not respond or lost response; 7.6% (70/1058) patients developed an adverse event leading to drug cessation and the drug was withdrawn in 13.4% (142/1058) patients for non-treatment failure reasons (physician recommendation: 78.2% (111/142), patient choice: 21.8% (31/142)).

Patient characteristics, stratified by the development of immunogenicity to their first anti-TNF, are shown in Table 1, Supplemental Tables 2 and 3. Multivariable logistic regression analyses confirmed that infliximab, compared with adalimumab, smoking, inflammatory disease (B1) in patients with Crohn's disease and anti-TNF therapy without an immunomodulator, but not dosing regimen or diagnosis, were independently associated with development of immunogenicity to first anti-TNF (Figure 2, Supplemental Figures 1 and 2).

Table 1: Variables associated with development of immunogenicity to first anti-TNF

		Immunogenicity to first anti-TNF	
Variable	Level	Yes (n = 803)	No (n = 255)
Gender	Male	76.7% (408/532)	23.3% (124/532)
	Female	75.1% (395/526)	24.9% (131/526)
Age (years)	Start first anti-TNF	29.2 (18.6 - 45.9)	29.5 (20.7 - 43)
	Paediatric (<18 years old)	82.3% (195/237)	17.7% (42/237)
Ethnicity	White: British	74.6% (647/867)	25.4% (220/867)
	Black: Caribbean	66.7% (4/6)	33.3% (2/6)
	Asian: Indian	76% (19/25)	24% (6/25)
Smoking	Current	83% (127/153)	17% (26/153)
Weight (kg)	Start first anti-TNF	68 (55 - 80.2)	70.2 (60 - 85.1)
Disease	Crohn's disease	75.8% (572/755)	24.2% (183/755)

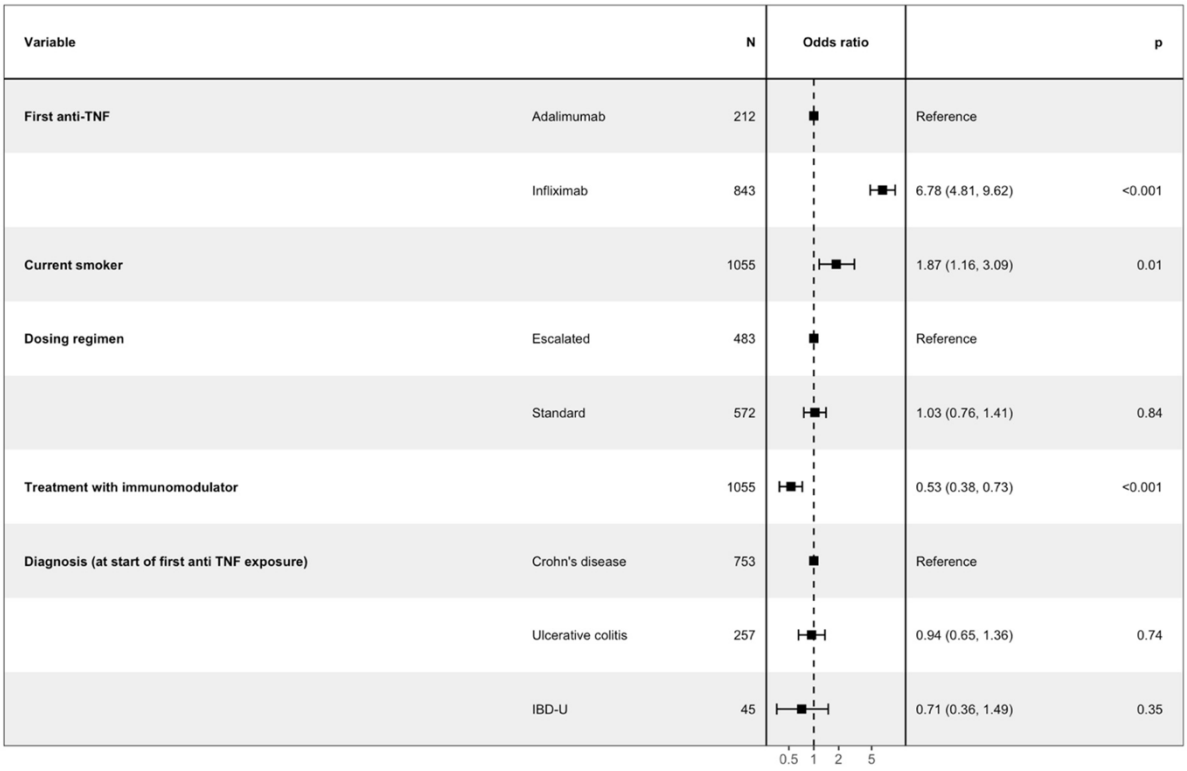
	Ulcerative colitis	77.5% (200/258)	22.5% (58/258)
	IBD-U	68.9% (31/45)	31.1% (14/45)
Location	L1	72.3% (141/195)	27.7% (54/195)
	L2	78.8% (149/189)	21.2% (40/189)
	L3	76.7% (277/361)	23.3% (84/361)
	L4	55.6% (5/9)	44.4% (4/9)
L4 modifier	True	74.2% (121/163)	25.8% (42/163)
Behaviour	B1	81.3% (370/455)	18.7% (85/455)
	B2	69.2% (108/156)	30.8% (48/156)
	B3	65.3% (94/144)	34.7% (50/144)
Perianal disease	True	76.5% (179/234)	23.5% (55/234)
Extent	E1	80.8% (21/26)	19.2% (5/26)
	E2	72.1% (93/129)	27.9% (36/129)

	E3	79.1% (117/148)	20.9% (31/148)
First anti-TNF	Infliximab	83.2% (704/846)	16.8% (142/846)
	Adalimumab	46.7% (99/212)	53.3% (113/212)
First anti-TNF indication	Luminal disease	75.7% (771/1019)	24.3% (248/1019)
	Extraintestinal	77.4% (24/31)	22.6% (7/31)
	Co-existing non-IBD diagnosis	55.6% (10/18)	44.4% (8/18)
Immunomodulator	Start first anti-TNF	73.3% (400/546)	26.7% (146/546)
Immunomodulator type	Azathioprine	72.1% (294/408)	27.9% (114/408)
	Mercaptopurine	73.3% (55/75)	26.7% (20/75)
	Tioguanine	100% (2/2)	0% (0/2)
	Methotrexate	80% (48/60)	20% (12/60)
Duration (years)	First anti-TNF	1.3 (0.7 – 2.6)	1.6 (0.7 – 3.4)
Dosing regimen[1]	Standard	75.4% (432/573)	24.6% (141/573)
	Escalated	76.5% (371/485)	23.5% (114/485)

Treatment outcome	Treatment failure	75.8% (641/846)	24.2% (205/846)
	Adverse event	70% (49/70)	30% (21/70)
	Non-treatment failure	79.6% (113/142)	20.4% (29/142)

[1]Dosing regimen was defined as standard if for infliximab-treated patients, treatment was 5 mg/kg, 8-weekly, and for adalimumab-treated patients, treatment was 40 mg, 2-weekly. Escalated dosing regimen was defined as, for infliximab-treated patients, an increase in dosing (for example, > 7.5 mg/kg) and/or shortening of interval (for example, < 7-weekly), and for adalimumab-treated patients, an increase in dosing (for example, 80 mg) and/or shortening of interval (for example, 1-weekly).

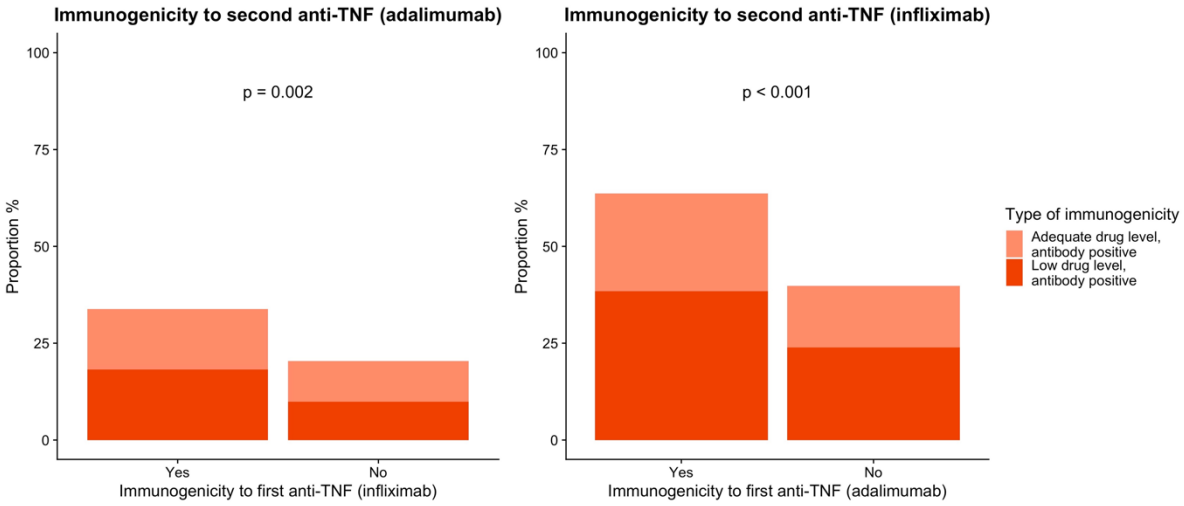
Figure 2: Forest plot showing the coefficients from a multivariable logistic regression model of associations with immunogenicity to first anti-TNF



Immunogenicity to a second anti-TNF drug

In patients treated with infliximab then adalimumab, patients who developed antibodies to infliximab were more likely to develop antibodies to adalimumab, compared to patients who did not develop antibodies to infliximab (odds ratio [OR] 1.99, 95% confidence interval [CI] 1.27 - 3.20, p = 0.002) (Figure 3). Similarly, in patients treated with adalimumab then infliximab, immunogenicity to adalimumab was associated with subsequent immunogenicity to infliximab (OR 2.63, 95% CI 1.46 - 4.80, p < 0.001).

Figure 3: Risk of immunogenicity to second anti-TNF, stratified by immunogenicity to first anti-TNF



***In patients treated with infliximab first, adalimumab second (left hand panel):**
Risk of developing 'immunogenic-pharmacodynamic failure' to adalimumab, having developed 'immunogenic-pharmacodynamic failure' to infliximab (light orange): OR 1.32 (95% CI 0.83 – 2.07, p = 0.243)
Risk of developing 'immunogenic-pharmacokinetic failure' to adalimumab, having developed 'immunogenic-pharmacodynamic failure' to infliximab (dark orange): OR 2.39 (95% CI 1.56 – 3.76, p <0.001)

***In patients treated with adalimumab first, infliximab second (right hand panel):**
Risk of developing 'immunogenic-pharmacodynamic failure' to infliximab, having developed 'immunogenic-pharmacodynamic failure' to adalimumab (light orange): OR 2.14 (95% CI 0.68 – 6.19, p = 0.150)
Risk of developing 'immunogenic-pharmacokinetic failure' to adalimumab, having developed 'immunogenic-pharmacodynamic failure' to infliximab (dark orange): OR 1.76 (95% CI 0.93 – 3.35, p = 0.066)

For each 10-fold increase in anti-infliximab antibody concentration, the odds of subsequently developing antibodies to adalimumab increased by 1.73 (95% CI 1.38 – 2.17, p<0.001). A similar observation was seen for patients who developed antibodies to adalimumab who were subsequently treated with infliximab (OR 1.99, 95%CI 1.34 – 2.99, p <0.001).

Sensitivity analyses according to drug clearance (undetectable anti-TNF drug levels, presence of antibodies) showed that patients who developed immunogenicity with undetectable drug levels to infliximab-first were more than twice as likely to then develop immunogenicity with undetectable drug levels to adalimumab-second (OR 2.37, 95% CI 1.39 – 4.19, p <0.001). This was not seen for patients treated with adalimumab-first and infliximab-second (OR 1.85, 95% CI

0.88 – 3.87, p = 0.10) (Supplemental Tables 4 and 5).

Youden's method demonstrated that the optimal anti-drug antibody titre cut-off to first anti-TNF to determine immunogenicity with undetectable drug level to second anti-TNF was 109 AU/mL for patients treated with infliximab first, with an area under the curve of 0.66 (95% CI 0.60 - 0.71). The sensitivity and specificity were 0.63 (95% CI 0.49 - 0.90) and 0.68 (95% CI 0.38 - 0.80), respectively. For patients treated with adalimumab first, the optimal anti-drug antibody titre cut-off was 11 AU/mL, with an area under the curve of 0.57 (95% CI 0.51 – 0.64). The sensitivity and specificity were 0.58 (95% CI 0.42 - 0.70) and 0.61 (95% CI 0.55 - 0.74), respectively.

Second anti-TNF treatment outcomes

Overall, 39.3% (416/1058) patients did not respond or lost response to the second anti-TNF, 4.3% (45/1058) patients developed an adverse drug reaction leading to drug cessation, and the drug was withdrawn electively in 4.3% (45/1058) patients.

Of the 846 patients who did not respond or who lost response to the first anti-TNF, 57.6% (487/846) and 18.2% (154/846) patients were classified with immunogenic-pharmacokinetic and immunogenic-pharmacodynamic failure, respectively. A further 6.9% (58/846) and 17.4% (147/846) patients were classified with nonimmunogenic-pharmacokinetic failure and nonimmunogenic-pharmacodynamic failure, respectively (Table 2).

Table 2: Variables associated with treatment failure to first anti-TNF, stratified by anti-TNF therapy and type of treatment failure to first anti-TNF

Infliximab as first anti-TNF						
Treatment failure to first anti-TNF	N	Immunomodulator status at start of infliximab Proportion (95% CI)	Antibody level[1] (IQR)	Drug level[2] (IQR)	Escalated dosing regimen[3] Proportion (95% CI)	Duration treated with infliximab [years (IQR)]
Immunogenic - pharmacokinetic Antibody present, low drug level	414	49.4% (95% CI 0.44 - 0.54)	102 (42 - 336.8)	<0.8	45.7% (95% CI 0.41 - 0.51)	1.2 (0.7 - 2.6)
Immunogenic - pharmacodynamic Antibody present, adequate drug level	136	53.7% (95% CI 0.45 - 0.62)	45.5 (16 - 72.8)	5.5 (4 - 9.1)	55.1% (95% CI 0.46 - 0.64)	1.9 (0.9 - 3.2)
Non-immunogenic - pharmacokinetic Antibody absent, low drug level	42	69% (95% CI 0.53 - 0.82)	5 (5 - 5)	2 (0.7 - 2.7)	45.2% (95% CI 0.30 - 0.62)	1.8 (0.9 - 4.3)
Non-immunogenic - pharmacodynamic Antibody absent, adequate drug level	64	70.3% (95% CI 0.57 - 0.81)	5 (5 - 5)	7.1 (4.4 - 13.9)	56.2% (95% CI 0.43 - 0.68)	2.1 (0.8 - 4.1)
Adalimumab as first anti-TNF						

Treatment failure to first anti-TNF	N	Immunomodulator status at start of adalimumab Proportion (95% CI)	Antibody level[1] (IQR)	Drug level[2] (IQR)	Escalated dosing regimen[3] Proportion (95% CI)	Duration treated with adalimumab [years (IQR)]
Immunogenic - pharmacokinetic Antibody present, low drug level	73	29.2% (95% CI 0.19 - 0.41)	201 (98 - 201)	<0.8	31.5% (95% CI 0.21 - 0.44)	1.4 (0.7 - 2.5)
Immunogenic - pharmacodynamic Antibody present, adequate drug level	18	55.6% (95% CI 0.31 - 0.78)	15.5 (11.2 - 62.8)	6.5 (5.9 - 11.4)	44.4% (95% CI 0.22 - 0.69)	1.4 (0.9 - 2.4)
Non-immunogenic - pharmacokinetic Antibody absent, low drug level	16	43.8% (95% CI 0.21 - 0.69)	5 (5 - 5)	4.2 (1.8 - 4.4)	37.5% (95% CI 0.16 - 0.64)	0.7 (0.5 - 1.8)
Non-immunogenic - pharmacodynamic Antibody absent, adequate drug level	83	44.6% (95% CI 0.34 - 0.56)	5 (5 - 5)	9.8 (8.2 - 13.7)	44.6% (95% CI 0.34 - 0.56)	1.5 (0.7 - 2.9)

[1]Threshold for presence of anti-TNF antibodies: infliximab 9 AU/mL and adalimumab 6 AU/mL

[2] Threshold for adequate anti-TNF drug level: infliximab > 3 mg/L and adalimumab > 7 mg/L

[3] Dosing regimen was defined as standard if for infliximab-treated patients, treatment was 5 mg/kg, 8-weekly, and for adalimumab-treated patients, treatment

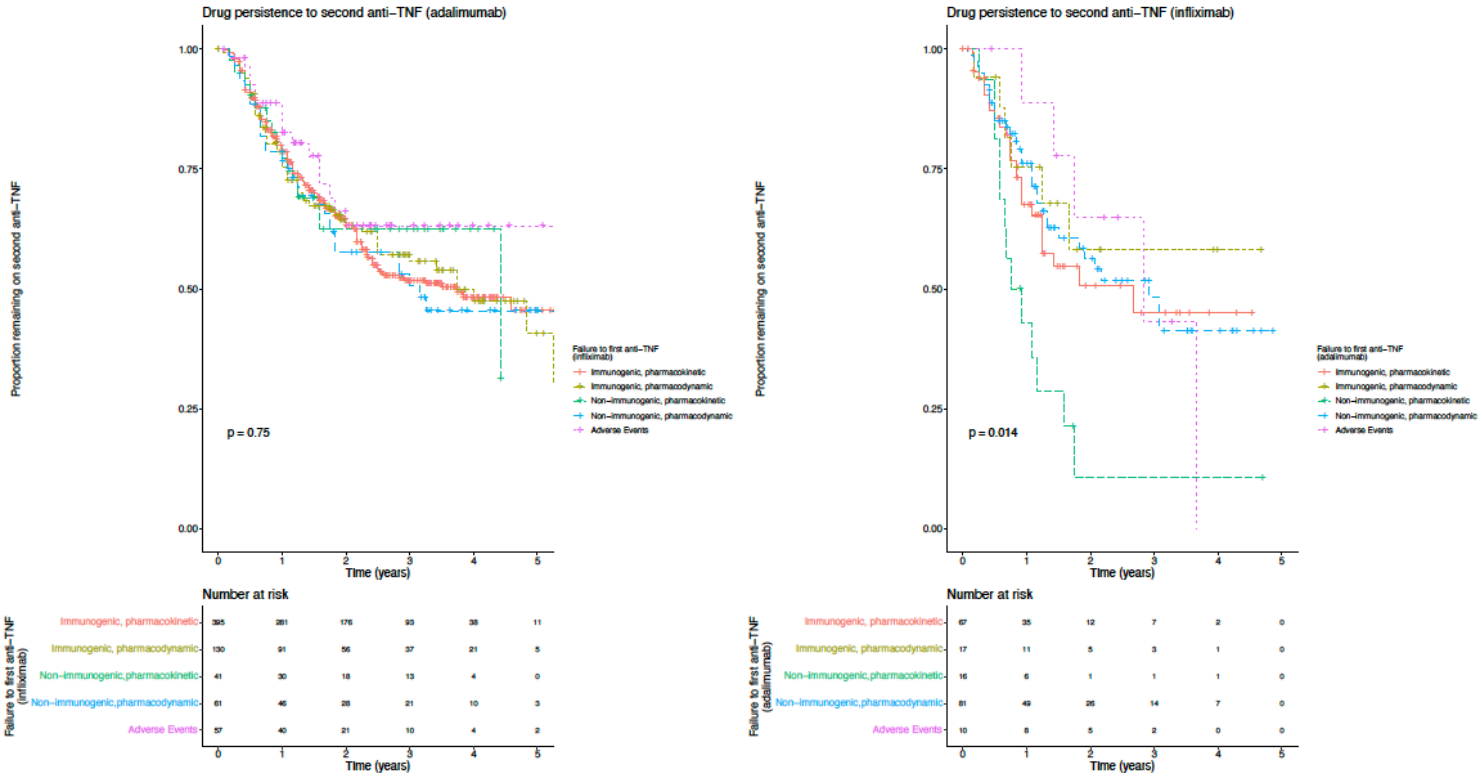
was 40 mg, 2-weekly. Escalated dosing regimen was defined as, for infliximab-treated patients, an increase in dosing (for example, > 7.5 mg/kg) and/or shortening of interval (for example, < 7-weekly), and for adalimumab-treated patients, an increase in dosing (for example, 80 mg) and/or shortening of interval (for example, 1-weekly)

The median duration of first anti-TNF treatment was similar between patients treated with infliximab as first anti-TNF and patients treated with adalimumab as first anti-TNF (infliximab: 1.3 years [IQR 0.6 - 2.7] vs adalimumab: 1.4 years [IQR 0.6 - 2.6], p = 0.56), however, more patients treated with infliximab as first anti-TNF were treated with a concomitant immunomodulator (infliximab: 53.4% [364/683] vs adalimumab: 40.5% [77/190], p = 0.002). Similar proportions of infliximab- and adalimumab- treated patients had their first anti-TNF dose escalated before switching drugs.

