Therapeutic Drug Monitoring of anti-TNF biologics in patients with Crohn's disease at Groote Schuur Hospital, Cape Town, South Africa

Student Name: Mohamed Yaaseen Sungay

Student number: SNGMOH003

Supervisor: Professor Gillian Watermeyer

Co Supervisor: Professor Mashiko Setshedi

A research report submitted to the Faculty of Health Sciences at the university of Cape Town, in partial fulfilment of the requirements for the degree of Masters of Medicine

FORM D19

Plagiarism Declaration

"This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor."

Name: Mohamed Yaaseen Sungay

Student Number: SNGMOH003

Signature Signed by candidate

Date:12 December 2023

Contents

Declaration

I, Dr Mohamed Yaaseen Sungay, hereby declare that this dissertation is based on my original research (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being or is intended to be submitted for another degree at this or any other university.

Signature:

Signed by candidate

Mohamed Yaaseen Sungay

Date: 12 December 2023

Cover letter

Full Title: Therapeutic Drug Monitoring of ant-TNF biologics in patients with Crohn's disease at Groote Schuur Hospital, Cape Town, South Africa

Student Name: Mohamed Yaaseen Sungay

Student Number: SNGMOH003

Cell phone number:0834237869

Email: ysungay@gmail.com, SNGMOH003@myuct.ac.za

Address: 7 Protea close Pinelands Cape Town

Supervisor: Professor Gillian Watermeyer

Position: Gastroenterology Consultant

Cell phone number: 083 571 7024

Email: gillian.watermeyer@uct.ac.za

Address: Department of Gastroenterology E23, Groote Schuur Hospital

Co Supervisor: Professor Mashiko Setshedi

Position: Gastroenterology Consultant and Head of department

Email: Mashiko.setshedi@uct.ac.za

Cell phone number:060 424 6664

Address: Department of Gastroenterology E23, Groote Schuur Hospital

Authors: Mohamed Yaaseen Sungay, Gillian Watermeyer, Mashiko Setshedi

Author affiliations:

1. Department of medicine, faculty of health science, university of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

2. Division of Gastroenterology, Department of medicine at Groote Schuur Hospital, Cape Town South Africa

Corresponding Author: Dr Mohamed Yaaseen Sungay

Email: ysungay@gmail.com

Word count

Abstract: 405

Manuscript: 7928

Keywords:

Biologics, infliximab, adalimumab, Inflammatory Bowel Disease, Crohn's disease,

Therapeutic Drug Monitoring.

Disclaimer:

All views expressed in this article are our own and not an official position of the institution or funder.

Abbreviations:

ADA Anti-Drug Antibodies

CD Crohn's disease

CRP C-reactive protein

HBI Harvey Bradshaw Index

IBD Inflammatory Bowel Disease

IMM immunomodulators

MMF Mycophenolate mofetil

MRI Magnetic resonance imaging

PNR Primary non-response

SLR secondary loss of response

TDM Therapeutic drug monitoring

TLs trough levels

TNF α Tissue necrosis factor α

UC Ulcerative colitis

Abstract

Monoclonal antibodies targeting Tumour Necrosis Factor-α (TNF α) have revolutionised the management of Inflammatory Bowel Disease (IBD) and have proved highly effective in both inducing and maintaining remission in both ulcerative colitis (UC) and Crohn's disease (CD). The advent of Therapeutic Drug monitoring (TDM) in recent years has allowed further optimisation of their use. TDM involves the measurement of serum trough levels (TLs) and anti-drug antibodies (ADAs), with higher serum drug concentrations and the absence of ADAs associated with favourable therapeutic outcomes. Adjustment of anti-TNF therapy based on reactive TDM in patients who are either primary non-responders or have secondary loss of response to these biologics is associated with superior clinical outcomes when compared to empiric escalation of therapy. In this study we explore the efficacy of TDM since it was first implemented in our practice and the impact on clinical outcomes in patients with CD in our resource limited setting.