Second anti-TNF drug persistence

At 4-year follow-up, patients treated with adalimumab as second anti-TNF were more likely to continue the anti-TNF therapy compared to patients treated with infliximab as second anti-TNF (adalimumab: 49.2% [95% CI 44.6 - 54.2] vs infliximab: 37.8% [95% CI 28.8 - 49.6], p = 0.005). No differences were seen in drug persistence in patients treated with adalimumab as second anti-TNF, according to infliximab treatment failure status (Figure 4). In patients treated with infliximab as second anti-TNF, patients who developed non-immunogenic-pharmacokinetic failure had lower drug persistence compared to all other treatment failure groups. Sensitivity analyses demonstrated no difference in drug persistence to second-anti TNF, when applying a stricter definition of immunogenic, pharmacokinetic failure of undetectable drug level in the presence of antibodies (Supplemental Figures 3 and 4).

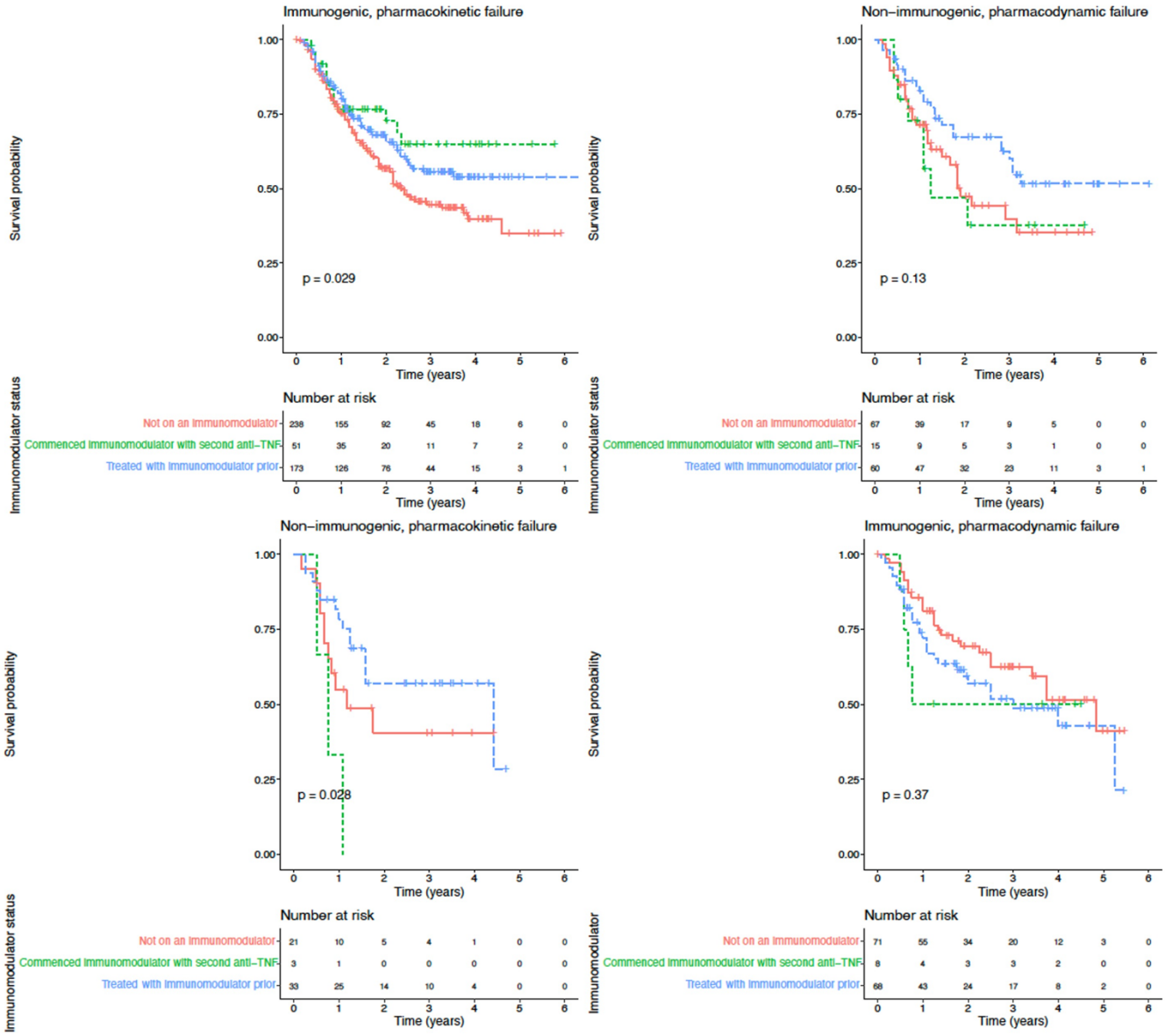
Figure 4: Drug persistence to second anti-TNF, stratified by first anti-TNF and type of failure to first anti-TNF



Impact of immunomodulator on drug persistence

Of patients who developed immunogenic, pharmacokinetic failure to their first anti-TNF, those who commenced an immunomodulator with the second anti-TNF, and those who were treated with an immunomodulator prior to starting second anti-TNF, experienced longer drug persistence than patients who were not treated with an immunomodulator at time of second anti-TNF ($p = 0.03$) (Figure 5). There was no difference in drug persistence in patients who commenced an immunomodulator at the time of second anti-TNF or those who were treated with an immunomodulator prior to starting second anti-TNF ($p = 0.36$). No other associations between type of treatment failure to first anti-TNF and immunomodulator status were observed.

Figure 5: Drug persistence to second anti-TNF, stratified by treatment failure to first anti-TNF and immunomodulator status with second anti-TNF



Adverse events

Patients who developed an adverse event had lower drug persistence to first anti-TNF than patients who developed treatment failure or non-treatment failure (adverse event: 0.6 years (0.3 - 1.2), treatment failure: 1.5 (0.7 - 2.8), non-

treatment failure: 1.4 years (0.8 - 3)).

Adverse events leading to withdrawal of the second anti-TNF occurred in 5.7% (12/212; 95% CI 3.1% - 9.9%) patients treated with infliximab and 3.9% (33/846; 95% CI 2.7% - 5.5%) patients treated with adalimumab. The most common adverse events were infusion and injection-site reactions (52.2%, 60/115), rash (23.5%, 27/115), arthritis (3.5%, 4/115), and viral infections (3.5%, 4/115) (Supplemental Table 6).

Infusion reactions to infliximab, which occurred after a median of a 20.4 weeks (IQR 14.4 - 58.5), were associated with subsequent injection site reactions to adalimumab, that occurred after a median of 30.5 weeks (IQR 6.8 - 49.2) [69% (40/58] vs 18.2% (6/33) p <0.001]. Overall, infusion reactions to infliximab were associated with higher anti-infliximab antibody levels; for every 10-fold increase in antibodies, there was an 8 times risk of having an infusion reaction (OR 8.57, 95% CI 4.38 - 18.73, p <0.001). No association was seen for injection site reactions and anti-adalimumab antibody levels.

Discussion

Irrespective of anti-TNF sequence, immunogenicity to the first anti-TNF, was associated with immunogenicity to the second anti-TNF. We report here that 34% (95% CI 30 – 37%) and 64% (95% CI 54 – 73%) patients subsequently developed anti-drug antibodies to adalimumab and infliximab, respectively. Patients who developed immunogenicity with undetectable drug levels to infliximab were more than twice as likely to develop immunogenicity with undetectable drug levels to adalimumab. Commencing an immunomodulator at the time of switching to the second anti-TNF was associated with improved drug persistence in patients with immunogenic-, but not pharmacodynamic-treatment failure to the first anti-TNF.

It is widely accepted that infliximab is more immunogenic than adalimumab. This has been attributed to the chimeric formulation of infliximab and the more variable drug levels and associated discontinuity of immune responses seen across the standard 8-week dosing interval[2,26]. Why some individuals have a propensity to develop antibodies to unrelated epitopes of infliximab and then adalimumab is unknown[27]. However, the dose-effect observed here between the magnitude of antibody responses to the first anti-TNF and the risk of developing antibodies to the second suggests that this association is not spurious.

We, like others, have shown previously that carriage of one or more HLA-DQA1*05 alleles confers an almost two-fold increased risk of immunogenicity to both infliximab and adalimumab, irrespective of concomitant immunomodulator use[3,4]. It is plausible then that some of the risk of sequential immunogenicity is explained by HLA-DQA1*05 carriage. We were unable to replicate the association reported

by Casteele et al[7], showing an association between drug level at time of switch and subsequent immunogenicity to the second anti-TNF. Our data argues against a mechanism common to both drugs accelerating clearance leading to subsequent immunogenicity. It is also possible that there is cross-reactivity between both antibody assays and unmeasured antibodies such as hinge autoantibodies, rheumatoid factor, human anti-mouse- or human anti-human antibodies[28–30].

We have replicated findings of a recent open-label randomized controlled trial (RCT) that demonstrated reduced clinical failure rates in 90 patients with immunogenic-pharmacokinetic treatment failure to first anti-TNF who commenced azathioprine at the time of switch to a second anti-TNF[8]. In our real-world cohort of 1058 patients, 20% of whom were treated with adalimumab where immunogenicity rates are lower than for infliximab-treated patients, we were powered to demonstrate the predictive risk of immunogenicity to patients treated with infliximab second-line. Unlike the RCT performed by Roblin X et al. which only included patients who had immunogenic-pharmacokinetic treatment failure, we were also able to demonstrate no additional benefit of an immunomodulator in patients who had pharmacodynamic failure to their first anti-TNF, including in those who developed anti-drug antibodies in the presence of adequate drug levels

Herein, at the time of first anti-TNF treatment failure about one in five patients had anti-drug antibodies that were detectable in the presence of drug[2,31,32]. Considerable uncertainty remains as to the function and relevance of these antibodies. Theoretically, they maybe neutralizing, transient or maturing, and in the future may lead to a more robust immune response, and clear drug[26]. Against

them being clinically relevant, however, we found no association with subsequent immunogenicity, drug level, or the duration of treatment with the second anti-TNF drug. Functional studies are required to better characterize these antibodies and to understand if they are clinically relevant.

As commonly performed in clinical practice, we incorporated use of TDM-based decision making in the setting of primary non-response or loss of response. Consistent with recently published systematic reviews[33,34], we stratified patients into one of four categories based on presence or absence of antibodies and anti-TNF drug concentration. Low anti-TNF drug level cut-offs were chosen based on the best available randomised controlled trial, prospective, or post-hoc analyses data that were associated with non-remission. During maintenance therapy, for infliximab, based on randomised controlled trial data[21], this was determined to be 3 mg/L, and for adalimumab, based on the DIAMOND trial[20], this was determined to be 5 mg/L.

We acknowledge, however, the following limitations. First, inherent to our retrospective study design, we have no data on patients who failed an anti-TNF drug but did not have TDM undertaken. Because of this we may have underestimated the rates of immunogenicity and overestimated drug persistence. This, and the lack of alternative biologic treatments during the timeframe of the study, probably accounts for why over half of patients, regardless of their immunogenicity status, were being treated with their second anti-TNF after four years. Second, our results are potentially subject to interpretation bias, and bias because of missing data, including anti-TNF and immunomodulator dose

optimization data.

Third, we accept that our data would have been strengthened by objective markers of disease activity and endoscopic outcomes. Fourth, this was an unselected TDM referral cohort and although we recommend blood sampling just before the next dose, inevitably, some non-trough samples will have been processed. Even drug-tolerant anti-drug antibody assays are not completely drug-tolerant and therefore we are likely to have underestimated the rates of immunogenicity[35]. This effect may be more important in adalimumab-treated patients where TDM testing is more often ad-hoc rather than immediately before administration as for infliximab.

Finally, although we were able to show that patients who developed immunogenicity with low drug levels to infliximab also developed this outcome to subsequent adalimumab, because only 20% of our cohort were treated with adalimumab first, we were probably underpowered to demonstrate this association for patients treated with second-line infliximab.

We collected data from multiple sites from across the UK, who, based on the variability in the numbers of tests per patient, used a range of TDM practices. However, because we were able to confirm associations with immunogenicity that we reported in the prospective UK-wide PANTS study[2], it is likely that our immunogenicity findings will be generalizable to other western populations. Whether sequential immunogenicity occurs in populations with low HLA-DQA1*05 carriage and lower rates of immunogenicity is unknown[4,20]. Further research is needed to elucidate if patients who develop immunogenicity to one or more anti-

TNF drugs are also at risk of developing anti-drug antibodies to the newer biologic therapies.

Conclusions

Patients who developed antibodies to their first anti-TNF were more likely to develop antibodies to their second anti-TNF, irrespective of drug sequence. Our findings support international recommendations for the management of anti-TNF treatment failure, to switch out of biologic class when drug levels are therapeutic, and within class with an immunomodulator when anti-TNF drug levels are low and associated with antibody development.

Role of the funding source

The study was funded by unrestricted grants from Janssen Pharmaceuticals and Cure Crohn's Colitis (Scottish-registered charity). The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

The choice of treatment, care, or services was that of the healthcare professional and patient. In line with Health Research Authority guidelines, formal ethics approval for our study and patient consent was not required. The sponsor of the study is the Royal Devon and Exeter NHS Foundation Trust.

Data availability statement

The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be directed to **tariq.ahmad1@nhs.net**. To gain access data requestors will need to sign a data access agreement.

Conflict of interest disclosure

I have nothing to declare. Simeng Lin reports non-financial support from Pfizer, non-financial support from Ferring, outside the submitted work. Nicholas A Kennedy reports grants from F. Hoffmann-La Roche AG, grants from Biogen Inc, grants from Celltrion Healthcare, grants from Galapagos NV, non-financial support from Immundiagnostik, during the conduct of the study; grants and non-financial

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Supplementary Table 1: IMSAT study collaborator list

* denotes principal investigator at collaborating site

Trust name	First name	Middle Initial	Surname	Title	Patients recruited
Alder Hey Children's NHS Foundation Trust					33
	*Marcus	K	Auth	Consultant paediatric gastroenterologist	
	Chai Leng		Lee	Specialist registrar in paediatric gastroenterology	
Ashford and St Peter's Hospitals NHS Foundation Trust					89
	*Helena		Robbins	Consultant gastroenterologist	
	Shi		Looi	Specialist registrar in gastroenterology	
Blackpool Teaching Hospitals NHS Foundation Trust					18
	*Senthil	V	Murugesan	Consultant gastroenterologist	

	Tom		Riley	Specialist registrar in gastroenterology	
Bradford Teaching Hospitals NHS Foundation Trust					8
	*Cathryn		Preston	Consultant gastroenterologist	
	Sophie		Stephenson	Gastroenterology research nurse	
	Wendy		Cardozo	Gastroenterology research nurse	
Calderdale and Huddersfield Royal Infirmary					21
	*Sunil	A	Sonwalkar	Consultant gastroenterologist	
	Mohammed		Allah-Ditta	Specialist pharmacist in gastroenterology	
	Lynne		Mansfield	IBD clinical nurse specialist	
Cardiff and Value University Health Board					32
	*Dharmaraj		Durai	Consultant gastroenterologist	
	Mark		Baker	Research nurse	
Countess of Chester Hospital NHS Foundation Trust					55

	*Ian		London	Consultant gastroenterologist	
	Emily		London	Clinical research fellow	
Croydon Health Services NHS Trust					14
	*Sanjay		Gupta	Consultant gastroenterologist	
Gloucestershire Hospitals NHS Foundation Trust					19
	*Alex		Di Mambro	Consultant gastroenterologist	
	Aisling		Murphy	Clinical fellow in gastroenterology	
Great Ormond Street Hospital					33
	*Edward		Gaynor	Consultant paediatric gastroenterologist	
	Kelsey	DJ	Jones	Consultant paediatric gastroenterologist	
Great Western Hospitals NHS Foundation Trust					12
	*Andrew		Claridge	Consultant gastroenterologist	
Hull University Teaching Hospitals NHS Trust					77

	*Shaji		Sebastian	Professor of gastroenterology	
	Sankaranarayanan		Ramachandran	Specialty doctor in gastroenterology	
Leeds Teaching Hospitals NHS Trust					24
	*Christian	P	Selinger	Consultant gastroenterologist	
Manchester University NHS Foundation Trust					13
	*Simon	P	Borg-Bartolo	Consultant gastroenterologist	
	Paul		Knight	Consultant gastroenterologist	
Mid Yorkshire Hospitals NHS Trust					39
	*Michael	B	Sprakes	Consultant gastroenterologist	
	Julie		Burton	Research nurse	
	Patricia		Kane	Research nurse	
	Stephanie		Lupton	Research nurse	
	Aimee		Fletcher	Clinical trials assistant	
NHS Greater Glasgow and Clyde – Glasgow Royal Infirmary					35
	*Daniel	R	Gaya	Consultant gastroenterologist	

	Roghan		Colbert	Senior clinical fellow in gastroenterology	
NHS Greater Glasgow and Clyde – Queen Elizabeth University Hospital					60
	*John Paul		Seenan	Consultant gastroenterologist	
	Jonathan		MacDonald	Consultant gastroenterologist	
	Lucy		Lynch	Specialist registrar in gastroenterology	
	Iain		McLachlan	Clinical development fellow	
	Stephanie		Shields	Clinical research fellow	
NHS Greater Glasgow and Clyde – Royal Hospital for Sick Children					10
	*Richard		Hansen	Consultant paediatric gastroenterologist	

	Lisa		Gervais	Inflammatory bowel disease/research nurse specialist	
	Mwansa		Jere	Clinical research fellow	
NHS Lanarkshire - University Hospital Wishaw					4
	*Muhammad		Akhtar	Consultant gastroenterologist	
	Karen		Black	Senior research nurse	
NHS Lothian - Royal Hospital For Sick Children					5
	*Paul		Henderson	Consultant paediatric gastroenterologist	
	Richard	K	Russell	Professor of paediatric gastroenterology	
NHS Lothian - Western General Hospital					47
	*Charlie	W	Lees	Professor of gastroenterology	

	Lauranne	AAP	Derikx	Senior clinical fellow in gastroenterology	
North Bristol NHS Trust					43
	*Melanie		Lockett	Consultant gastroenterologist	
	Frederica		Betteridge	Specialist registrar in gastroenterology	
Royal Berkshire NHS Foundation Trust					14
	*Aminda		De Silva	Consultant gastroenterologist	
	Arif		Hussenbux	Specialist registrar in gastroenterology	
Royal Cornwall Hospitals NHS Trust					51
	*John		Beckly	Consultant gastroenterologist	
	Oliver		Bendall	Specialist registrar in gastroenterology	
Royal Devon and Exeter NHS Foundation Trust					106
	James	W	Hart	Consultant paediatrician	

	Amanda		Thomas	Senior clinical fellow in gastroenterology	
	Ben		Hamilton	Specialist registrar in gastroenterology	
	Claire		Gordon	Senior clinical fellow in gastroenterology	
	Desmond		Chee	Senior clinical fellow in gastroenterology	
	Marian		Parkinson	Research administrator	
	Helen		Gardner Thorpe	Research administrator	
Shrewsbury and Telford Hospital NHS Trust					49
	*Jeff	R	Butterworth	Consultant gastroenterologist	
	Asima		Javed	Specialty registrar in gastroenterology	

	Sarah		Al-Shakhshir	Specialist registrar in gastroenterology	
	Rekha		Yadagiri	Specialty doctor in gastroenterology	
	Sebrene		Maher	Medical student	
St George's University Hospitals NHS Foundation Trust					46
	*Richard	CG	Pollok	Professor of gastroenterology	
	Tze		Ng	Clinical fellow in gastroenterology	
	Priscilla		Appiahene	Physician associate	
	Fiona		Donovan	Inflammatory bowel disease nurse specialist	
	James		Lok	Specialist registrar in gastroenterology	
St Helens and Knowsley Teaching Hospitals NHS Trust					56
	*Rajiv		Chandy	Consultant gastroenterologist	

	*Reema		Jagdish	Specialty doctor in gastroenterology	
	Daniyal		Baig	Specialist registrar in gastroenterology	
Stockport NHS Foundation Trust					15
	*Zahid		Mahmood	Consultant gastroenterologist	
	Liane		Marsh	Clinical research practitioner	
Taunton and Somerset NHS Foundation Trust					22
	*Alison		Moss	Research nurse	
	Amin		Abdulgader	Clinical fellow in gastroenterology	
	Angus		Kitchin	Clinical fellow in gastroenterology	
Torbay and South Devon NHS Foundation Trust					39
	*Gareth	J	Walker	Consultant gastroenterologist	
	Becky		George	Inflammatory bowel disease clinical nurse specialist	

	Yuen-Hui		Lim	Specialist registrar in gastroenterology	
	James		Gulliver	Specialist registrar in gastroenterology	
University College London Hospitals NHS Foundation Trust					157
	*Stuart		Bloom	Professor of gastroenterology	
	Holly		Theaker	Clinical fellow in gastroenterology	
	Sean		Carlson	Internal medical trainee	
University Hospital Southampton NHS Foundation Trust					24
	*JR	Fraser	Cummings	Consultant gastroenterologist	
	Robert		Livingstone	Clinical fellow in gastroenterology	
University Hospitals Bristol and Weston NHS Foundation Trust - Bristol					45
	*Amanda		Beale	Consultant gastroenterologist	
	Josiah	O	Carter	Specialist registrar in gastroenterology	

University Hospitals Bristol and Weston NHS Foundation Trust - Weston					14
	*Andrew		Bell	Consultant gastroenterologist	
	Archibald		Coulter	Specialist registrar in gastroenterology	
University Hospitals Dorset NHS Foundation Trust					72
	*Jonathon		Snook	Consultant gastroenterologist	
	Helen		Stone	Specialist registrar in gastroenterology	

Supplemental Table 2: Variables associated with development of immunogenicity to first anti-TNF, stratified by biologic drug (infliximab as first anti-TNF)

		Immunogenicity to first anti-TNF	
Variable	**Level**	**Yes** (n = 704)	**No** (n = 142)
Gender	Male	82.8% (357/431)	17.2% (74/431)
	Female	83.6% (347/415)	16.4% (68/415)
Age (years)	At first anti-TNF	29 (18 - 46)	26 (18 - 43)
	Paediatric (<18 years old)	83.9% (182/217)	16.1% (35/217)
Ethnicity	White: British	83% (571/688)	17% (117/688)
	Black: Caribbean	80% (4/5)	20% (1/5)
	Asian: Indian	76.2% (16/21)	23.8% (5/21)
Smoking	Current Smoker	87.1% (101/116)	12.9% (15/116)
Disease	Crohn's disease	83.1% (493/593)	16.9% (100/593)
	Ulcerative colitis	83.9% (183/218)	16.1% (35/218)
	IBD-U	80% (28/35)	20% (7/35)
Location	L1	81.9% (118/144)	18.1% (26/144)
	L2	87.9% (131/149)	12.1% (18/149)
	L3	81.9% (240/293)	18.1% (53/293)
	L4	66.7% (4/6)	33.3% (2/6)
L4 modifier	True	80.2% (105/131)	19.8% (26/131)
Behaviour	B1	88.2% (321/364)	11.8% (43/364)

	B2	76.2% (93/122)	23.8% (29/122)
	B3	73.8% (79/107)	26.2% (28/107)
Perianal disease	True	81.1% (163/201)	18.9% (38/201)
Extent	E1	86.4% (19/22)	13.6% (3/22)
	E2	81.7% (85/104)	18.3% (19/104)
	E3	84.3% (107/127)	15.7% (20/127)
First anti-TNF indication	Luminal disease	83% (679/818)	17% (139/818)
	Extraintestinal	78.9% (15/19)	21.1% (4/19)
	Co-existing non-IBD diagnosis	100% (5/5)	0% (0/5)
Immunomodulator	Start TNF1	78.8% (364/462)	21.2% (98/462)
Immunomodulator type	Azathioprine	78.1% (235/301)	21.9% (66/301)
	Mercaptopurine	83.8% (62/74)	16.2% (12/74)
	Tioguanine	0% (0/0)	0% (0/0)
	Methotrexate	81.1% (43/53)	18.9% (10/53)
Duration (years)	TNF1	1.3 (0.7 - 2.7)	1.9 (0.8 - 4.3)
Drug level	TNF1	0.7 (0.7 - 3.2)	4.2 (2.6 - 7.6)
Dosing regimen	Escalated	83% (338/407)	17% (69/407)
	Standard	83.4% (366/439)	16.6% (73/439)
Treatment outcome	Treatment failure	83.8% (550/656)	16.2% (106/656)
	Adverse event	81.4% (48/59)	18.6% (11/59)
	Non-treatment failure	80.9% (106/131)	19.1% (25/131)

Supplemental Table 3: Variables associated with development of immunogenicity to first anti-TNF, stratified by biologic drug (adalimumab as first anti-TNF)			
		Immunogenicity to first anti-TNF	
Variable	**Level**	**Yes** (n = 99)	**No** (n = 113)
Gender	Male	50.5% (51/101)	49.5% (50/101)
	Female	43.2% (48/111)	56.8% (63/111)
Age (years)	At first anti-TNF	34 (23 - 47)	33 (24 - 44)
	Pediatric (<18 years old)	65% (13/20)	35% (7/20)
Ethnicity	White: British	42.5% (76/179)	57.5% (103/179)
	Black: Caribbean	0% (0/1)	100% (1/1)
	Asian: Indian	75% (3/4)	25% (1/4)
Smoking	Current	70.3% (26/37)	29.7% (11/37)
Weight (kg)	Start TNF1	77.6 (66 - 90.5)	72 (63.8 - 87)
Disease	Crohn's disease	48.8% (79/162)	51.2% (83/162)
	Ulcerative colitis	42.5% (17/40)	57.5% (23/40)
	IBD-U	30% (3/10)	70% (7/10)
Location	L1	45.1% (23/51)	54.9% (28/51)
	L2	45% (18/40)	55% (22/40)
	L3	54.4% (37/68)	45.6% (31/68)
	L4	33.3% (1/3)	66.7% (2/3)
L4 modifier	True	50% (16/32)	50% (16/32)

Behaviour	B1	53.8% (49/91)	46.2% (42/91)
	B2	44.1% (15/34)	55.9% (19/34)
	B3	59.5% (22/37)	40.5% (15/37)
Perianal disease	True	48.5% (16/33)	51.5% (17/33)
Extent	E1	50% (2/4)	50% (2/4)
	E2	32% (8/25)	68% (17/25)
	E3	47.6% (10/21)	52.4% (11/21)
First anti-TNF indication	Luminal disease	45.8% (92/201)	54.2% (109/201)
	Extraintestinal	75% (9/12)	25% (3/12)
	Co-existing non-IBD diagnosis	38.5% (5/13)	61.5% (8/13)
Immunomodulator	Start TNF1	42.9% (36/84)	57.1% (48/84)
Immunomodulator type	Azathioprine	37.3% (22/59)	62.7% (37/59)
	Mercaptopurine	50% (7/14)	50% (7/14)
	Tioguanine	0% (0/0)	0% (0/0)
	Methotrexate	60% (6/10)	40% (4/10)
Duration (years)	TNF1	1.4 (0.7 - 2.5)	1.2 (0.6 - 2.7)
Drug level	TNF1	1.1 (0.7 - 3.9)	9 (6.5 - 11.9)
Dosing regimen	Escalated	42.3% (33/78)	57.7% (45/78)
	Standard	49.3% (66/134)	50.7% (68/134)
Treatment outcome	Treatment failure	47.9% (91/190)	52.1% (99/190)
	Adverse event	9.1% (1/11)	90.9% (10/11)

	Non-treatment failure	63.6% (7/11)	36.4% (4/11)

Supplemental Table 4: Antibody concentration and drug level profiles, of patients who developed immunogenic-pharmacokinetic and immunogenic-pharmacodynamic failure to first anti-TNF, stratified by anti-TNF

Infliximab as first anti-TNF			
Treatment failure to first anti-TNF		**Immunogenic - pharmacokinetic** Antibody present, low drug level (n = 414)	**Immunogenic - pharmacodynamic** Antibody present, adequate drug level (n = 136)
Antibody concentration (AU/mL) (IQR)		102 (42 – 336.8)	45.5 (16 – 72.8)
Antibody concentration quartiles	1	2.7% (11/414)	5.9% (8/136)
	2	26.1% (108/414)	52.2% (71/136)
	3	36.2% (150/414)	31.6% (43/136)
	4	35% (145/414)	10.3% 9 (14/136)
Drug level (mg/L) (IQR)		0.7 (0.7 – 0.7)	5.5 (4 – 9.1)
Drug level quartiles (mg/L)	1	43.2% (179/414)	0% (0/136)
	2	40.3% (167/414)	0% (0/136)
	3	16.4% (68/414)	48.5% (66/136)
	4	0% (0/414)	51.5% (70/136)
Treatment failure to first anti-TNF		**Immunogenic - pharmacokinetic** Antibody present, low drug level	**Immunogenic - pharmacodynamic** Antibody present, adequate drug level

		(n = 73)	(n = 18)
Antibody concentration (AU/mL) (IQR)		201 (98 – 201)	15.5 (11.2 – 62.8)
Antibody concentration (AU/mL) quartiles	1	1.4% (1/73)	11.1% (2/18)
	2	15.1% (11/73)	61.1% (11/18)
	3	23.3% (17/73)	22.2% (4/18)
	4	60.3% (44/73)	5.6% (1/18)
Drug level (mg/L) (IQR)		0.7 (0.7 – 1.8)	6.5 (5.9 – 11.4)
Drug level quartiles (mg/L)	1	35.6% (26/73)	0% (0/18)
	2	43.8% (32/73)	0% (0/18)
	3	20.5% (15/73)	5.6% (1/18)
	4	0% (0/73)	94.4% (17/18)

Supplemental Table 5: Risk of developing immunogenicity and immunogenic-pharmacokinetic failure (undetectable drug level) to second anti-TNF, stratified by condition

	Crohn's disease	
	Infliximab-first, adalimumab-second (n = 593)	Adalimumab-first, Infliximab-second (n = 162)
Risk of developing immunogenicity	OR 2.52 (95% CI 1.43 - 4.68)	OR 2.94 (95% CI 1.48 – 5.94)
Risk of developing immunogenic-pharmacokinetic failure (undetectable drug level)	OR 2.61 (95% CI 1.35 – 5.27)	OR 1.65 (95% CI 0.70 – 3.78)
	Ulcerative colitis	
	Infliximab-first, adalimumab-second (n = 218)	Adalimumab-first, Infliximab-second (n = 40)
Risk of developing immunogenicity	OR 1.16 (95% CI 0.50 – 2.89)	OR 0.85 (95% CI 0.18 – 3.71)
Risk of developing immunogenic-pharmacokinetic failure (undetectable drug level)	OR 2.26 (95% CI 0.80 – 7.34)	OR 4.14 (95% CI 0.59 – 30.06)

Supplemental Table 6: Adverse Events leading to drug cessation					
	First anti-TNF		**Second anti-TNF**		**Total**
	Adalimumab (n = 12)	**Infliximab** (n = 58)	**Adalimumab** (n = 33)	**Infliximab** (n = 12)	
Adverse event					
Infusion reaction	0% (0/12)	69% (40/58)	0% (0/33)	66.7% (8/12)	41.7% (48/115)
Psoriasiform dermatitis	0% (0/12)	8.6% (5/58)	24.2% (8/33)	8.3% (1/12)	12.2% (14/115)
Rash (not otherwise specified)	25% (3/12)	6.9% (4/58)	15.2% (5/33)	8.3% (1/12)	11.3% (13/115)
Injection site reaction	50% (6/12)	0% (0/58)	18.2% (6/33)	0% (0/12)	10.4% (12/115)
Arthritis	16.7% (2/12)	0% (0/58)	3% (1/33)	8.3% (1/12)	3.5% (4/115)
Viral infection	0% (0/12)	1.7% (1/58)	9.1% (3/33)	0% (0/12)	3.5% (4/115)
Deranged liver function tests	0% (0/12)	3.4% (2/58)	3% (1/33)	0% (0/12)	2.6% (3/115)
Lupus-like syndrome	0% (0/12)	1.7% (1/58)	3% (1/33)	8.3% (1/12)	2.6% (3/115)
Headache	0% (0/12)	1.7% (1/58)	3% (1/33)	0% (0/12)	1.7% (2/115)
Leukocytoclastic vasculitis	0% (0/12)	1.7% (1/58)	3% (1/33)	0% (0/12)	1.7% (2/115)

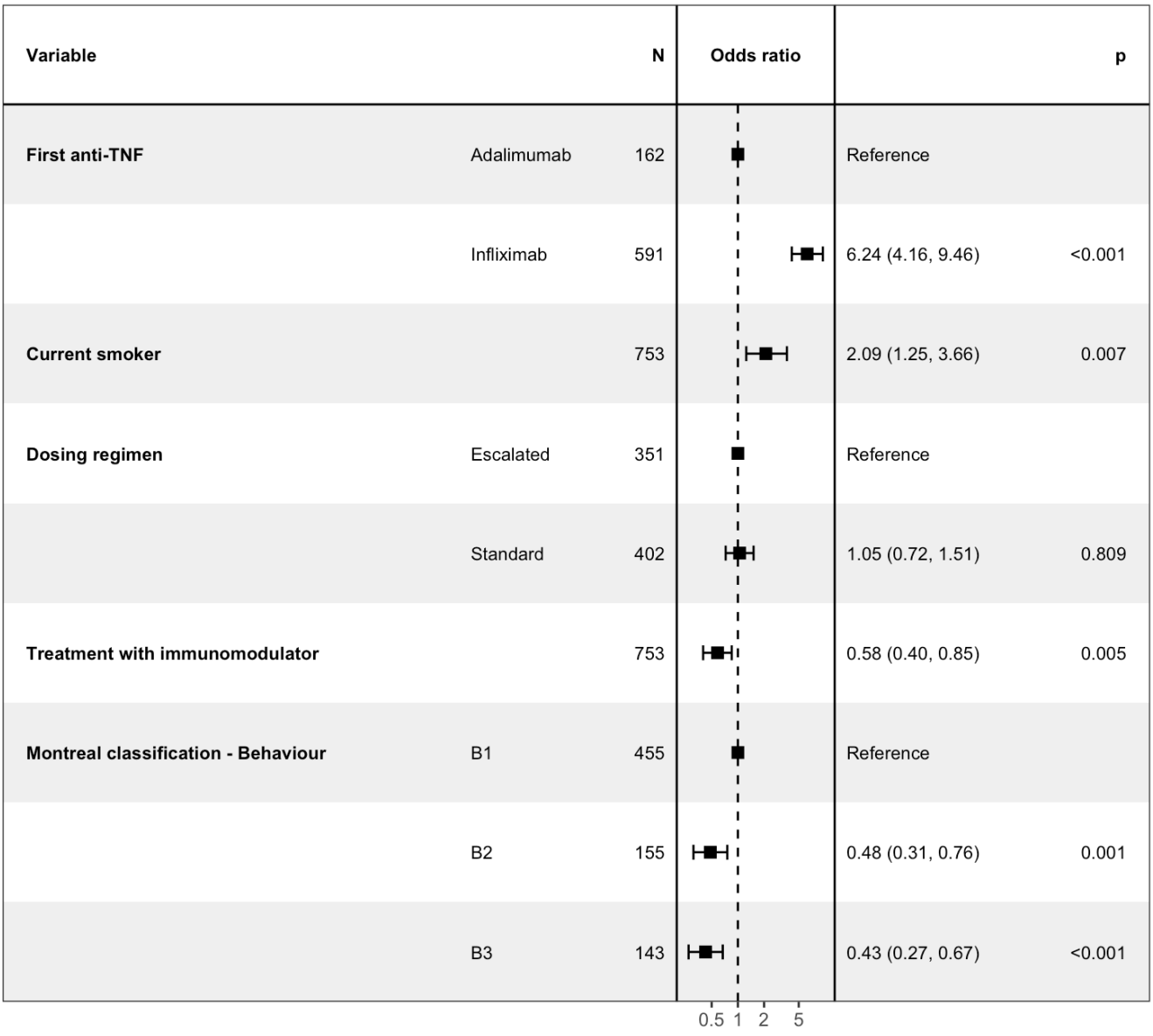
Clinically isolated syndrome	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Deranged renal function	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Interstitial lung disease	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Night sweats	0% (0/12)	1.7% (1/58)	0% (0/33)	0% (0/12)	0.9% (1/115)
Oral granulomatosis	8.3% (1/12)	0% (0/58)	0% (0/33)	0% (0/12)	0.9% (1/115)
Peripheral neuropathy	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Small bowel adenocarcinoma	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Systemic inflammatory response syndrome	0% (0/12)	1.7% (1/58)	0% (0/33)	0% (0/12)	0.9% (1/115)
Tuberculosis (miliary)	0% (0/12)	0% (0/58)	3% (1/33)	0% (0/12)	0.9% (1/115)
Vasculitis	0% (0/12)	1.7% (1/58)	0% (0/33)	0% (0/12)	0.9% (1/115)
Serious					
Yes	0% (0/12)	5.2% (3/58)	24.2% (8/33)	0% (0/12)	9.6% (11/115)
No	100% (12/12)	94.8% (55/58)	75.8% (25/33)	100% (12/12)	90.4% (104/115)

Severity					
Mild	33.3% (4/12)	36.2% (21/58)	36.4% (12/33)	33.3% (4/12)	35.7% (41/115)
Moderate	66.7% (8/12)	63.8% (37/58)	57.6% (19/33)	66.7% (8/12)	62.6% (72/115)
Severe	0% (0/12)	0% (0/58)	6.1% (2/33)	0% (0/12)	1.7% (2/115)
Causality					
Not related	0% (0/12)	0% (0/58)	0% (0/33)	0% (0/12)	0% (0/115)
Unlikely	8.3% (1/12)	0% (0/58)	6.1% (2/33)	8.3% (1/12)	3.5% (4/115)
Possibly	16.7% (2/12)	17.2% (10/58)	33.3% (11/33)	8.3% (1/12)	20.9% (24/115)
Probably	25% (3/12)	37.9% (22/58)	39.4% (13/33)	41.7% (5/12)	37.4% (43/115)
Definitely	50% (6/12)	44.8% (26/58)	21.2% (7/33)	41.7% (5/12)	38.3% (44/115)

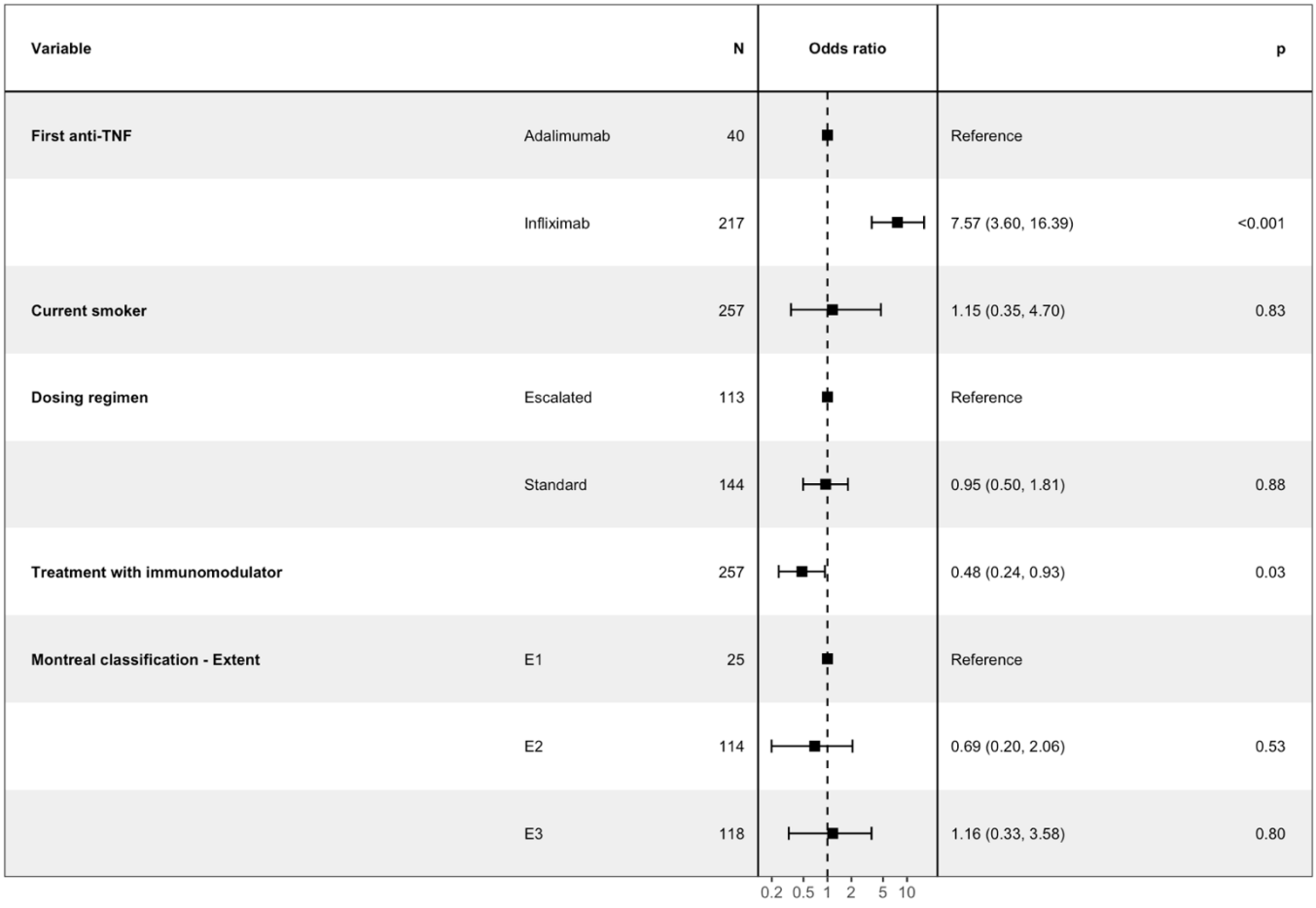
Supplemental Table 7: Therapies following cessation to second anti-TNF

Therapy	Number of patients (n = 1058)
Anti-TNF therapy	16 (1.5)
Infliximab	6 (0.6)
Adalimumab	2 (0.2)
Certolizumab	2 (0.2)
Golimumab	6 (0.6)
Vedolizumab	173 (16.4)
Ustekinumab	198 (18.7)
Tofacitinib	16 (1.5)
5 ASA-monotherapy	5 (0.5)
Thiopurine monotherapy	15 (1.4)
Methotrexate monotherapy	7 (0.7)
Exclusive enteral nutrition	2 (0.2)
Long-term corticosteroids	4 (0.4)
Ciclosporin	0 (0)
Metronidazole	1 (0.1)
Thalidomide and sirolimus	1 (0.1)
Cannabidiol oil	1 (0.1)
Clinical trial	1 (0.1)
Surgery	32 (3)
No treatment	34 (3.2)
Remained on second anti-TNF	552 (52.2)