Method

A retrospective cohort study was performed on all patients with CD treated with an anti-TNF biologic, either infliximab or adalimumab, who underwent TDM since it was first implemented in the IBD clinic at Groote Schuur hospital, Cape Town, South Africa between July 2018, and March 2023. Hospital records were analysed, and relevant demographic variables and clinical outcomes were extracted.

Results

Sixty-nine patients with Crohn's Disease started treatment with an Anti TNF, of which 53 were identified to have had undergone reactive TDM. Forty seven (90%) were treated with an immunomodulator prior to starting anti TNF therapy. The median time from initiation of anti-TNFs to first TDM was 9.5 months (IQR5-35); 35 patients (67%) had sub-therapeutic trough levels at that time. No significant predictors of sub-therapeutic trough levels were identified, notably there was no association with disease activity, behaviour, location, or the presence of perianal disease. Adjustment of anti-TNF therapy based on reactive TDM was only performed in 24 patients (45.3%); in all other patients there was no adjustment to the anti TNF therapy. Escalation of biologic therapy based on TDM results in patients with sub-therapeutic TL and no ADAs did not impact clinical remission or response rates at 3 or 6 months of follow up.

Conclusion

In our cohort most patients that underwent TDM had sub-therapeutic trough levels; no significant predictors of sub-therapeutic trough levels were identified. Neither dose optimisation or switching to a 2nd anti-TNF proved effective in achieving clinical remission at either 3 or 6 months of follow up.

Literature review

Introduction

The term Inflammatory bowel disease (IBD) refers primarily to two conditions, ulcerative colitis (UC) and Crohn's disease (CD). Both diseases are chronic inflammatory disorders of the gastrointestinal tract.[1] Failure to treat these conditions effectively can lead to progressive and irreversible damage which may result in hospitalisation, surgery, and ultimately have a profound negative impact on quality of life. The precise cause of IBD remains unclear, however it is accepted as multifactorial involving various factors such as genetic predisposition, mucosal barrier dysfunction, the gastrointestinal microbiome, a dysregulated immune response, and environmental triggers.[2]

With the exception of backwash ileitis, UC is limited to the colon, and in most cases involves the rectum with varying degrees of proximal extension. In contrast, CD can affect the GIT at any point between the mouth and the anus, with a predilection for the terminal ileum and caecum.[1]

Crohn's disease

The presentation of CD is protean and is currently classified by the Montreal classification according to disease behaviour, disease location, and age at onset (Figure 1). At diagnosis most patients present with uncomplicated inflammatory luminal disease (B1).[3] Over time however the majority will progress to complicated penetrating (B3) or stricturing phenotypes (B2), often requiring surgery and hospitalisation.[4]

Figure 1. Montreal Classification for Crohn's disease.[5]

Montreal Classification for Crohn's disease		
Crohn's disease		
Age of onset	≤16 years old	A1
	17-40 years old	A2
	> 40 years old	A3
Location	Terminal Ileum	L1
	Colon	L2
	Ileocolonic	L3
	*Upper Gastrointestinal	L4
Behaviour	Non-stricturing, non-penetrating	B1
	Stricturing	B2
	Penetrating	B3
	**Perianal	P
*L4 can be added to L1, L2 and L3 if Upper Gastrointestinal disease is also present as a modifier		
**P can be added to B1, B2 and B3 if perianal disease is also present as a modifier		

Crohn's disease can affect any part of the gastrointestinal tract, most commonly the terminal ileum and colon.[4] In general disease location remains stable over time, but disease behaviour often evolves to become either stricturing or penetrating in nature, regardless of age at diagnosis or disease location.[3] During the first few years of disease the inflammatory B1 phenotype predominates, but over time most patients experience complications and are classified as having penetrating or stricturing disease. [3] The location of disease is often associated with a specific disease phenotype; ileal disease is frequently stricturing in nature, while colonic/ileocolonic disease is more likely to manifest with penetrating disease. [3]

Most patients with luminal CD have a chronic intermittent disease course, with 13% having an unremitting disease course, and 10% having disease remission.[3]