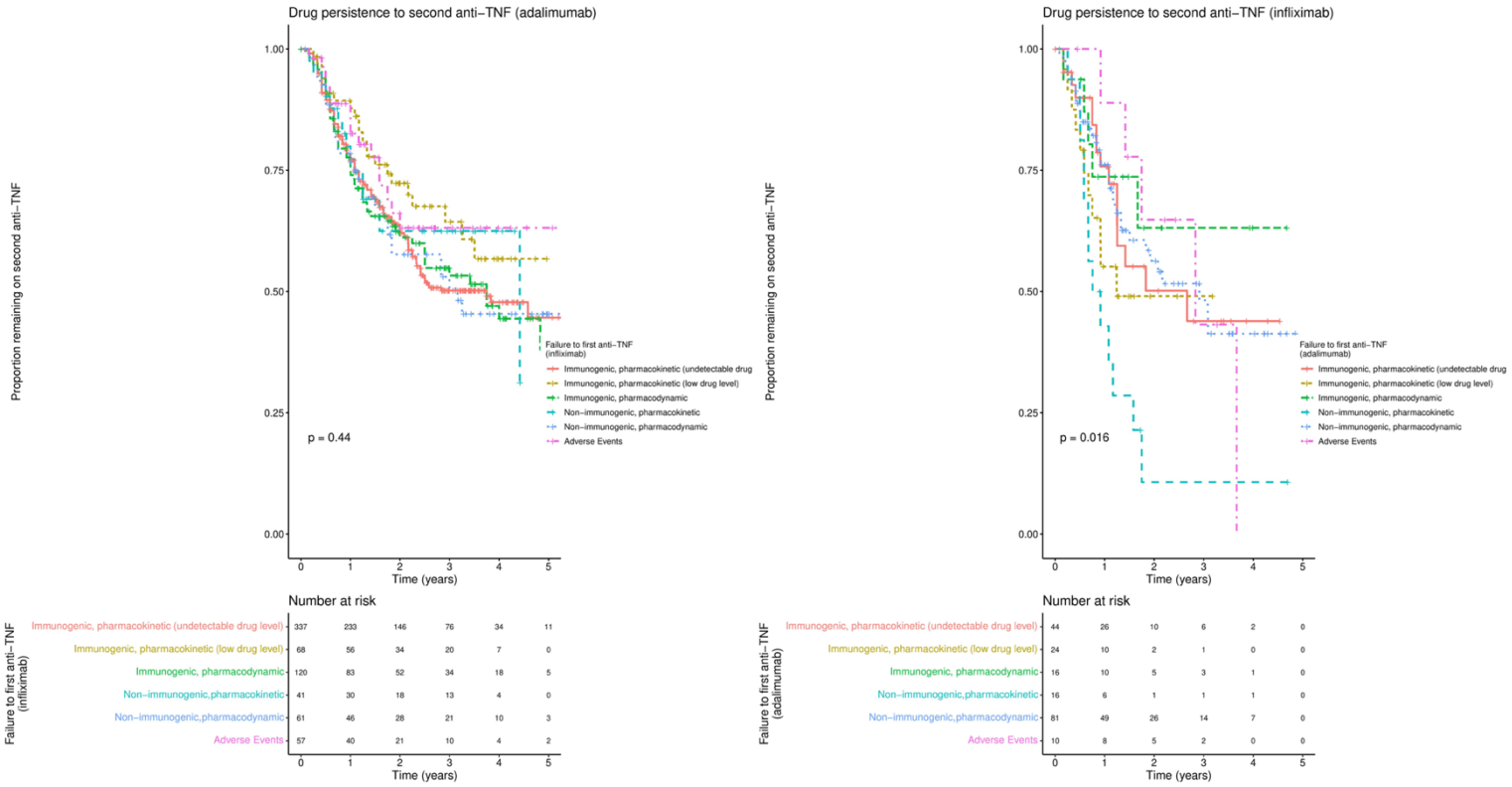
Supplemental Figure 1: Forest plot showing the coefficients from a multivariable logistic regression model of associations with immunogenicity first anti-TNF, stratified by condition (Crohn's disease)



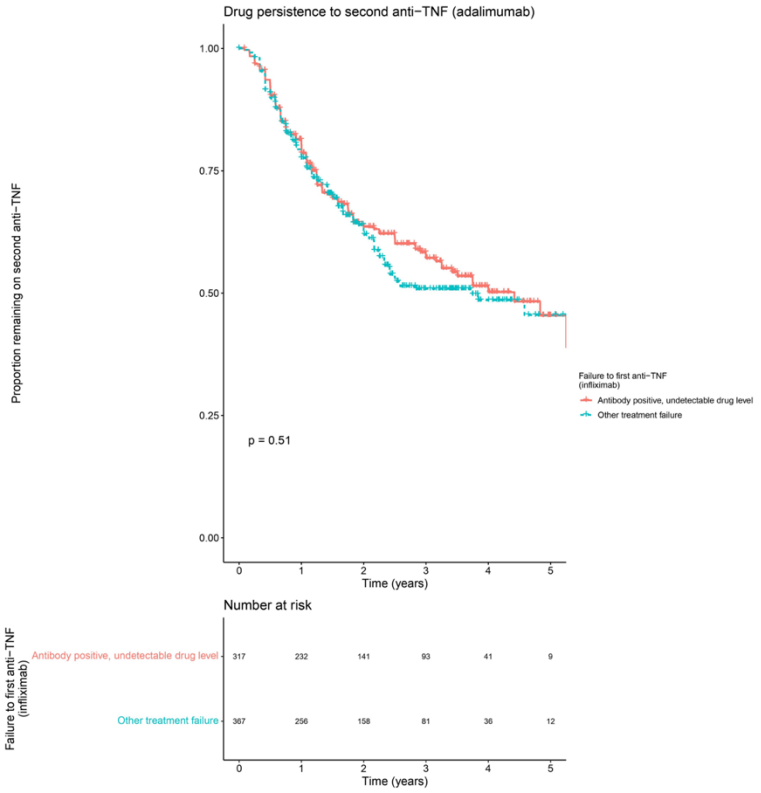
Supplemental Figure 2: Forest plot showing the coefficients from a multivariable logistic regression model of associations with immunogenicity first anti-TNF, stratified by condition (ulcerative colitis)

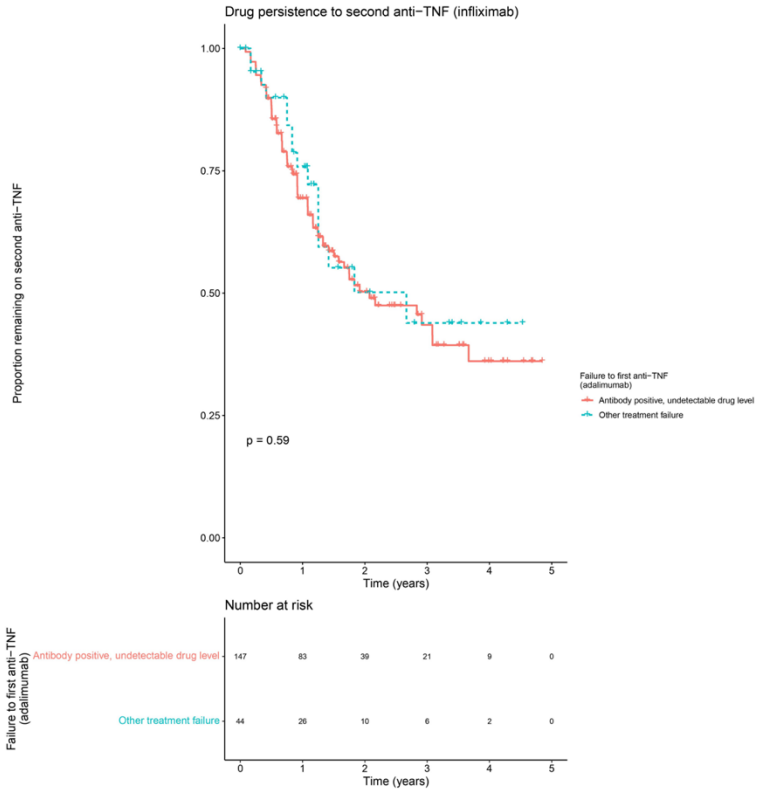


Supplemental Figure 3: Drug persistence to second anti-TNF, including stricter definition of immunogenic, pharmacokinetic failure (antibody present, undetectable drug level), stratified by first anti-TNF and type of failure to first anti-TNF



Supplemental Figure 4: Drug persistence to second anti-TNF, comparing patients who developed immunogenic-pharmacokinetic failure (antibody present, undetectable drug level) to patients who developed treatment failure for other reasons





Chapter 8: Adalimumab and infliximab impair SARS-CoV-2 antibody responses: results from a therapeutic drug monitoring study in 11422 biologic-treated patients

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Abstract

Background and aims

Infliximab attenuates serological responses to SARS-CoV-2 infection. Whether this is a class effect, or if anti-TNF level influences serological responses, remains unknown.

Methods

Seroprevalence and the magnitude of SARS-CoV-2 nucleocapsid antibody responses were measured in surplus serum from 11422 (53.3% (6084) male; median age 36.8 years) patients with immune-mediated inflammatory diseases, stored at six therapeutic drug monitoring laboratories between 29th January and 30th September 2020. Data were linked to nationally-held SARS-CoV-2 PCR results to 4th May 2021.

Results

Rates of PCR confirmed SARS-CoV-2 infection were similar across treatment groups. Seroprevalence rates were lower in infliximab- and adalimumab- than vedolizumab-treated patients (infliximab: 3% (178/5893), adalimumab: 3% (152/5074), vedolizumab: 6.7% (25/375), p = 0.003). The magnitude of SARS-CoV-2 reactivity was similar in infliximab- vs adalimumab-treated patients (median 4.30 cut-off index (COI) (1.94 – 9.96) vs 5.02 (2.18 – 18.70), p = 0.16), but higher in vedolizumab-treated patients (median 21.60 COI (4.39 - 68.10, p = 0.004). Compared to patients with detectable infliximab and adalimumab drug levels, patients with undetectable drug levels (<0.8 mg/L) were more likely to be seropositive for SARS-CoV-2 antibodies. One-third of patients who had PCR testing prior to antibody testing failed to seroconvert, all were anti-TNF treated.

Subsequent positive PCR-confirmed SARS-CoV-2 was seen in 7.9% (12/152) patients after a median time of 184 days (130 – 235), without differences between drugs.

Conclusion

Anti-TNF treatment is associated with lower SARS-CoV-2 nucleocapsid seroprevalence and antibody reactivity when compared to vedolizumab-treated patients. Higher seropositivity rates in patients with undetectable anti-TNF levels supports a causal relationship, although confounding factors, such as combination therapy with immunomodulator, may have influenced the results.

Study aim

To determine whether adalimumab attenuates serological responses, and whether anti-TNF drug level influences serological responses, to SARS-CoV-2 infection.

My role in the study

I was study coordinator and lead, designing the research question, methodology, sample management for SARS-CoV-2 testing (ie – receipt, processing, analysis, and communicating to laboratories). Importantly, I was responsible for obtaining ethical approval for the study, and patient data was provided from public health bodies to the Royal Devon University Healthcare NHS Foundation Trust (Exeter) under Regulation 3 (4) of the Health Service Control of Patient Information (COPI) Regulations 2002 to facilitate a COVID-19 research purpose. I analysed all the data, and authored the manuscript that was published.

Findings

Seroprevalence rates were lower in infliximab- and adalimumab- than vedolizumab-treated patients. The magnitude of SARS-CoV-2 reactivity was similar in infliximab- vs adalimumab-treated patients, but higher in vedolizumab-treated patients. Compared to patients with detectable infliximab and adalimumab drug levels, patients with undetectable drug levels (<0.8 mg/L) were more likely to be seropositive for SARS-CoV-2 antibodies. One-third of patients who had PCR testing prior to antibody testing failed to seroconvert, all were anti-TNF treated. Subsequent positive PCR-confirmed SARS-CoV-2 was seen in 8% patients, without differences between drugs.

Relevance and impact on my learning

Generating a clinically important research question and designing a robust study during the COVID-19 pandemic was a unique challenge. Navigating the legal and ethical framework that allows one to carry out topical, pandemic-related research in the UK quickly was a new experience for me as an early-career researcher. Generating comprehensive applications to public health bodies to acquire and maintain data was another new skill that I learnt.

I coordinated sample management from multiple laboratories, which required liaising with local principle investigators and scientists. This allowed me to gain a more in-depth understanding in to how to different laboratories process serum samples for therapeutic drug monitoring across the UK, and how laboratory differ across the country. Conducting this experiment sent precedent for how different laboratories might work together in the future to generate large datasets on patients with IBD, mapped to TDM results, and potentially clinical outcomes.

Furthermore, as lead author of this study which took place early during my PhD, I demonstrated practical skills of manuscript writing, editing, submission, and how to respond to editorial and peer review comments' appropriately. This has been hugely advantageous to my academic career, and I will continue to grow these skills as I develop my research career.

Acknowledgements of co-authors and contributions to paper

Myself, Simeng Lin, Claire Bewshea, Shaji Sebastian, Nick Powell, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad participated in the conception and design of this study. Claire Bewshea was the project manager. Rachel Nice and Timothy J McDonald coordinated all biochemical analyses and central laboratory aspects of the project. Sample collection and coordination was done by Rachel Nice, Zehra Arkir, Claire Bewshea, Bessie Cipriano, Allan Dunlop, Louise Greathead, Rachel L Griffiths, Peter Kelleher, Klaartje B Kok, Jonathan MacDonald, Timothy J McDonald, and Peter M Irving. Myself, Simeng Lin, Desmond Chee, Rachel Nice, Hajir Ibraheim, Philip J Smith, Peter M Irving, Nick Powell, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad were involved in the acquisition, analysis, or interpretation of data. Data analysis was done by myself, Simeng Lin, and Nicholas A Kennedy. Drafting of the manuscript was done by myself, Simeng Lin, Lauranne AAP Derikx, Nicholas A Kennedy, James R Goodhand, and Tariq Ahmad. Tariq Ahmad obtained the funding for the study. All the authors contributed to the critical review and final approval of the manuscript. Myself, Simeng Lin, Nicholas A Kennedy, and Tariq Ahmad have verified the underlying data.

Introduction

The increased transmissibility of the dominant delta variant of SARS-CoV-2 means that >80% of the UK population will need to be fully vaccinated to achieve herd immunity[1]. Anti-TNF drugs impair protective immunity following pneumococcal[2], influenza[3,4], and viral hepatitis[5] vaccinations and increase the risk of serious respiratory infections[6]. By suppressing immune responses, biologic and immunosuppression therapies increase the reservoir for viral transmission and have been implicated in the evolution and emergence of novel variants of SARS-CoV-2[7].

We have recently reported that seroprevalence, seroconversion rates, and the magnitude of SARS-CoV-2 nucleocapsid (N) antibodies following SARS-CoV-2 infection are reduced in patients with inflammatory bowel disease (IBD) treated with infliximab compared to vedolizumab[8]. Vedolizumab is a gut-selective anti-integrin α4β7 monoclonal antibody and, unlike anti-TNF therapy, is not associated with increased susceptibility to systemic infection or attenuated serological responses to SARS-CoV-2 vaccination[9]. Because we observed similar rates of SARS-CoV-2 infection and hospitalisations between infliximab- and vedolizumab-treated patients, our findings suggest that infliximab directly influences the serological response to SARS-CoV-2 infection. In the same cohort of IBD patients, SARS-CoV-2 spike (S) antibody levels and rates of seroconversion were also lower after a single-dose of either the BNT162b2 (Pfizer) or ChAdOx1 nCoV-19 (AstraZeneca/Oxford) vaccines in patients treated with infliximab than

vedolizumab[10].

Whether antibody responses following SARS-CoV-2 infection are also impaired in patients treated with other biopharmaceuticals, including other anti-TNF therapies such as adalimumab, and if biologic drug levels influence the magnitude of SARS-CoV-2 (N) antibody responses, remain unknown.

Objectives

In patients with immune-mediated inflammatory diseases (IMIDs), we aimed to define whether biologic class impacted the:

i) seroprevalence of SARS-CoV-2 antibodies

ii) magnitude of SARS-CoV-2 antibodies, stratified by biologic drug levels

iii) seroconversion and subsequent positive PCR-confirmed SARS-CoV-2

Methods

Study design and population

CLARITY IBD is a UK wide, multicentre, observational cohort study investigating the impact of biologics and/or concomitant immunomodulators on SARS-CoV-2 acquisition, illness, and immunity in patients with IBD (www.clarityibd.org).

Here, we report data from a retrospective cohort of patients with IMIDs who had serum stored following routine therapeutic drug monitoring (TDM) tests as part of clinical care during the early phase of the COVID-19 pandemic. Surplus serum samples were obtained from six UK laboratories (Barts Health NHS Trust, NHS Greater Glasgow and Clyde, Guy's and St Thomas' NHS Foundation Trust, North West London Pathology NHS Trust, Royal Devon and Exeter NHS Foundation Trust, and Royal Wolverhampton NHS Trust) who offer TDM for infliximab, adalimumab, ustekinumab, or vedolizumab. Samples archived between 29th January 2020, shortly after the first case of COVID-19 was reported in the UK[11], to 30th September 2020, were included.

Surplus samples were transferred to the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust and serum was tested for SARS-CoV-2 nucleocapsid (N) antibodies. Samples with adequate linked clinical data, of more than 150 microliters, the minimum volume required to undertake the assay, and not contaminated by haemolysis, were processed.

Outcomes

The primary outcome was the proportion of patients with a positive SARS-CoV-2 (N) antibody test. Secondary outcomes were the impact of biologic drug levels on

seropositivity and the magnitude of SARS-CoV-2 antibodies, and seroconversion and rates of subsequent positive PCR-confirmed SARS-CoV-2.

Variables and case definition

We recorded the patient's national patient identifier (National Health Service (NHS) number or Community Health Index (CHI)), sex, date of birth, postcode, date of serum sample, and referring hospital. Where missing, these data were obtained from the NHS Digital Data Access Request Service. The following variables, where available from the TDM requisition form, were also recorded: diagnosis (IBD (Crohn's disease, ulcerative colitis, or IBD-unclassified), non-IBD (ankylosing spondylitis, Bechet's disease, hidradenitis suppurativa, juvenile idiopathic arthritis, malignancy, psoriatic arthritis, psoriasis, rheumatoid arthritis, sarcoidosis, or systemic lupus erythematosus), treatment, and results from TDM (biologic drug and anti-drug antibody testing) performed at the referring site.

We linked our data by NHS number or CHI to data held by Public Health England, Scotland, and Wales, who archive dates and results of SARS-CoV-2 PCR tests undertaken. Confirmed cases were patients with a positive PCR test to SARS-CoV-2. Due to differences in nationally held public health databases, we received: all negative and positive PCR test results from Public Health Wales (23rd March 2020 to 4th May 2021) and Public Health Scotland (14th March 2020 to 11th July 2021), and all negative PCR test results up to and including the first positive PCR test result from Public Health England (26th February 2020 to 18th April 2021).

Laboratory methods

We used the Roche Elecsys Anti-SARS-CoV-2 nucleocapsid (N) immunoassay to detect antibodies to SARS-CoV-2. This sandwich electrochemiluminescence immunoassay uses a recombinant protein of the nucleocapsid antigen for determination of antibodies against SARS-CoV-2[12]. The electrochemiluminescence signal from a negative and positive calibrator are assigned a value of 0.8 and 1.2, respectively, and a cut-off index (COI) is set at a signal equivalent to 1. The manufacturer reports clinical sensitivity of 99.5% (97 - 100) $\geq$ 14 days post PCR confirmation and specificity of 99.8% (95% CI 99.7 - 99.9)[12].

In-house assay validation experiments demonstrated the intra- and inter-assay coefficient of variation were 2.2 and 7%, respectively. No effect was observed on recovery of SARS-CoV-2 antibodies following four freeze/thaw cycles. SARS-CoV-2 antibodies were stable in uncentrifuged blood and serum at ambient temperature for up to seven days permitting postal transport from research sites to the central laboratory. No analytical interference was observed for the detection of SARS-CoV-2 antibodies with infliximab, adalimumab, or vedolizumab up to 10000 mg/L, 8000 g/L, and 60000 mg/L, respectively, or with anti-drug antibodies to infliximab, adalimumab, or vedolizumab up to 400 AU/mL, 200 AU/mL, and 38 AU/mL respectively. For anti-TNF-treated patients, absence of drug was defined using a cut-off of <0.8 mg/L[13]. For vedolizumab-treated patients, absence of drug was defined using a cut-off of <3.1 mg/L. Anti-drug antibody levels, recorded as positive or negative, were supplied by referring laboratory.

Statistical analysis

A priori sample size calculations were not undertaken for this study, rather we collected all available samples saved through the early phase of the pandemic.

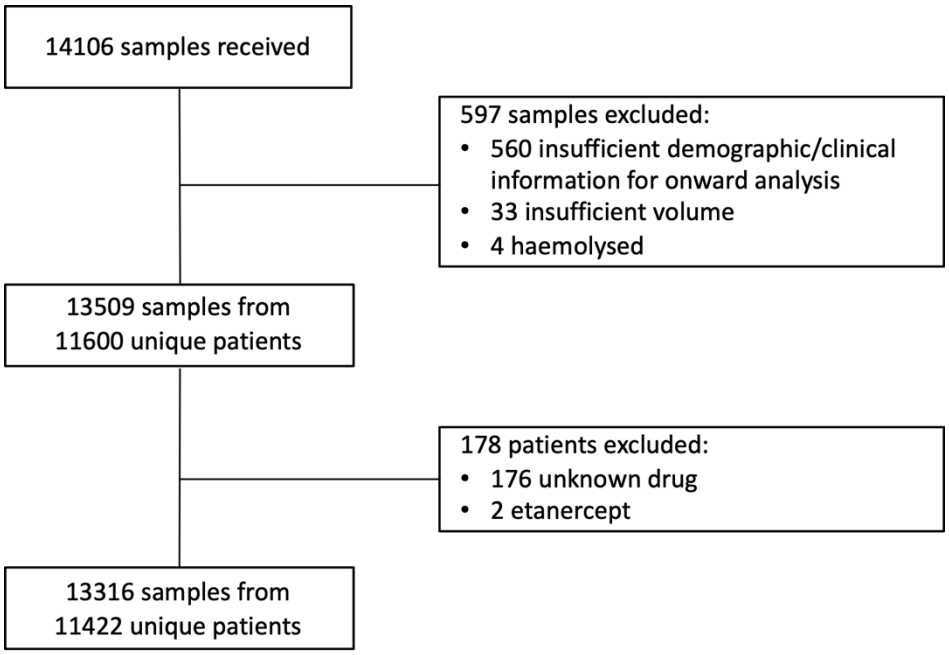
Statistical analyses were undertaken in R 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two tailed and p-values <0.05 were considered significant. We included patients with missing clinical data in analyses for which they had data and have specified the denominator for each variable. Continuous data were reported as median and interquartile range, and discrete data as numbers and percentages, unless otherwise stated. We used patients' postcodes to assign them to one of ten UK administrative regions and present seroprevalence rates mapped to these regions. We also used postcodes to derive patients' income and employment deprivation scores using combined English and Welsh data from 2019[14] and Scottish data from 2020[15].

Seroprevalence of SARS-CoV-2 antibodies was estimated as the proportion of samples with a positive SARS-CoV-2 antibody result. Univariable analyses, using Fisher's exact and Mann-Whitney U tests, were used to identify demographic and treatment related factors, including TDM, associated with SARS-CoV-2 seropositivity. We explored the magnitude of antibody reactivity using density plots, stratified by drug exposure among patients with a positive SARS-CoV-2 antibody result. We performed a sensitivity analysis restricting the cohort to patients treated with an anti-TNF who were known to have IBD, and all vedolizumab-treated patients, which is only licensed in the UK for treatment of IBD.

Results

14106 surplus samples were received; 4.2% samples (597/14106) were excluded because of insufficient demographic or clinical information, insufficient volume, or haemolysis, leaving 13509 samples from 11600 patients to be analysed. Of these, 1.5% (178/11600) patients did not have adequate treatment details (n = 176) or were treated with etanercept (n = 2), and therefore excluded. 13316 samples from 11422 unique patients were included in the final analysis (Figure 1, Supplementary Figure 1 and 2).

Figure 1: Study profile



Patient characteristics

Overall, 53.3% (6084/11422) patients were male with a median age of 36.8 years (IQR 25.5 – 51.5). The median income deprivation score was 0.10 (IQR 0.06 – 0.17). Diagnosis was not recorded in 79.3% (9061/11422) patients; 19.5%

(2231/11422) patients had IBD and 1.1% (130/11422) had a non-IBD diagnosis. 51.6% (5893/11422) patients were treated with infliximab, 44.4% (5074/11422) adalimumab, 3.3% (375/11422) vedolizumab, and 0.7% (80/11422) ustekinumab. Baseline characteristics stratified by biologic drug are shown in Table 1.

60.2% (6875/11422) patients had undergone PCR testing across England, Scotland, and Wales, of whom 11.2% (770/6875) had a positive PCR test. No differences were observed in the proportion of patients who tested positive for SARS-CoV-2 (infliximab: 11.2% (402/3600), adalimumab: 11.4% (342/2990), ustekinumab: 4.2% (2/48), vedolizumab: 10.1% (24/237), p = 0.467).

Table 1: Baseline characteristics of patients, stratified by biologic therapy.

IBD=inflammatory bowel disease.

Variable		**Adalimumab**	**Infliximab**	**Ustekinumab**	**Vedolizumab**	**Total**
Sex	Female	49.7% (2520/5074)	44.2% (2607/5893)	48.8% (39/80)	42.1% (158/375)	46.6% (5324/11422)
	Male	50.2% (2549/5074)	55.7% (3282/5893)	51.3% (41/80)	56.5% (212/375)	53.3% (6084/11422)
	Unknown	0.1% (5/5074)	0.1% (4/5893)	0% (0/80)	1.3% (5/375)	0.1% (14/11422)
Diagnosis	IBD	18.2% (923/5074)	19.6% (1153/5893)	56.3% (45/80)	29.3% (110/375)	19.5% (2231/11422)
	Non-IBD	2% (101/5074)	0.5% (29/5893)	0% (0/80)	0% (0/375)	1.1% (130/11422)
	Unknown	79.8% (4050/5073)	79.9% (4711/5893)	43.8% (35/80)	70.7% (265/375)	79.3% (9061/11422)
Age (years)		39 (27 - 52)	35 (23 - 50)	37 (25 - 58)	40 (30 - 58)	37 (26 - 51)
Income deprivation score		0.1 (0.1 - 0.2)	0.1 (0.1 - 0.2)	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.2)	0.1 (0.1 - 0.2)
UK region	East Midlands	8.6% (422/4921)	6.5% (373/5732)	0% (0/79)	0.8% (3/366)	7.2% (798/11098)
	East of England	12.2% (599/4921)	10.6% (609/5732)	10.1% (8/79)	6.8% (25/366)	11.2% (1241/11098)

	London	12.5% (614/4921)	18.6% (1063/5732)	5.1% (4/79)	36.9% (135/366)	16.4% (1816/11098)
	North East	2.9% (142/4921)	1.9% (109/5732)	1.3% (1/79)	0% (0/366)	2.8% (252/11098)
	North West	9.3% (459/4921)	8.3% (476/5732)	0.% (0/79)	2.7% (10/366)	8.5% (945/11098)
	Scotland	26.2% (1289/4921)	19.2% (1102/5732)	0% (0/79)	0% (0/366)	21.5% (2391/11098)
	South East	9.3% (457/4921)	12.1% (692/5732)	10.1% (8/79)	6.8% (25/366)	10.7% (1182/11098)
	South West	7.8% (385/4921)	9.2% (527/5732)	53.2% (42/79)	16.9% (62/366)	9.2% (1016/11098)
	Wales	2% (99/4921)	2.2% (124/5732)	0% (0/79)	0.8% (3/366)	2.1% (226/11098)
	West Midlands	6.3% (312/4921)	6.1% (348/5732)	0% (0/79)	16.7% (61/366)	6.5% (721/11098)
	Yorkshire and the Humber	2.9% (143/4921)	5.4% (309/5732)	20.3% (16/79)	11.5% (42/366)	4.6% (510/11098)

SARS-CoV-2 seroprevalence

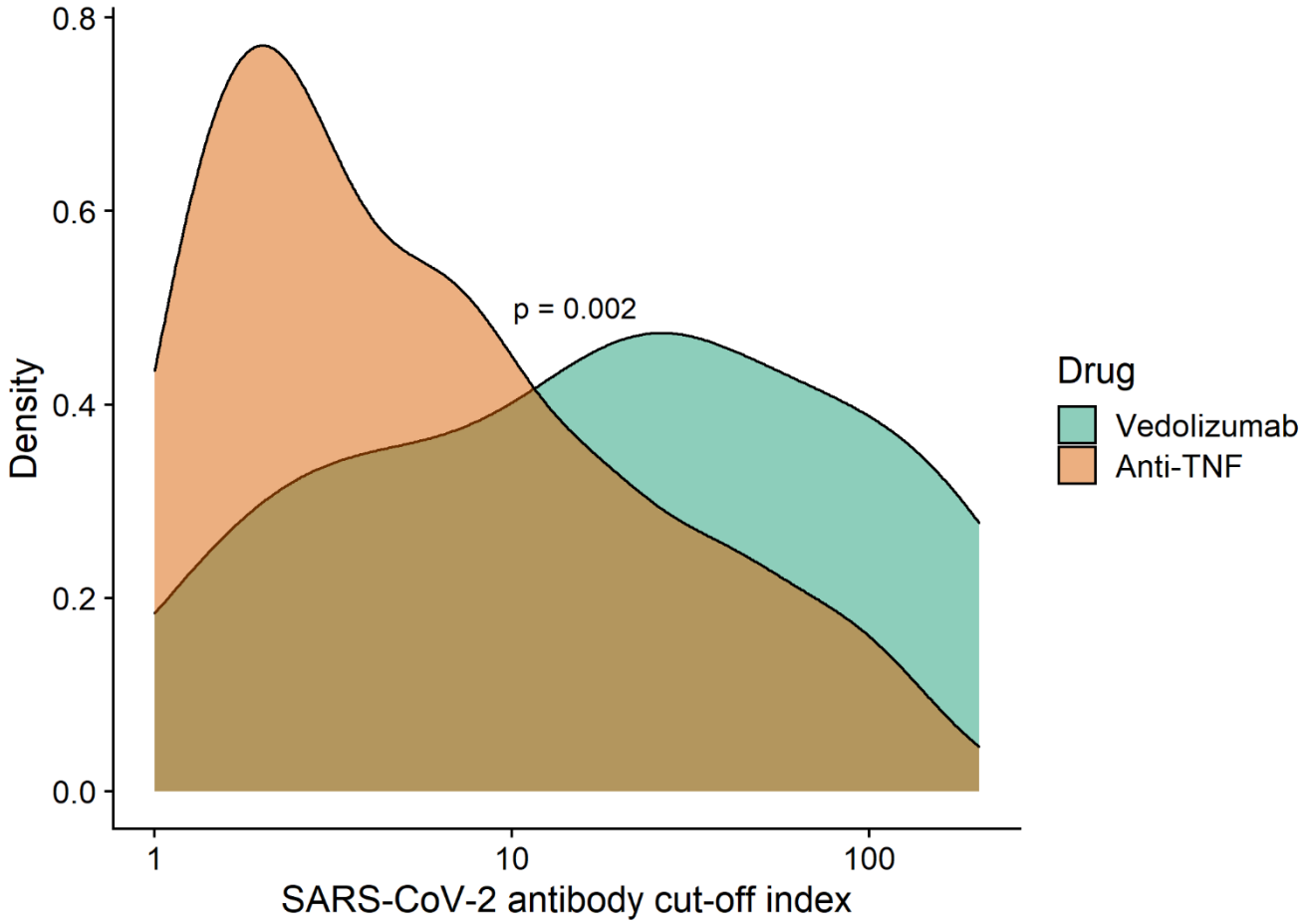
Seropositivity to SARS-CoV-2 was first observed on 3rd February 2020 and seroprevalence increased to 3.1% by 30th September 2020 (Supplementary Figure 3). Univariable analyses demonstrated that the proportion of patients with a positive SARS-CoV-2 antibody test was lower in anti-TNF- and ustekinumab-patients than vedolizumab- treated patients (infliximab: 3% (178/5893), adalimumab: 3% (152/5074), ustekinumab: 1.3% (1/80), vedolizumab: 6.7% (25/375), p = 0.003) (Table 2). The magnitude of SARS-CoV-2 reactivity was similar in infliximab- vs adalimumab-treated patients (median 4.30 COI (1.94 – 9.96) vs 5.02 (2.18 – 18.70), p = 0.16), and for both drugs, lower than the vedolizumab-treated group (median 21.60 COI (4.39 - 68.10), p = 0.004) (Figure 2A, Figure 3). Seropositivity was also associated with UK region and calendar month (Table 2, Supplementary Figure 4).

Table 2: Seroprevalence of SARS-CoV-2 antibodies, stratified by baseline characteristics. *IBD=inflammatory bowel disease.*

Variable	Seroprevalence	P value
Biologic therapy		
Adalimumab	3% (152/5074)	0.003
Infliximab	3% (178/5893)	
Ustekinumab	1.3% (1/80)	
Vedolizumab	6.7% (25/375)	
Sex		
Female	3% (158/5324)	0.27
Male	3.2% (197/6084)	
Unknown	7.1% (1/14)	
Diagnosis		
IBD	2.4% (53/2231)	0.07
Non-IBD	3.1% (4/130)	
Unknown	3.3% (299/9061)	
Region		
East Midlands	1.9% (15/798)	<0.001
East of England	2.7% (34/1241)	
London	7.9% (144/1816)	
North East	2.4% (6/252)	
North West	3.3% (31/945)	
Scotland	1.4% (34/2391)	
South East	1.9% (22/1182)	

South West	1.5% (15/1016)	
Wales	2.2% (5/226)	
West Midlands	4.7% (34/721)	
Yorkshire and the Humber	2.2% (11/510)	
Income score	0.1 (0.06 - 0.19)	0.05
Age >70	1.8% (10/555)	0.08
Calendar month sample tested		
January	0% (0/51)	<0.001
February	0.6% (2/330)	
March	0.2% (1/491)	
April	3.5% (20/566)	
May	3.7% (38/1015)	
June	4.6% (87/1893)	
July	3.2% (82/2570)	
August	2.2% (49/2225)	
September	3.4% (77/2282)	

Figure 3: Density plot of the magnitude of SARS-CoV-2 antibody reactivity, stratified by biologic therapy



Sensitivity analysis

The diagnosis of IBD was recorded in 19.6% (1153/5893) and 18.2% (923/5074) of infliximab- and adalimumab-treated patients, respectively. Univariable analyses demonstrated that the proportion of patients with a positive SARS-CoV-2 antibody test was lower in anti-TNF- than vedolizumab- treated patients (infliximab: 2.3% (27/1153); adalimumab: 2.3% (21/923); vedolizumab: 6.7% (25/375), p <0.001). The magnitude of SARS-CoV-2 reactivity was similar in infliximab- vs adalimumab-treated patients (median 2.66 COI (1.65 – 7.29) vs 4.38 (2.31 – 16.20), p = 0.13),

and for both drugs, lower than the vedolizumab-treated group (median 21.60 COI (4.39 - 68.10), p = 0.004).

Impact of biologic drug level on seropositivity and magnitude of SARS-CoV-2 antibodies

Of 11422 patients in the study, 95.6% (5636/5893) infliximab-, 97% (4923/5074) adalimumab-, and 89.1% (334/375) vedolizumab-treated patients had biologic drug level data available for analysis. Overall, 12.1% (681/5636) of infliximab- and 7% (347/4923) of adalimumab-treated patients had undetectable drug level, of which 54.8% (373/681) and 39.2% (136/347) had detectable anti-infliximab and anti-adalimumab antibodies, respectively. 10.8% (36/334) of vedolizumab-treated patients had undetectable drug level.

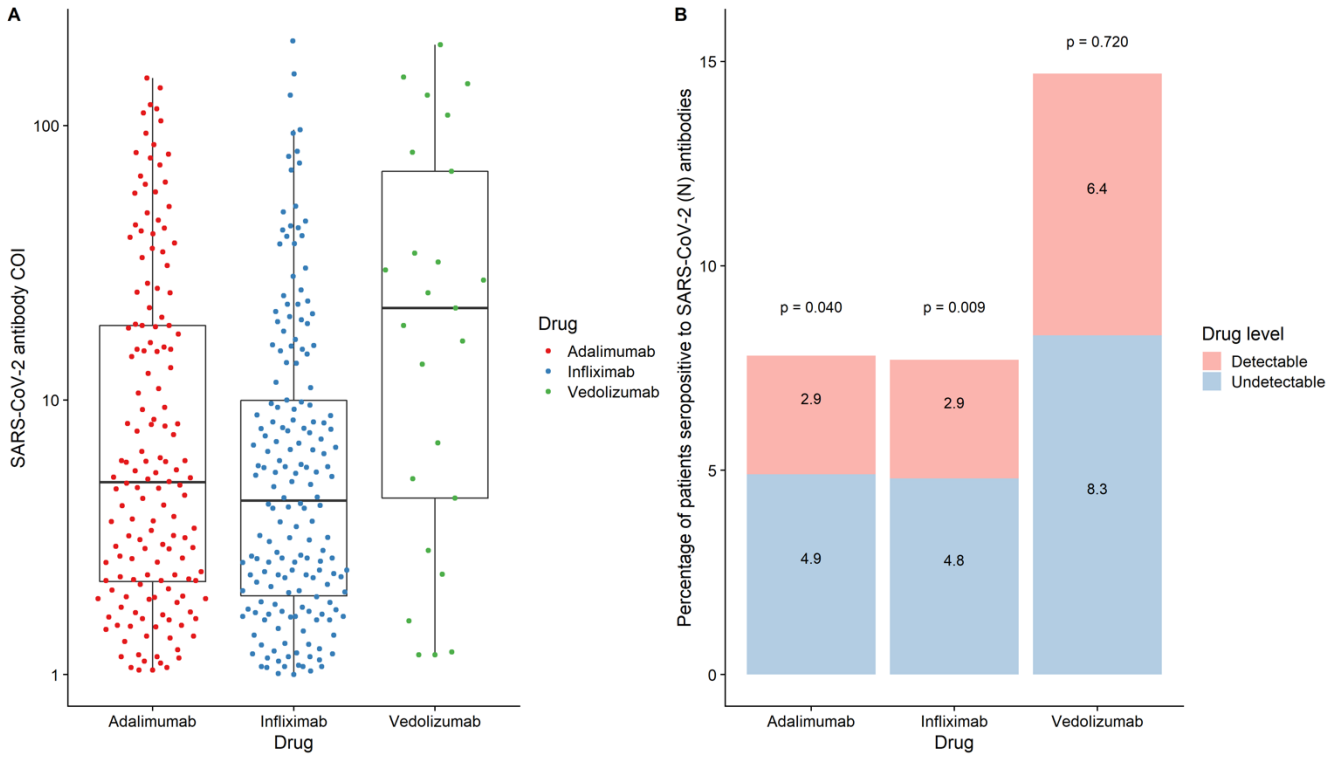
Compared to patients with detectable infliximab drug levels, patients with undetectable drug levels (<0.8 mg/L) were more likely to be seropositive for SARS-CoV-2 antibodies (OR 1.73, 95%CI 1.13 – 2.56, p = 0.01) (Figure 2B) and had a higher magnitude of SARS-CoV-2 antibodies (median COI 7.72 (3.05 – 41.6) vs 3.54 (1.77 – 8.70), p = 0.002). Sensitivity analyses showed that the effect size was greater when only patients with undetectable drug and an anti-infliximab antibody were included (OR 2.02, 95%CI 1.20 – 3.26, p = 0.007; median COI 9.26 (5.47 – 44.80), p = 0.001).

Similarly, compared to patients with detectable adalimumab drug levels ($\geq$ 0.8 mg/L), patients with undetectable drug levels were more likely to be seropositive for SARS-CoV-2 antibodies (OR 1.72, 95%CI 0.96 – 2.90, p = 0.05) (Figure 2B), but

there was no difference in the magnitude of SARS-CoV-2 antibodies (median COI 8.49 (3.21 – 25.5) vs 4.80 (1.93 – 18.5), p = 0.11).

There was no association between vedolizumab drug levels and seropositivity or magnitude of SARS-CoV-2 antibodies (Figure 2B). Compared to vedolizumab-treated patients, infliximab- and adalimumab- treated patients with undetectable drug levels had similar seropositivity rates (infliximab: 4.9% (33/681), adalimumab: 4.9% (17/347), vedolizumab: 6.7% (25/375), p = 0.43) and magnitude of SARS-CoV-2 titres (infliximab: median COI 7.72 (3.05 – 41.6), adalimumab: median COI 8.49 (3.21 – 25.5), vedolizumab: median COI 21.6 (4.39 – 68.1), p = 0.38).

Figure 2: (A) Boxplot of the magnitude of SARS-CoV-2 antibody reactivity, stratified by biologic therapy (B) Percentage of patients with seropositivity, defined by a SARS-CoV-2 nucleocapsid antibody concentration ≥10 U/mL, stratified by biologic drug and drug level. P value above each bar represents within-drug comparison between patients with detectable or undetectable drug level and SARS-CoV-2 antibodies.



Seroconversion and subsequent positive PCR-confirmed SARS-CoV-2

Overall, 1.6% (23/1428) patients had a positive PCR test prior to collection of the sample used for SARS-CoV-2 antibody testing. Of those with a positive PCR test, all of whom were treated with an anti-TNF drug, 65% (15/23) patients seroconverted. There was no difference in seroconversion, stratified by time from PCR testing to SARS-CoV-2 (N) antibody testing (positive antibody: 100 days (76 –

146) vs negative antibody: median 64 days (28 – 129), p = 0.42). Moreover, there was no correlation between time to SARS-CoV-2 (N) antibody test and magnitude of SARS-CoV-2 antibodies (Spearman's rho R = 0.03, p = 0.88).

Subsequent positive PCR-confirmed SARS-CoV-2 was seen in 7.9% (12/152) patients. The median magnitude of SARS-CoV-2 antibody reactivity prior to a positive PCR test was 1.74 COI (1.14 – 15.48), with a median time from positive SARS-CoV-2 antibody to positive PCR test of 184 days (130 – 235). There was no association between biologic class (anti-TNF 7.4% (10/135) vs vedolizumab 11.8% (2/17), p = 0.35), or magnitude of SARS-CoV-2 antibody reactivity (p = 0.13), and a subsequent positive PCR test.

Discussion

We have shown that patients with IMIDs treated with infliximab and adalimumab have attenuated serological responses to SARS-CoV-2 infection with lower seroprevalence and antibody reactivity when compared to vedolizumab-treated patients. Amongst patients treated with adalimumab and infliximab, seropositivity rates were highest in patients with undetectable drug levels and were similar to those observed in patients treated with vedolizumab. One-third of our cohort who had PCR-confirmed SARS-CoV-2 infection, all of whom were treated with anti-TNF therapy, subsequently did not develop SARS-CoV-2 antibodies. Subsequent positive PCR-confirmed SARS-CoV-2 was observed in 8% patients.

Like infliximab[8], adalimumab impairs antibody responses following SARS-CoV-2 infection, and we observed that higher SARS-CoV-2 antibody levels were associated with undetectable infliximab and adalimumab drug levels. This is biologically plausible since anti-TNF drugs directly impede the immune mechanisms responsible for generating antibody responses including maturation of antigen presenting cells and co-stimulation of antigen-specific T-cells.[16–18] TNF neutralization, or genetic ablation, results in reduced B-cells in primary follicles in germinal centres and the periphery, and B-cell immunoglobulin synthesis[16]. In keeping with this hypothesis, in infliximab-treated patients the highest SARS-CoV-2 antibody concentrations were seen in patients with undetectable drug levels in the presence of anti-infliximab antibodies where drug is absent[19,20]. It is possible that this cohort of patients were less likely to be treated with an immunomodulator, which we have previously shown is independently associated with SARS-CoV-2 seroconversion in infliximab-treated patients with IBD.[21] An alternative explanation

for our results is that anti-TNF agents in IMIDs prevent severe COVID-19 infection and consequently immune responses.[22] Against this postulate, we previously observed no difference in rates of hospitalisation for confirmed COVID-19 amongst infliximab- compared to vedolizumab-treated patients with IBD, and that vaccine responses were similarly impaired in anti-TNF treated patients.[8,10]

Even after PCR-confirmed infection, one-third of patients who were subsequently tested for SARS-CoV-2 antibodies, and all of whom were treated with either adalimumab or infliximab, failed to mount an antibody response. Whilst this might be explained by antibody decay in the period between the positive PCR test and SARS-CoV-2 antibody test, we reported similar findings in our prospective cohort of patients with IBD, where 52% (42/81) infliximab-treated patients did not mount an antibody response following PCR-confirmed infection[8]. Whether a failure to seroconvert after infection predisposes people to recurrent SARS-CoV-2 infection cannot be determined in this cohort because of a paucity of PCR testing in the early phase of the pandemic. However, following a positive SARS-CoV-2 antibody test, over 7% patients subsequently had PCR-confirmed SARS-CoV-2. We acknowledge that none of these 12 patients had a positive PCR test prior to their initial SARS-CoV-2 antibody test and it is therefore possible that these patients may have had false positive antibody tests. An alternative explanation is that these patients may have failed to clear a primary SARS-CoV-2 infection or had a second infection.

The main strength of this study was analysis of SARS-CoV-2 antibodies on more than 13,000 samples from 11422 unique patients with IMIDs treated with biologic

therapy during the early phase of the pandemic. Other strengths include correlation with comprehensive biologic drug level data, and linkage with SARS-CoV-2 public health testing data. We acknowledge, however, the following limitations. Firstly, because this was an analysis of surplus serum, clinical details were infrequently entered on requisition forms. We did not, therefore, have access to comprehensive clinical data for study subjects including comorbidities, ethnicity, diagnosis, symptoms of suspected COVID-19, and indications for, and duration of, biologic and concomitant therapies. Secondly, as serum samples for this study were collected early in the pandemic a limited number of subjects had PCR-confirmed infection. Thirdly, our ability to interpret SARS-CoV-2 antibody durability and risk of re-infection was limited by the duration of follow-up, frequency of sampling, and the availability of the first positive PCR test results conducted in England. Finally, as this study involved surplus serum samples used for TDM, limited data were available for patients treated with therapies for which TDM is not widely used, including ustekinumab.

From a public health perspective, attention has turned from natural infection to vaccine effectiveness in the face of novel SARS-CoV-2 variants. Several groups have shown that most patients with IBD can mount an effective immune response in the short-term following both licensed doses of SARS-CoV-2 vaccine [23–28]. Urgent research is needed to understand the factors linked to vaccine non-response. For patients who need to start anti-TNF therapy, they and their families should receive SARS-CoV-2 vaccines without a delay between vaccine doses, wherever possible before anti-TNF therapies are started. Whether timing booster doses towards the end of an anti-TNF treatment cycle when drug levels are

lowest[29], and/or the temporary discontinuation of immunomodulators[30], potentiate long-term immunogenicity warrants further study. So too does the use of higher-dose vaccines[3], adjuvants including the influenza vaccines (ComFluCOV)[31] and/or switching between vaccines with different mechanisms of action[32].

Conclusions

Patients with IMIDs treated with infliximab and adalimumab have attenuated serological responses to SARS-CoV-2 when compared to vedolizumab-treated patients. Seropositivity rates were highest in patients with undetectable drug levels and were similar to those observed in patients treated with vedolizumab, supporting a causal relationship between anti-TNF use and attenuated antibody responses to infection, although confounding factors, such as combination therapy with immunomodulator, may have influenced the results.

Role of the funding source

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Data were provided to the Royal Devon and Exeter NHS Foundation trust under Regulation 3 (4) of the Health Service Control of Patient Information (COPI) Regulations 2002 to facilitate a COVID-19 research purpose. The Surrey Borders Research Ethics committee approved the study (REC reference: REC 20/HRA/3114) in September 2020. The sponsor was the Royal Devon and Exeter NHS Foundation Trust. The protocol is available online at https//www.clarityibd.org. The study was registered with the ISRCTN registry, ISRCTN45176516.

Data sharing

The study protocol including the statistical analysis plan is available at www.clarityibd.org. Individual participant de-identified data that underlie the results reported in this article will be available immediately after publication for a period of 5 years. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be

directed to tariq.ahmad1@nhs.net; to gain access data requestors will need to sign a data access agreement.

Declarations of interest

Dr. Lin reports non-financial support from Pfizer, non-financial support from Ferring, outside the submitted work. Dr. Chee reports non-financial support from Ferring, personal fees and non-financial support from Pfizer, outside the submitted work. Dr. Derikx has served on advisory board for Sandoz, outside the submitted work. Dr Kelleher reports financial support from Pfizer, Wellcome Trust, UKRI and non-financial support from Oxford Immunotech outside the submitted work. Dr. Kok reports personal fees from Janssen, personal fees from Takeda, personal fees from PredictImmune, personal fees from Amgen, outside the submitted work. Dr. Lees reports personal fees from Abbvie, personal fees from Janssen, personal fees from Pfizer, personal fees from Takeda, grants from Gilead, personal fees from Gilead, personal fees from Galapagos, personal fees from Iterative Scopes, personal fees from Trellus Health, personal fees from Celltion, personal fees from Ferring, personal fees from BMS, during the conduct of the study. Dr. Macdonald reports grants and personal fees from Takeda Pharmaceuticals, grants and personal fees from Biogen, personal fees and non-financial support from AbbVie, personal fees from Grifols, personal fees from Sandoz, personal fees from Celltrion, personal fees and non-financial support from Janssen, personal fees from Vifor Pharmaceuticals, personal fees from Predictimmune, personal fees from Bristol Myers Squibb, non-financial support from Ferring Pharmaceuticals, outside the submitted work. Dr.Shaji reports grants from Takeda, Abbvie, AMGEN, Tillots Pharma, personal fees from Jaansen, Takeda, Galapagos, Celltrion, Falk Pharma,

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No conflicts of interest:

Myself, Ben Hamilton, Rachel Nice, Zehra Arkir, Claire Bewshea, Bessie Cipriano, Allan Dunlop, Louise Greathead, Rachel Griffiths, Hajir Ibraheim, Timothy J McDonald.

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Patient involvement

The Exeter IBD Patient Panel reviewed the study protocol. A member of the Exeter IBD Patient Panel sits on the study management committee, ensuring patient involvement in all aspects of study delivery, data analysis and dissemination of findings.

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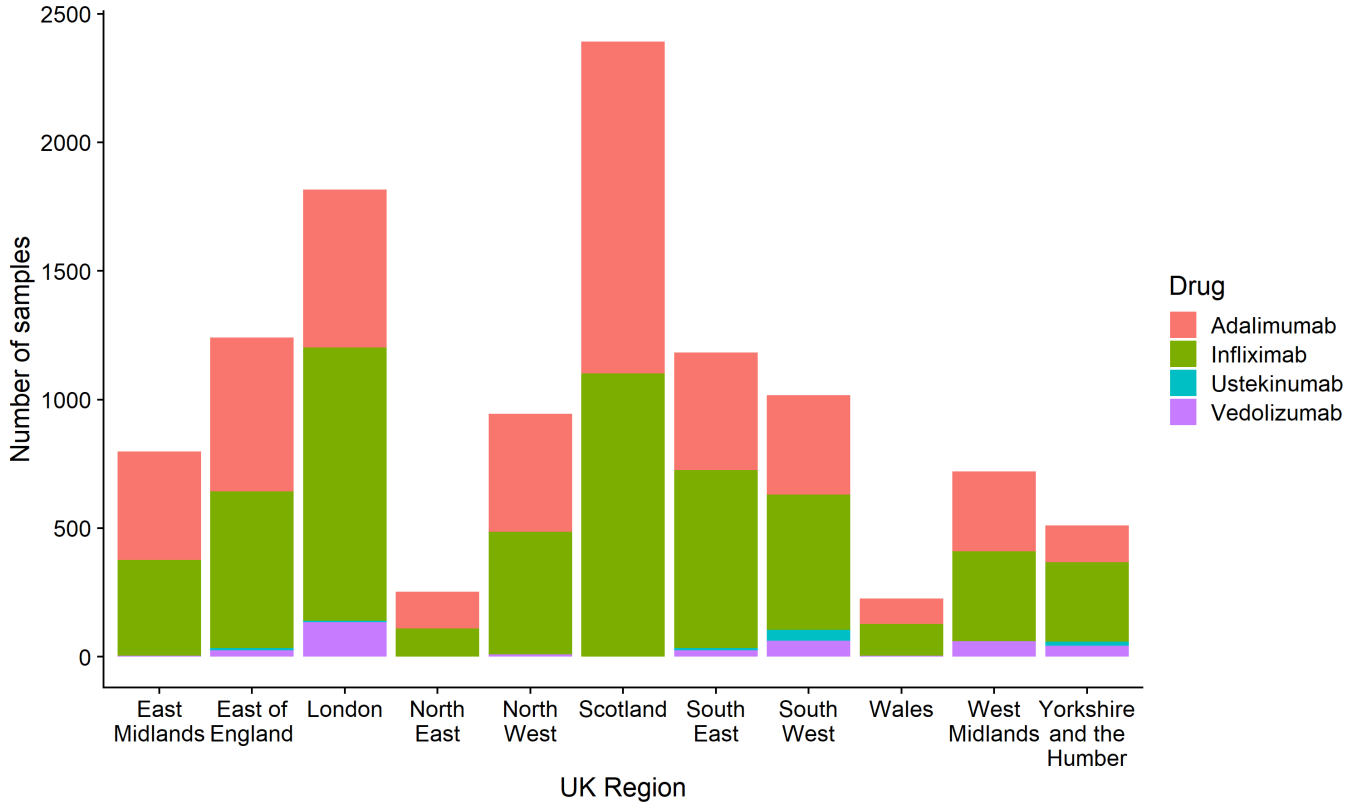
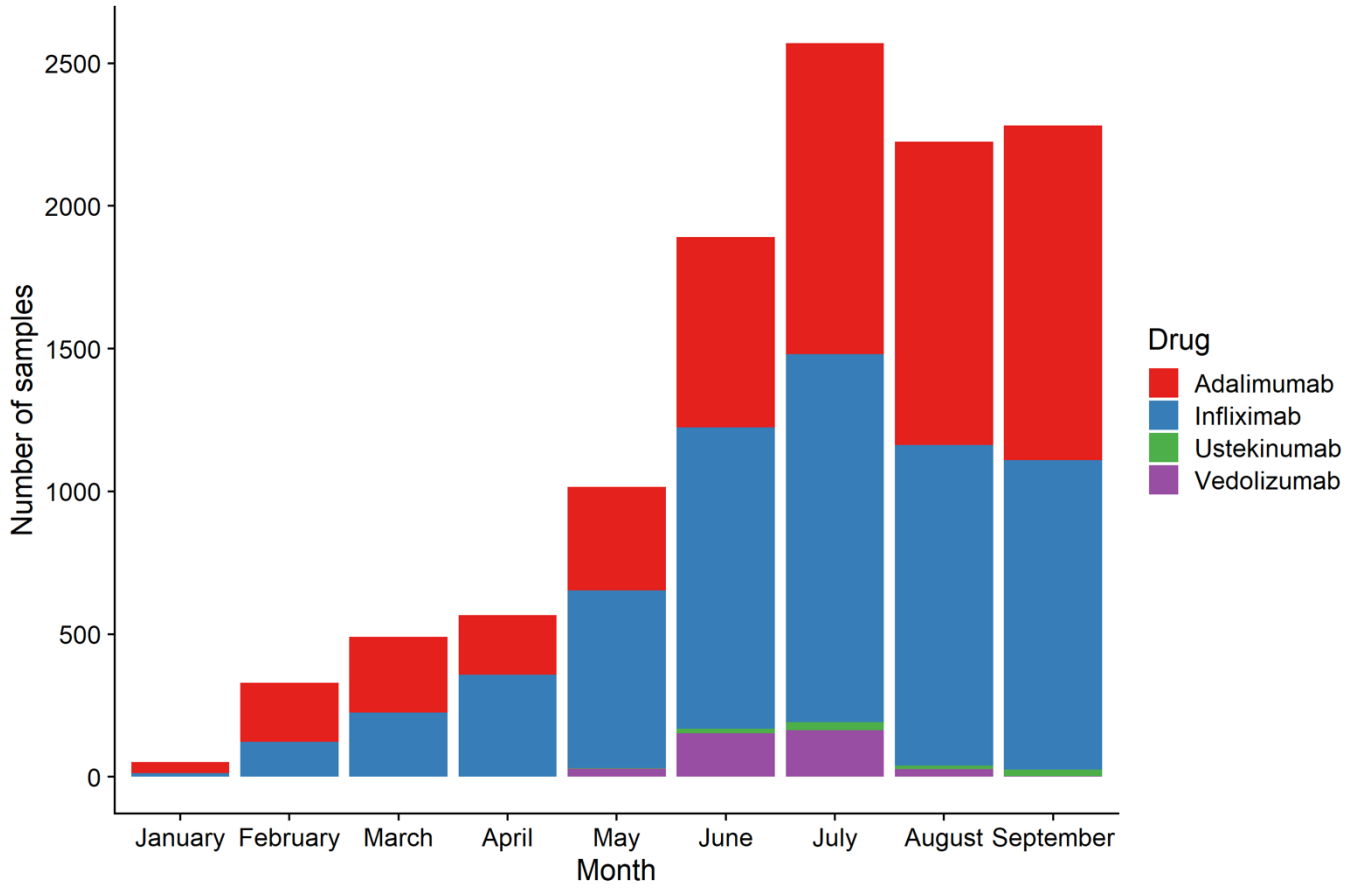
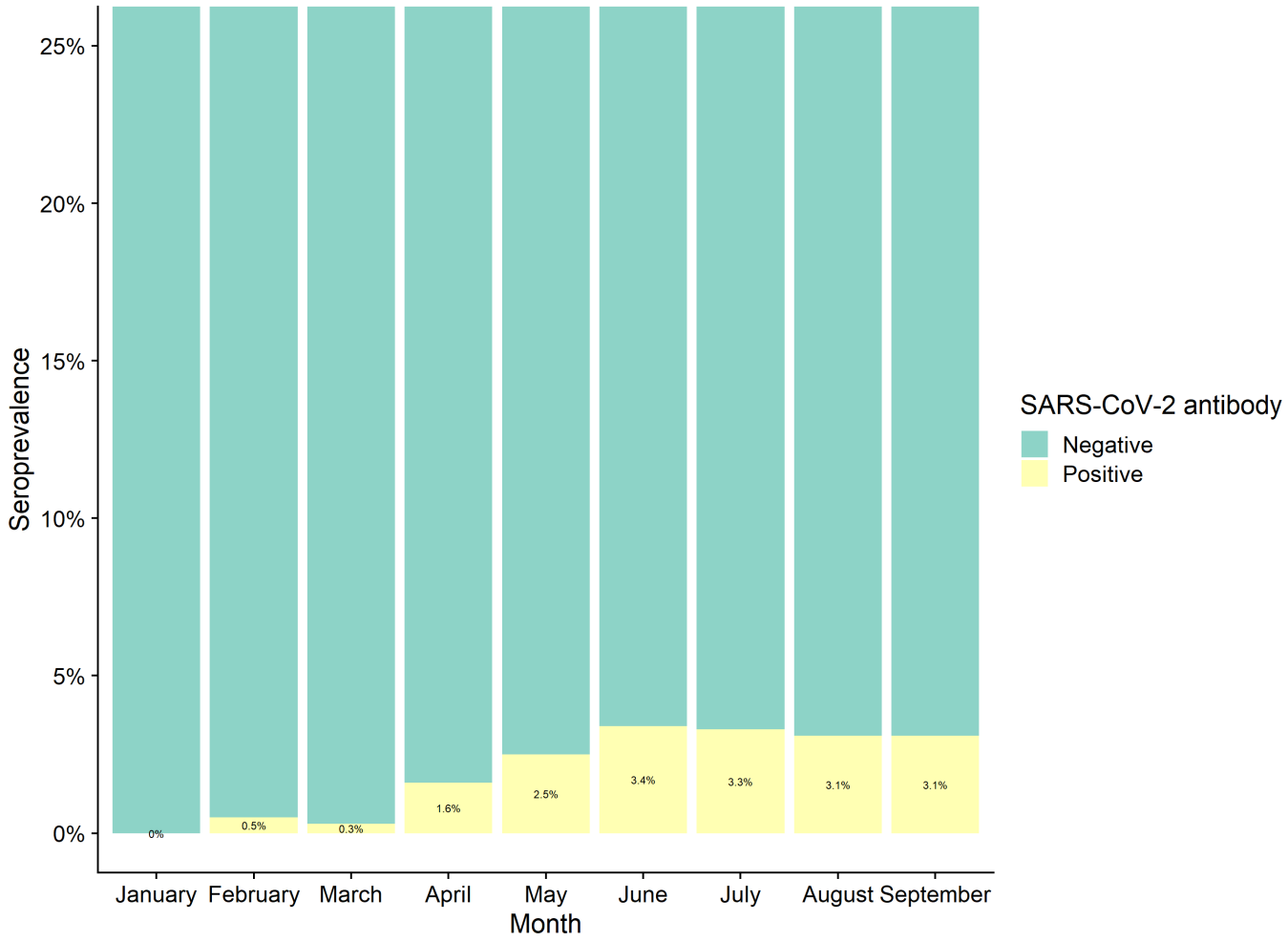
Supplementary Figure 1: Number of samples analysed across UK regions, by biologic therapy



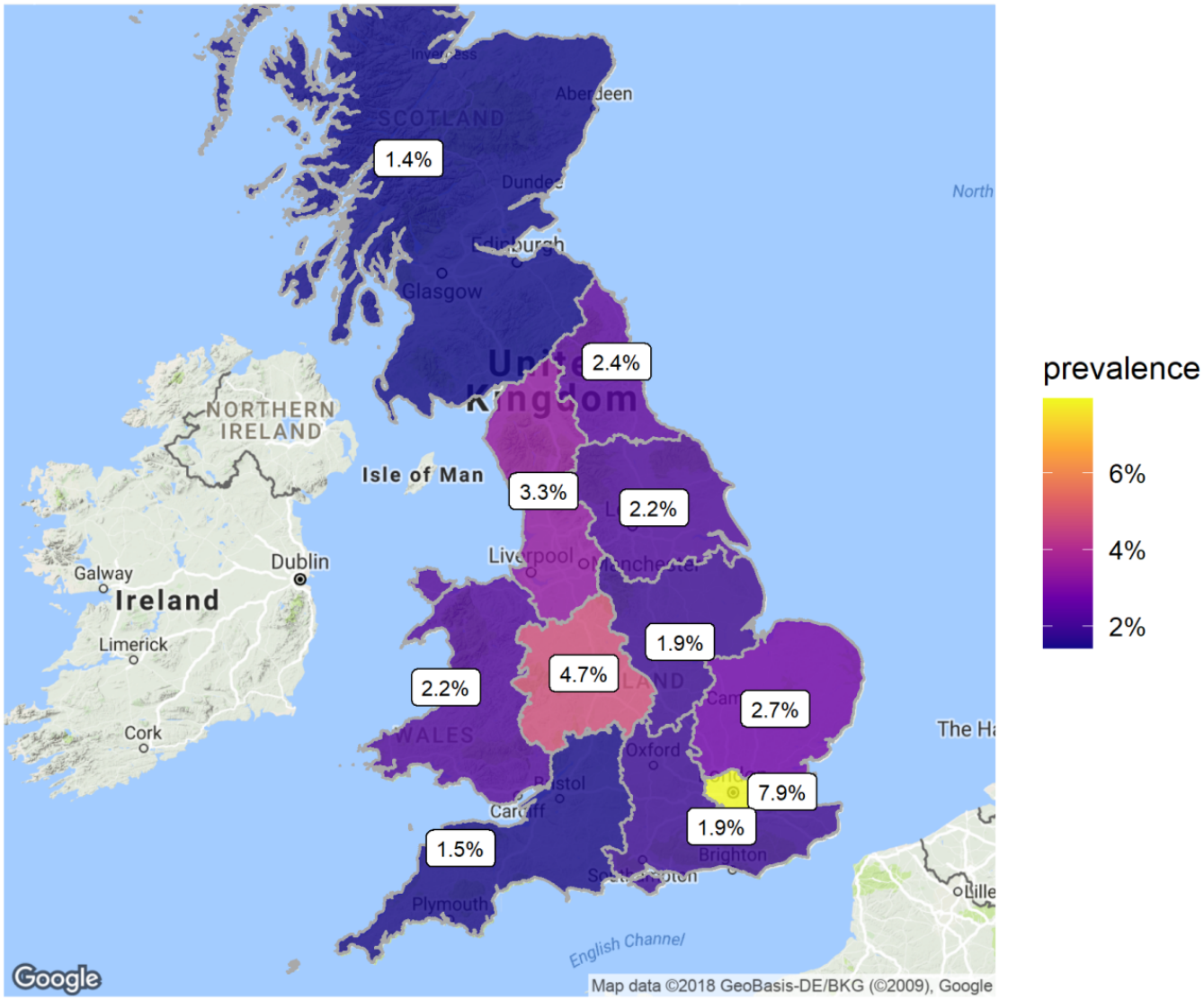
Supplementary Figure 2: Number of samples analysed across time, by biologic therapy



Supplementary Figure 3: Cumulative seroprevalence of SARS-CoV-2 antibodies by month (January 2020 – September 2020)



Supplementary Figure 4: Regional seroprevalence of SARS-CoV-2 by NUTS1 region



Chapter 9: Discussion

Anti-TNF medications are the most commonly prescribed class of medications worldwide for moderate to severe IBD. The work presented in this thesis highlights patient-, disease-, and drug-related factors that are implicated in anti-TNF treatment failure, and identifies strategies that healthcare professionals might adopt to mitigate and manage this complication. I have shown how anti-TNF therapy can both induce and attenuate immune responses to antigens, eliciting anti-drug antibodies. The arrival of the COVID-19 pandemic stimulated investigation of the impact of immunosuppressive medications on antibody responses following SARS-CoV-2 infection and vaccination in patients with IBD. As a result of work carried out during this PhD, I have generated robust, large-scale and long-term data that supports international recommendations on managing anti-TNF treatment failure, and COVID-19 international guidelines on vaccination [2–4].

This chapter provides a brief overview of the main findings of the thesis and discusses the work's conclusions, implications for clinical practice, and potential areas for further research.

8.1 Chapter 3: Validating the positivity thresholds of drug tolerant anti-infliximab and anti-adalimumab antibody assays

8.1.1 Conclusions

In this study, I found that the positivity thresholds of the drug-tolerant anti-infliximab and anti-adalimumab antibody enzyme-linked immunosorbent assays (ELISA) were lower than the manufacturer's suggested thresholds. When applying the newly derived antibody thresholds to a national TDM cohort, I found that anti-drug antibodies were more common in infliximab-, compared to adalimumab-, treated patients. In this cohort, adalimumab-treated patients who were re-classified as being anti-drug antibody positive had an intermediate drug concentration. For both drugs, the new thresholds increased the rates of persistent, non-clearing anti-drug antibodies. When applied to a cohort of IBD patients newly starting anti-TNF, the lowered adalimumab positivity threshold was associated with lower week 14 drug concentrations, treatment failure at the end of induction, and non-remission at the end of one year of treatment. It remains unclear whether these results are generalisable to other anti-drug antibody assays as this study assessed the IDKmonitor [5,6] ELISA assay only. Other limitations include the potential to underestimate rates of immunogenicity due to sample timing and lack of standardised follow-up, and lack of endoscopic data to look at whether the association was seen for the outcome of mucosal healing.

8.1.2 Implications for clinical practice

I recommend that clinical laboratories should independently derive antibody positivity thresholds for assays they use. Because manufacturers define

positivity thresholds in small cohorts of healthy individuals, independent assessment of whether the positivity threshold is different from that recommended is likely to be beneficial. Reporting a positivity threshold with greater precision may add value to the test by increasing the rate of antibody detection, which may indicate emergent treatment failure. This might therefore offer clinicians an opportunity to act early, and institute a course of action to mitigate loss of response, such as optimizing immunomodulator use, anti-TNF dose intensification, or switch to alternative agent.

8.1.3 What I have learnt

By performing this study, I have gained a deeper understanding of the theory underlying drug level and anti-drug antibody detection, in addition to the practical understanding and limitations of performing TDM. I have gained a more in-depth understanding of development and validation of assays, diagnostic accuracy of biochemical testing, and measures of performance including limit of blank, detection, and quantitation.

By working collaboratively alongside scientists from the department of biochemistry, as well as in conjunction with researchers from the EXTEND study, I was able to able to obtain findings from each stage of this triple-cohort study.

Through this study, I was able to demonstrate the utility of validating anti-drug assays within large patient cohorts. Doing so will help healthcare professionals, scientists, and drug manufacturers better understand how to derive clinically meaningful anti-drug antibody thresholds, and how to use their results in clinical

practice. With respect to my clinical career, I will liaise with TDM laboratories that generate results for patients I manage to better understand which anti-drug antibody assay they utilize and how they have set their positivity thresholds. I would advocate, where possible, for validation of the thresholds to be undertaken in real-world clinical cohorts, using similar methodology used in this study, in order to optimise patients' results and subsequent clinician action.

8.1.4 Future research

There remains a lack of standardised antibody testing material, which limits guidance offered to manufacturers on how to assess positivity thresholds of anti-drug antibody assays accurately [7,8]. The Anti-Biopharmaceutical Immunization Prediction and Clinical Relevance to Reduce the Risk Consortium strives to improve information and guidance on assessment of immunogenicity for commercial companies [9], and I will continue to produce results as a researcher that may inform future guidelines.

Future research should also focus on the clinical implications of the anti-drug antibodies detected, particularly the function and natural history of non-clearing anti-drug antibodies remains poorly elucidated. One way of advancing this is to better understand the characterisation of function of clearing versus sustaining antibodies. To do this, I may develop a project whereby I assess binding anti-drug antibodies using a cell-based luciferase reporter assay (iLite) to determine whether they are neutralising or non-neutralising, depending on their binding site [10–12]. Further characterisation experiments, including drug-spiking experiments, may then be undertaken for antibodies that are non-neutralising (ie - rheumatoid factor, heterophilic antibodies, human anti-mouse, and human

anti-human antibodies). Clinically, neutralising anti-drug antibodies are thought to reduce the therapeutic activity of anti-TNF therapy, whereas non-neutralising antibodies are thought to accelerate clearance of the agent [13]. To better understand this relationship, I would then correlate the type of antibody to treatment outcome, for example to patients from the PANTS study for whom outcome data has been obtained.

8.2 Chapters 4, 5, and 6

Chapter 4: Understanding anti-TNF treatment failure: Does serum triiodothyronine-to-thyroxine (T3/T4) ratio predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease?

Chapter 5: Pre-treatment vitamin D concentrations do not predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease

Chapter 6: Understanding the mechanisms and management of loss of response to anti-TNF therapy: three-year data from the PANTS study

8.2.1 Conclusions

In these studies, I aimed to determine the factors associated with anti-TNF treatment failure in patients who participated in the prospective, observational UK-wide Personalised anti-TNF therapy in Crohn's disease study (PANTS) study.

In Chapter 4, I found that lower baseline serum free triiodothyronine-to-thyroxine (fT3/T4) ratio was associated with female sex, corticosteroid use, and Crohn's disease activity, and predicted primary non-response to anti-TNF treatment at week 14, but not non-remission or changes in faecal calprotectin concentrations at week 54. In Chapter 5, I found that vitamin D deficiency is common in patients with active Crohn's disease. Unlike previous studies, pre-treatment serum 25-hydroxyvitamin D concentration did not predict primary non-response to anti-TNF treatment at week 14 or non-remission at week 54. Vitamin D deficiency was, however, associated with a longer time to immunogenicity in patients treated with infliximab, but not adalimumab.

In Chapter 6, I found that about one-third of patients with active luminal Crohn's disease treated with an anti-TNF drug were estimated to be in remission at the end of two and three years. This was predicted by remission status at the end of treatment induction and year one. For both infliximab and adalimumab, low week 14 anti-TNF concentrations and presence of immunogenicity, were predictive of lower year two and year three remission rates. Two-thirds of initial responders experienced loss of response events by the end of year three. Loss of response events, for both infliximab and adalimumab-treated patients, were predicted by low anti-TNF concentrations at week 14, and for infliximab-treated patients, lower thiopurine dose quartiles. Drug-clearing anti-drug antibodies, detected in almost a half of infliximab-treated patients and one fifth of adalimumab-treated patients by three years, were associated with loss of response or treatment failure. Concomitant use of an immunomodulator, started prior to, or on the day of the first infliximab infusion, was associated with increased survival without the development of drug-clearing antibodies. Infliximab dose intensification in the setting of immune-mediated pharmacokinetic failure was associated with low rates of drug persistence.

These analyses have multiple limitations. During the PANTS study, I used pragmatic definitions of treatment response and loss of response, combining corticosteroid use, biochemical results, and clinical, but not endoscopic outcomes, which would have strengthened the results. Conducting long-term analyses in patients recruited to the PANTS study was limited by observation bias after the first year of study because about one-third of participants declined to participate in the extension phase of the study. Follow-up during the extension phase was limited to 6-monthly intervals, thereby potentially leading

to underestimation of immunogenicity rates. Furthermore, the study protocol, as initially written in 2012, observed use of standard anti-TNF dosing as the appropriate starting regime for all patients, with observation of dose intensification only in the setting of loss of response or treatment failure. Use of 'standard' anti-TNF treatment regimens do not reflect current clinical practice anymore, and most clinicians have deviated from a 'one-size fits all' approach. Therefore, as lessons have been learnt during the conduct of the study, direct correlation of the results from the study with current practice have been growing apart.

8.2.2 Implications for clinical practice

Serum fT3/T4 to predict primary non-response lacked diagnostic accuracy and is unlikely to be a clinically useful predictor. Currently, there is no additional justification for use of vitamin D supplementation in anti-TNF treatment over current indications.

Loss of response events and non-remission in years two and three of anti-TNF treatment are predicted by low drug concentrations at week 14 and week 52. Achieving higher drug concentrations during year one, particularly during induction may lead to better long-term outcomes. Because most loss of response events occurred in the first year of treatment, the benefit of proactive TDM is likely to be limited after year one and reactive TDM in the setting of treatment failure is then likely to be more cost-effective.

I found that infliximab-treated patients in the highest weight-based quartile of thiopurine dosing were least likely to experience loss of response, and

demonstrated that use of a concomitant immunomodulator reduces the risk of developing drug clearing immunogenicity to both infliximab and adalimumab. With the increasingly early introduction of infliximab, commencement of a concomitant thiopurine may be delayed whilst waiting on a thiopurine methyltransferase laboratory result, or to allow steroid taper to minimise the risks of triple immunosuppression. My data suggests this delay may increase the risk of immunogenicity to infliximab and should be avoided.

In the setting of immune-mediated pharmacokinetic failure (undetectable drug concentration with antibodies), dose intensification resulted in shorter drug persistence compared to patients with non-immune mediated pharmacokinetic failure (undetectable or subtherapeutic drug concentration without antibodies). These observations support the current practice of dose intensification in the setting of low drug concentrations without immunogenicity [4,14].

8.2.3 What I have learnt

By conducting these experiments, I learnt about the importance of conducting research using homogenous cohorts, and how known and unknown confounders (ie - age, sex, disease behaviour, genetic risk, steroid use) can affect the association between exposure and outcome. Given that anti-TNF treatment response is multifactorial and occurs via different physiological pathways, understanding the effect of one exposure on outcome remains very challenging in clinical research. One way to overcome this is to aim to replicate findings via muti-omic analyes, which will help improve our understanding of different contribution of genetic variants to anti-TNF treatment response.

Publishing these papers taught me how to engage with other groups within my research field, and how I can scientifically debate and challenge other groups' works and findings using an academic process. For two of these chapters, I analysed the results of this study questions independently, and sought review of my code/analyses at a later stage compared to work carried out earlier in my PhD, thereby demonstrating increased confidence and competency in using R and performing statistical analyses.

Conclusions from these studies were different to the conclusions from smaller, mostly retrospective studies that investigated the same research question, demonstrating to me the benefit of using large, well-powered, prospective cohorts to replicate results from smaller cohorts. From this, I have learnt the value of carrying out multi-site research by assimilating real-world cohorts of patients to answer clinical questions. Data from these cohorts can complement findings from clinical trials, and are increasingly additive to decision-making in clinical practice. They do, however, require substantial resources, including research staff, digital and face to face infrastructure, and engagement of patients with research teams, to be successful. My role as study lead for these chapters has taught me the importance of data cleaning and engaging study sites with data clarification queries as they arise, throughout the conduct of the study (compared to at the end of the study) is integral to obtaining high-quality, accurate data.

8.2.4 Future research

Further work using endoscopic outcomes by disease and biologic type is needed to confirm or refute the usefulness of the fT3/T4 ratio to predict anti-

TNF treatment outcomes. Although my results do not suggest fT3/fT4 ratio as a predictor of anti-TNF response is clinically useful, it may have a role in a larger panel including pharmacokinetic variables such as drug concentration, and emergent molecular biomarkers. Whilst vitamin D deficiency did not predict primary non-response to anti-TNF treatment, whether low vitamin D levels protect against the development of anti-drug antibodies requires further study.

There are limited data regarding the optimal dose of thiopurines in anti-TNF combination therapy, although several studies small, largely retrospective studies have suggested that a lower thioguanine nucleotide concentration may be effective in reducing immunogenicity to infliximab therapy [15–17]. My findings of high-dose, early use of thiopurine to reduce drug-clearing antibodies should be further investigated, ideally by well-powered interventional studies.

My findings that remission rates and loss of response events to anti-TNF therapy were predicted by low anti-TNF concentrations at week 14 and week 52 suggests that currently recommended drug concentration targets are too low [14,18]. Low anti-TNF drug concentrations may also explain why proactive TDM, particularly during induction of therapy, is likely to be important. Point-of-care, dashboard-driven TDM to guide clinician action is likely to facilitate more effective decision making and immediate action of loss of response events. We await the results from studies currently underway that are investigating these research questions [19,20].

With respect to my future work in this area, I would like to continue to identify and better understand potential clinical and biochemical predictors of anti-TNF

treatment failure or loss of response. To do this, large-scale, prospective clinical cohorts are required, such as the size of the PANTS study. At study design, I would perform a sample size calculation of the cohort to look for a genetic predictor of anti-TNF treatment failure, as that might be the most clinically useful biomarker that can be undertaken at diagnosis. Given the challenges I faced with ascertaining treatment outcome during the conduct of Chapters 3, 4, and 5, I would aim to collect faecal calprotectin and endoscopic/histological data following anti-TNF treatment; these were clear limitations of the PANTS cohort that were not collected due to pragmatic reasons, but would have added a wealth of clinically useful data and better informed the research question.

Fostering good working relationships with healthcare professionals, scientists, patient-facing charities, and funders is extremely important to generating good research and accurate results from clinical studies. The PANTS study was heavily supported by multiple pharmaceutical, non-pharmaceutical, and charitable funders, and I would aim to emulate such a model in my future research because of the benefits I have seen come from multi-stakeholder involvement. Carrying out research that is not only important to healthcare professionals, but also to patients is a priority for me when developing my research career. Along these lines, and in accordance with the James Lind Alliance priorities in the treatment of IBD, I would aim to answer research questions related to anti-TNF treatment failure that patients have been involved in formulating [21,22]. I have learnt about the importance of patient and public involvement at all stages of study conduct, but most importantly, during the study design phase so that patients can contribute to research in a meaningful way that they deem is acceptable.

8.3 Chapter 7: Implications for sequencing of biologic therapy and choice of second anti-TNF in patients with inflammatory bowel disease: results from the Immunogenicity to Second Anti-TNF Therapy (IMSAT) therapeutic drug monitoring study

8.3.1 Conclusions

In this study, I found that, irrespective of anti-TNF sequence, immunogenicity to the first anti-TNF was associated with immunogenicity to the second anti-TNF, and that patients who developed immunogenicity with undetectable drug levels to infliximab as first anti-TNF were more than twice as likely to develop immunogenicity with undetectable drug levels to adalimumab as second anti-TNF. Commencing an immunomodulator at the time of switching to the second anti-TNF was associated with improved drug persistence in patients with immunogenic-, but not pharmacodynamic-treatment failure to the first anti-TNF. My study was limited by a retrospective cohort design, and I was unable to collect data on patients who failed an anti-TNF but did not have TDM undertaken, thereby potentially underestimating rates of immunogenicity and over-estimating drug persistence rates. Furthermore, missing data may, particularly related to dose optimisation of immunomodulator and anti-TNF therapy, may have led to interpretation bias.

8.3.2 Implications for clinical practice

I confirmed associations with immunogenicity that were reported in the prospective UK-wide PANTS study [23] as well as those reported in an open-label randomised controlled trial [24]. My findings support international recommendations for the management of anti-TNF treatment failure, to switch

out of biologic class when drug levels are therapeutic, and within class with an immunomodulator when anti-TNF drug levels are low and associated with antibody development [14,18].

8.3.3 What I have learnt

As study lead, I was responsible for authoring the study protocol, authoring the submission to the Integrated Research Application System, and navigating the processes by which a research study is approved in the UK – a skill that has led me to becoming an independent researcher.

I worked collaboratively with departments of biochemistry locally and nationally to recruit UK sites to join the study. I held 36 site initiation visits independently; a strategy that I believe led to higher quality data being entered into the database, compared to if I held the visits via video recording only. Therefore, I fostered a working relationship with personnel at each study site, meaning they felt open to communicating with me via email during the conduct of their data entry and asking me questions to aid their own understanding of the study and how to submit patient data. Furthermore, learning how to code and analyse this breadth and complexity of data was a new skill that took many months to perform competently, but one that I will use increasingly during my research career.

This is one of the largest scale studies to investigate this research question, and link clinical data to TDM results. It replicated findings from other large-scale laboratory studies, but added novelty with respect to clinical outcomes following

treatment with second anti-TNF. Because of its large sample size, it refuted findings from smaller studies for which there was clinical uncertainty.

8.3.4 Future research

Consistent with previous studies, I reported that, at the time treatment failure to first anti-TNF, about one in five patients had anti-drug antibodies that were detectable in the presence of drug [23,25,26]. Considerable uncertainty remains as to the function and relevance of these antibodies. They maybe neutralizing, transient or maturing, and in the future may lead to a more robust immune response, and clear drug [27] Functional studies are required to better characterize these antibodies and to understand if they are clinically relevant. Whether sequential immunogenicity occurs in populations with low HLA-DQA1*05 carriage and lower rates of immunogenicity is unknown [28,29]. Further research is needed to elucidate if patients who develop immunogenicity to one or more anti-TNF drugs are also at risk of developing anti-drug antibodies to the newer biologic therapies.

With respect to my own research in this field, due to how data was collected by study sites, I was limited in how I was able to answer some nuanced clinical questions, specifically timing of immunomodulator and anti-TNF, and dosing regimens at the time of TDM. Furthermore, there were fewer adalimumab-treated first patients compared to infliximab-treated patients in the study; a limitation that meant I had to analyse both groups together for the primary and secondary analyses, leading to use of heterogenous drug exposures. To address these limitations, if I were to carry out such a study on a different cohort in the future, I would ask study sites to submit more granular data regarding

patients' treatment regimens. Also, repeating this study in a few years' time may mean that more adalimumab-treated first patients are potentially recruitable and would therefore make up a more balanced proportion of total subjects included, thereby allowing a more refined assessment of risk for these patients. Ideally, I would obtain prospective approval for serum sampling to evaluate potential genetic risk factors for the development of immunogenicity, such as carriage of the HLA-DQA1*05 risk variant collected in the PANTS cohort.

8.4 Chapter 8: Adalimumab and infliximab impair SARS-CoV-2 antibody responses: results from a therapeutic drug monitoring study in 11422 biologic-treated patients

8.4.1 Conclusions

In this study, I found that patients with immune-mediated inflammatory diseases, including IBD, treated with the anti-TNF therapies infliximab and adalimumab had attenuated serological responses to SARS-CoV-2 infection with lower seroprevalence rates and antibody reactivity, when compared to vedolizumab-treated patients. The magnitude of SARS-CoV-2 reactivity was similar in infliximab- vs adalimumab-treated patients, but higher in vedolizumab-treated patients. Compared to patients with detectable infliximab and adalimumab drug levels, patients with undetectable drug levels were more likely to be seropositive for SARS-CoV-2 antibodies, and in this cohort, seropositivity rates were similar to those observed in patients treated with vedolizumab. I also found that one-third of patients who had SARS-CoV-2 testing prior to antibody testing failed to seroconvert, all of whom were treated with anti-TNF therapies. Importantly, because this was a therapeutic drug monitoring study analysing surplus serum, data was limited by lack of comprehensive clinical details including comorbidities, ethnicity, diagnosis, symptoms of suspected COVID-19, and indications for, and duration of, biologic and concomitant therapies. In addition, data on SARS-CoV-2 antibody durability and risk of re-infection was limited by duration of follow-up, frequency of sampling, and availability of testing results from UK governmental agencies.

8.4.2 Implications for clinical practice

Our group had previously demonstrated that infliximab impaired antibody responses following SARS-CoV-2 infection and vaccination in patients with IBD [3,30,31]. This contribution of this study demonstrated that the effects were similar for both infliximab and adalimumab, and likely across other immune-mediated inflammatory conditions. Furthermore, my finding that anti-TNF level influenced SARS-CoV-2 serological responses may support a causal relationship between anti-TNF use and attenuated antibody responses to infection. Serological testing should therefore be considered to detect acute and chronic SARS-CoV-2 infection in patients with immune-mediated inflammatory conditions, and assessment of viral surveillance and evolution in these patients to inform public health policy.

8.4.3 What have I learnt

Generating a clinically important research question and designing a robust study during the COVID-19 pandemic was a unique challenge. Navigating the legal and ethical framework that allows one to carry out topical, pandemic-related research in the UK quickly was a new experience for me as an early-career researcher. Generating comprehensive applications to public health bodies to acquire and maintain data was another new skill that I learnt.

I coordinated sample management from multiple laboratories, which required liaising with local principle investigators and scientists. This allowed me to gain a more in-depth understanding in to how to different laboratories process serum samples for therapeutic drug monitoring across the UK, and how laboratory differ across the country. Conducting this experiment sent precedent for how

different laboratories might work together in the future to generate large datasets on patients with IBD, mapped to TDM results, and potentially clinical outcomes.

From a translational impact perspective, this study was the first to demonstrate that both infliximab and adalimumab treatment was associated with lower SARS-CoV-2 nucleocapsid seroprevalence and antibody reactivity when compared to vedolizumab-treated patients. It was hypothesised that higher seropositivity rates in patients with undetectable anti-TNF levels supported a causal relationship, however, this was challenged by a larger cohort study carried out later in the pandemic [31]. From this study, I learnt about how researchers can identify patients opportunistically (via stored surplus serum samples). This might be one avenue to obtain laboratory/sample data to explore further when considering projects related to audit or service evaluation, for which patient consent may not be necessary (https://www.hra-decisiontools.org.uk/research/).

8.4.4 Future research

We need to better understand the relationship between biologic therapy and SARS-CoV-2 natural infection and effectiveness of SARS-CoV-2 vaccination in patients with IBD. During the COVID-19 pandemic, future studies went on to, and continue, assess factors linked to vaccine non-response, the impact, timing, and dosing booster doses, and whether temporary discontinuation of multiple immunosuppressive therapies impacted long-term immunogenicity of SARS-CoV-2 vaccines [32–36].

Publishing research during the COVID-19 pandemic was a unique experience, in part because of the changing, dynamic landscape at which information and data was being shared, often lacking peer review or scientific scrutiny, and made up from small cohorts. Through conducting the CLARITY IBD studies, including Chapter 8 of this thesis, I learned about the importance of collaboration across the UK and worldwide, replicating results in larger cohorts, and the utility of meta-analysing cohorts, to make data more valid and enriched.

I also experienced first-hand of the importance of electronic patient consent forms, thereby reducing minimising use of resources and reducing patient burden for face-to-face research visits (https://www.nihr.ac.uk/case-studies/clarity-ibd-changing-the-way-we-do-research/30021). Other digital and decentralised delivery techniques to maximise patient engagement with the CLARITY studies I was involved with included communicating to patients via 1:1 phone calls (preferred to in-person visits), use of encrypted electronic database servers (such as REDCap) to facilitate multi-site research and communicate with patients directly via email, and remote therapeutic drug monitoring using postal-based kits. I will integrate these core set of innovative research methods when designing studies in the future as an independent researcher.

8.5 Final remarks

The studies presented in this thesis were designed to inform healthcare professionals how best to personalise anti-TNF therapy for patients with IBD. In this field, few of the so-called precision medicine biomarkers to facilitate the right drug, to the right patient, at the right time have translated to clinical care [37–40].

Through the data generated in this thesis, I have been able to address analytical aspects of anti-TNF antibody level testing that have clarified current uncertainties regarding their measurement and clinical utility permitting more meaningful application in clinical practice (Chapter 3). I studied short-term (serum free triiodothyronine-to-thyroxine ratio [Chapter 4] and pre-treatment 25-hydroxyvitamin D concentrations [Chapter 5]) and long-term (Chapter 6) predictors of anti-TNF treatment failure in patients recruited to the PANTS study. This allowed me to better understand what serological markers for anti-TNF treatment failure have clinical and diagnostic utility, as well as provide long-term estimates of anti-TNF remission, loss of response events, and immunogenicity rates, stratified by anti-TNF concentrations and anti-drug antibodies. Through these analyses, I was able to postulate the optimal dosing and timing of immunomodulatory co-therapies needed to prevent immunogenicity, as well as the optimal management strategies for patients who develop immunogenicity and treatment failure. In Chapter 7, I provided more evidence on how best to sequence biologic medication in the setting of treatment failure to an initial anti-TNF therapy. These data have supported current international guidelines and will inform the generation of updated recommendations.

Half of this thesis was complete in a time when the global COVID-19 pandemic occurred. As laboratory work was heavily restricted for most of 2020 - 2021, I had to modify experiments and project plans that were initially intended to be carried out at the beginning of my PhD fellowship. As a result, I carried out a COVID-19 related experiment (Chapter 8). Through this project, I was able to identify the impact of anti-TNF therapy on SARS-CoV-2 infection, and for the first time, understand the potential impact of anti-TNF drug concentrations of SARS-CoV-2 seroconversion rates and antibody durability.

The practical achievements of this thesis are laid out in Appendix 1. Five chapters have been published and one is currently being reviewed for publication. All data have been presented internationally as oral abstracts or invited presentations.

In addition to improving patient care, my thesis has met its aims of providing insights into the molecular and cellular mechanisms of anti-TNF treatment failure and loss of response, informing the development of new or repositioning of old therapies that reduce the risk of immunogenicity and treatment failure, and aiding healthcare professionals' and patients' understanding of the impact of immunosuppressive medications on SARS-CoV-2 infection. Through this body of work, we are closer to developing predictive clinical decision tools that will help us select the right drug, or combination of drugs, to provide safer, longer-lasting anti-TNF therapy for patients with IBD than what is currently achieved.

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Appendix 1: List of awards and publications arising from my doctoral studies

Award	Year	Awarding Body
BMJ Open Gastroenterology best clinical science abstract – BSG Live conference	2023	British Society of Gastroenterology
Top 10 Digital Oral Presentation – 16th Congress of ECCO	2021	European Crohn's Colitis Organisation
Research Team making an outstanding contribution to the NIHR portfolio studies	2021	British Society of Gastroenterology and NIHR Clinical Research Network
Sam Tucker Fellowship – Commitment to an academic career in paediatrics	2020	Royal Society of Medicine
Best Poster (STEMM) - Postgraduate Research Showcase	2019	University of Exeter (Doctoral College)
Richard Driscoll IBD Research Fellowship (inaugural recipient)	2018	Crohn's and Colitis UK

Publications

Original Research

1. Bai BYH, Reppell M, Smaoui N, Waring JF, Pivorunas V, Guay H, Lin S, **Chanchlani N**, Bewshea C, Goodhand JR; UK Inflammatory Bowel Disease Pharmacogenetics Study Group; Kennedy NA, Ahmad T, Anderson CA. Baseline expression of immune gene modules in blood is associated with primary response to anti-TNF therapy in Crohn's disease

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3. Hanić M, Vučković F, Deriš H, Bewshea C, Lin S, Goodhand JR, Ahmad T, Trbojević-Akmačić I, Kennedy NA, Lauc G; **PANTS Consortium (listed as collaborator)**. Anti-TNF Biologicals Enhance the Anti-Inflammatory Properties of IgG N-Glycome in Crohn's Disease. Biomolecules. 2023 Jun 7;13(6):954. doi: 10.3390/biom13060954.
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COVID-19 vaccine (CLARITY IBD): an analysis of a prospective multicentre cohort study. Lancet Gastroenterol Hepatol. 2022 Dec 5:S2468-1253(22)00389-2.

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9. Lin S*, Kennedy NA*, Saifuddin A*, Sandoval DM*, Reynolds CJ, Seoane RC, Kottoor SH, Pieper FP, Lin KM, Butler DK, **Chanchlani N**, Nice R, Chee D, Bewshea C, Janjua M, McDonald TJ, Sebastian S, Alexander JL, Constable L, Lee JC, Murray CD, Hart AL, Irving PM, Jones GR, Kok KB, Lamb CA, Lees CW, Altmann DM, Boyton RJ*, Goodhand JR*, Powell N*, Ahmad T*; CLARITY IBD study. Antibody decay, T cell immunity and breakthrough infections following two SARS-CoV-2 vaccine doses in inflammatory bowel disease patients treated with infliximab and vedolizumab. Nature Communications. 2022 Mar 16;13(1):1379.
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12. Kennedy NA*, Lin S*, Goodhand JR*, **Chanchlani N**, Hamilton B, Bewshea C, Nice R, Chee D, Cummings JF, Fraser A, Irving PM, Kamperidis N, Kok KB, Lamb CA, Macdonald J, Mehta S, Pollok RC, Raine T, Smith PJ, Verma AM, Jochum S, McDonald TJ, Sebastian S, Lees CW, Powell N*, Ahmad T*. Contributors to the CLARITY IBD study. Infliximab is associated with attenuated immunogenicity to BNT162b2 and ChAdOx1 nCoV-19 SARS-CoV-2 vaccines in patients with IBD. Gut. 2021 Apr 26:gutjnl-2021-324789.
13. Kennedy NA*, Goodhand JR*, Bewshea C, Nice R, Chee D, Lin S, **Chanchlani N**, Butterworth J, Cooney R, Croft NM, Hart AL, Irving PM, Kok KB, Lamb CA, Limdi JK, Macdonald J, McGovern DP, Mehta SJ, Murray CD, Patel KV, Pollok RC, Raine T, Russell RK, Selinger CP, Smith PJ, Bowden J, McDonald TJ, Lees CW, Sebastian S, Powell N*, Ahmad T*; Contributors to the CLARITY IBD study. Anti-SARS-CoV-2 antibody responses are attenuated in patients with IBD treated with infliximab. Gut. 2021 May;70(5):865-875.

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15. Gordon C, Chee D, Hamilton B, Heerasing NM, Hendy P, **Chanchlani N**, Lin S, Wesley E, Daniels IR, Silva N, Osborne M, Kennedy NA, Goodhand JR, Ahmad T. Root-cause analyses of missed opportunities for the diagnosis of colorectal cancer in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2020 Nov 7.
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22. Kennedy NA, Heap GA, Green HD, Hamilton B, Bewshea C, Walker GJ, Thomas A, Nice R, Perry MH, Bouri S, **Chanchlani N**, Heerasing NM, Hendy P, Lin S, Gaya DR, Cummings JRF, Selinger CP, Lees CW, Hart AL, Parkes M, Sebastian S, Mansfield JC, Irving PM, Lindsay J, Russell RK, McDonald TJ, McGovern D, Goodhand JR, Ahmad T; UK Inflammatory Bowel Disease Pharmacogenetics Study Group. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active

luminal Crohn's disease: a prospective, multicentre, cohort study. Lancet Gastroenterol Hepatol. 2019 Feb 26. pii: S2468-1253(19)30012-3.

Non-research articles, including comment, analysis, and education

23. Lin S*, Lau LH*, **Chanchlani N***, Kennedy NA, Ng SC. Recent advances in clinical practice: management of inflammatory bowel disease during the COVID-19 pandemic. Gut. 2022 Apr 27:gutjnl-2021-326784.
24. **Chanchlani N**, Russell RK. Life expectancy in patients with inflammatory bowel disease: time will tell if biologics are the answer. CMAJ. 2021 Mar 15;193(11):E380.
25. **Chanchlani N,** Jarvis P, Hart JW, McMillan CH, Moudiotis CR. Adolescent with abdominal pain poorly responsive to analgesia. Arch Dis Child Educ Pract Ed. 2020 Dec 16:edpract-2020-320772.
26. **Chanchlani N**, Walters TD, Russell RK. Managing non-specific abdominal pain in children and young people. CMAJ. 2020 Nov 30;192(48):E1639-E1640.

Invited presentations

27. **Chanchlani N.** Can we predict anti-TNF treatment failure in our patients? ESPGHAN 54th Annual meeting. Copenhangen, Denmark. June 2022.
28. **Chanchlani N**. An update on therapeutic drug monitoring. South-west (regional) paediatric gastroenterology, hepatology, and nutrition monthly teaching. May 2022. Online.

29. **Chanchlani N**. Using therapeutic drug monitoring in paediatric IBD: Part 2. BSPGHAN Educational Series. April 2022. Online.
30. **Chanchlani N**, Lin S. Understanding the mechanisms of anti-TNF treatment failure in patients with Crohn's Disease: A proteomic analysis of the PANTS cohort. 8th Y-ECCO Basic Science Workshop. February 2022. Vienna, Austria.
31. **Chanchlani N**. Predicting response to anti-TNF therapy. ESPGHAN 6th IBD Masterclass. November 2021. Bristol, UK.
32. **Chanchlani N**. Predicting response to anti-TNF therapy. Scottish Society of Paediatric Gastroenterology, Hepatology, and Nutrition Annual Conference. November 2021. Online.

Oral presentations

33. **N Chanchlani**, S Lin, B Hamilton, C Bewshea, A Thomas, R Smith, C Roberts, R Nice, T J McDonald, J R Goodhand, T Ahmad, N A Kennedy, PANTS Consortium, O59 Understanding anti-TNF treatment failure: mechanisms and management of loss of response to anti-TNF therapy, three-year data from the PANTS study, Gut 2023;72:A34-A35. BSG Live 2023, Liverpool, UK. June 2023. (awarded 'BMJ Open Gastroenterology' abstract award)
34. Z Liu, K Le, X Zhou, J Alexander, S Lin, C Bewshea, **N Chanchlani**, R Nice, T McDonald, C Lamb, S Sebastian, K Kok, C Lees, A Hart, R Pollok, R Boyton, D Altmann, K Pollock, J Goodhand, N Kennedy, T Ahmad, N Powell, CLARITY IBD, OP24 Neutralising antibody potency against SARS-CoV-2 wild-type and Omicron BA.1 and BA.4/5 variants in infliximab and vedolizumab treated patients with Inflammatory Bowel

Disease after three doses of COVID-19 vaccine: a prospective multicentre cohort study, *Journal of Crohn's and Colitis*, Volume 17, Issue Supplement_1, February 2023, Pages i32–i34. ECCO, Copenhagen, Denmark. Mar 2023.

35. S Lin, E Hannon, J F Waring, M Reppell, N Smaoui, V L Pivorunas, H Guay, **N Chanchlani**, C Bewshea, B Y H Bai, N A Kennedy, J R Goodhand, J Mill, T Ahmad, DOP88 Understanding the molecular mechanisms of anti-TNF treatment failure: Whole blood DNA methylation changes associated with primary non-response to anti-TNF treatment in patients with Crohn's disease, *Journal of Crohn's and Colitis*, Volume 17, Issue Supplement_1, February 2023, Pages i164–i166. ECCO, Copenhagen, Denmark. Mar 2023.
36. Lin S*, Kennedy NA*, Saifuddin A*, Sandoval DM*, Reynolds CJ, Seoane RC, Kottoor SH, Pieper FP, Lin K, Butler DK, **Chanchlani N**, Nice R, Chee D, Bewseha C, Janjua M, McDonald TJ, Sebastian S, Alexander JL, Constable L, Lee JC, Murray CD, Hart AL, Irving PM, Jones G, Kok KB, Lamb CA, Lees CW, Altmann DM, Boyton RJ*, Goodhand JR*, Powell N*, Ahmad T*, Contributors of the CLARITY IBD study. OP22 Antibody decay, T cell immunity and breakthrough infections following SARS-CoV-2 vaccination in infliximab- and vedolizumab-treated patients, J Crohn's and Colitis, Volume 16, Issue Supplement_1, Jan 2022, Pages i023-i)25. ECCO, Virtual. Feb 2022
37. Kennedy NA, Goodhand JR, Bewshea C, Nice R, Chee D, Lin S**,** **Chanchlani N**, Butterworth J, Cooney R, Croft N, Hart A, Irving P, Kok K, Lamb C, Limdi J, MacDonald J, McGovern D, Mehta S, Murray C, Patel K, Pollok R, Raine R, Russell R, Selinger C, Smith P, Bowden J,

McDonald T, Lees C, Sebastian S, Powell N, Ahmad T, CLARITY IBD Investigators, OP03 Anti-SARS-CoV2 antibody responses are attenuated in patients with Inflammatory Bowel Disease treated with infliximab, J Crohn's and Colitis, Volume 15, Issue Supplement_1, May 2021, Pages S002–S004. ECCO, Virtual. June 2021 (awarded best investigator-initiated study)

38. **Chanchlani N**, Lin S, Thomas A, Hamilton B, Nice R, Chee D, Kennedy NA, Goodhand JR, Ahmad T, IMSAT study investigators, DOP62 Immunogenicity to second anti-TNF therapy (IMSAT): Implications for sequencing of biologic therapy, *J Crohn's and Colitis*, Volume 15, Issue Supplement_1, May 2021, Pages S097–S098. ECCO, Virtual. June 2021 (awarded 'Top 10 Digital Oral Presentation' prize)
39. Nice R*, **Chanchlani N*,** Green H, Bewshea C, Kennedy N, Ahmad T, Goodhand JR, McDonald TJ, Perry MH. Positivity thresholds of total infliximab and adalimumab anti-drug antibody assay: The prevalence of clearing and transient anti-drug antibodies in a national therapeutic drug monitoring service. J Crohn's Colitis 2020;14(Supplement_1):S018–9. ECCO, Vienna. February 2020.
40. Lin S, **Chanchlani N**, Invergo BM, Anderson MW, Guay HM, Reppell M, Butler JW, Goodhand JR, Ahmad T, Kenneddy NA. Understanding the molecular mechanisms of anti-TNF treatment failure in patients with Crohn's disease: A pilot serum proteomic analysis of the PANTS cohort. J Crohn's Colitis 2020;14(Supplement_1):S067–8. ECCO, Vienna. February 2020.
41. **Chanchlani N**, Kennedy NA, Lin S, PANTS Investigator Consortium, Russell RK, Goodhand JR, Ahmad T. Treatment failure, safety, and

immunogenicity of anti-TNF treatment in biologic-naïve children and adolescents with Crohn's Disease: a subgroup analysis of the PANTS cohort. J Pediatr Gastroenterol Nutr 2019;68;Suppl 1; G-0-052. Presented at: BSPGHAN Annual Conference, Oxford, UK. January 2019, ESPGHAN Annual Conference, Glasgow UK. June 2019.

Poster presentations

42. R Smith, Z Liu, K Laskey, S Lin, N Chanchlani, R Nice, C Roberts, J Alexander, Timothy J McDonald, Claire Bewshea, N Kennedy, J Goodhand, N Powell, T Ahmad. P115 Vitamin D concentrations are not associated with anti-SARS-CoV-2 antibody responses following mRNA COVID-19 vaccine in IBD patients treated with infliximabGut 2023;72:A114-A115. BSG Live 2023, Liverpool, UK. June 2023.
43. I Carbery*, S Lin*, **N Chanchlani***, M Janjua, R Nice, T J McDonald, C Bewshea, N A Kennedy, T Ahmad, J R Goodhand, C P Selinger, P649 Does serum triiodothyronine-to-thyroxine (T3/T4) ratio predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease?, *Journal of Crohn's and Colitis*, Volume 17, Issue Supplement_1, February 2023, Page i778. ECCO, Copenhagen, Denmark. Mar 2023.
44. **N Chanchlani**, S Lin, B Hamilton, C Bewshea, A Thomas, R Smith, C Roberts, R Nice, T J McDonald, J R Goodhand, T Ahmad, N A Kennedy, PANTS Consortium, P798 Understanding anti-TNF treatment failure: mechanisms and management of loss of response to anti-TNF therapy, three-year data from the PANTS study, *Journal of Crohn's and Colitis*,

Volume 17, Issue Supplement_1, February 2023, Pages i928–i930. ECCO, Copenhagen, Denmark. Mar 2023.

45. S Lin*, **N Chanchlani*,** R Smith, C Roberts, R Nice, T J McDonald, B Hamilton, C Bewshea, N A Kennedy, J R Goodhand, T Ahmad, PANTS consortium, P662 Pre-treatment vitamin D concentrations do not predict therapeutic outcome to anti-TNF therapies in biologic-naïve patients with active luminal Crohn's disease, *Journal of Crohn's and Colitis*, Volume 17, Issue Supplement_1, February 2023, Pages i791–i793. ECCO, Copenhagen, Denmark. Mar 2023. Also presented at BSG Live 2023, Liverpool, UK. June 2023.
46. **Chanchlani N**, Lin S, Thomas A, Hamilton B, Nice R, Chee D, Kennedy NA, Goodhand JR, Ahmad T, IMSAT study investigators. HMO-4 Immunogenicity to second anti-TNF therapy (IMSAT): implications for sequencing of biologic therapy. Gut. 70(Suppl 4):A31.2-A32, BSG Annual Conference. November 2021. (Highly Commended).
47. Windak H, Cairnes V, **Chanchlani N**, Desmond C, Hamilton B, Heerasing N, Hendy P, Lin S, Kennedy NA, Goodhand JR, Ahmad T, Gordon C. Outcomes of a GP outreach programme to offer colonoscopic surveillance for IBD patients being managed in primary care. J Crohn's Colitis 2020;**14**(Supplement_1):S668–9. ECCO, Vienna. February 2020.
48. Gordon C, Chee D, Hamilton B, **Chanchlani N**, Heerasing NM, Hendy P, Lin S, Wesley E, Daniels I, Goodhand JR, Kennedy NA, Ahmad T. Root cause analysis to identify missed opportunities for the diagnosis of colorectal cancer in inflammatory bowel disease. J Crohn's Colitis 2020;**14**(Supplement_1):S273–5. ECCO, Vienna. February 2020.

49. Walker G, Lin S, Thomas A, **Chanchlani N,** Moore L, Hendy P, Heerasing N, Green H, Bewshea C, Goodhand J, Kennedy N, Mole S, Ahmad T. Prospective cohort to identify factors associated with diagnostic delay in patients with inflammatory bowel disease. J Crohn's Colitis 2019;**13**(Supplement_1):S181. ECCO, Denmark. March 2019. BSG, Glasgow. June 2019.