It is clinically important to determine not only disease location and behaviour, but also the severity of disease as this will guide initiation and/or escalation of therapy appropriately. [3]

The Harvey Bradshaw Index (HBI) is a simplified disease activity score, which uses patients' symptoms and signs to define disease activity. [6] By calculating the sum of five parameters, disease activity is categorised as remission, mild, moderate, or severe disease

(Figure 2). The HBI also incorporates extraintestinal manifestations. It is important to note that disease activity should not be confused with disease severity and the HBI index is limited because its score is largely symptom based.[7]

Figure 2. Harvey Bradshaw index -for disease activity in Crohn's disease.[6]

Harvey Bradshaw index -for disease activity in Crohn's disease		
Parameters	Severity	Score
General well Being	Very well	0
	Slightly below average	1
	Poor	2
	Very poor	3
	Terrible	4
Abdominal Pain	None	0
	Mild	1
	Moderate	2
	Severe	3
Number of liquid stools		1 for each liquid stool per day
Abdominal mass	None	
	Dubious	
	Definite	
	definite with abdominal tenderness	
Complications	Arthralgia	1 point each
	Aphthous Ulcers	
	Abscess	
	Anal Fissure	
	Erythema nodosum	
	New fistula	
	Pyoderma Gangrenosum	
	Uveitis	
Total score calculated	<5	Remission
	5 to7	Mild disease
	8 to 16	Moderate Disease
	>16	Severe disease

At the time of a first flare of luminal CD most patients have mild to moderate disease clinical disease activity. [3]

However, there is poor correlation between symptoms and objective markers of active inflammation; patients with CD that are in clinical remission may well have unknown active, sub-clinical CD.[7]

Fistulizing disease is an important complication in patients with CD. A fistula represents a tract between two surfaces lined by epithelial cells and are usually filled with cell debris, erythrocytes, and inflammatory cells.[8] These tracts are thought to arise due to acute inflammation with infection and suppuration.[8] The cellular composition of fistula in CD compared to non-CD fistula differs markedly and the presence of epithelial lining in a subgroup of fistula may be important for the management and healing process.[9]

In a population-based study, one third of patients with Crohn's disease developed a fistula at least once during the disease course, of which perianal disease is the most common.[8] The cumulative incidence of perianal fistulas is 12% at 1 year, 15% after 5 years, 21% after 10 years, and 26% after 20 years.[8] The incidence of perianal fistulas depends on the disease location and is more common in patients with colonic CD with rectal involvement and rare in patients with isolated ileal disease.[8] Most patients have both luminal and perianal CD with only 5 % of patients having isolated perianal disease without luminal involvement. [8] Perianal disease is associated with significant impairment in quality of life and is an independent predictor of IBD related work disability.[10]

Perianal fistulas are classified as simple or complex. Simple fistulas are those with a single external opening and no evidence of abscess formation, no rectovaginal fistula, no inflammation of the rectum or anorectal stricture.[8] Simple fistulas have a higher rate of healing and may be amenable to surgical intervention.[8] A low fistula is one where the tract penetrates the lower third of the external anal sphincter.[10,11] Active rectal disease associated with complex fistulas complicates management. [10,12] Malignant transformation of fistula tracts is a concerning rare feature of fistulizing CD. [8]

The diagnosis of fistulizing Crohn's disease requires different techniques from luminal Crohn's disease, which include examination under anaesthesia and imaging such as endoscopic ultrasonography or magnetic resonance imaging (MRI).[8] Contrast enhanced pelvic MRI should be the first procedure to assess perianal fistulizing Crohn's disease.[8] The benefits of examination under anaesthesia has the advantage of performing concomitant surgical procedures.[8] Treatment of fistulizing Crohn's disease requires a multidisciplinary

team. Treatment often includes antibiotics, Immunomodulators, Anti TNF therapy and surgery. [8]

Conventional therapies for CD

Pharmacotherapy remains a foundation in the treatment of patients with inflammatory bowel disease, with most patients requiring lifelong therapy due to the chronic nature of the disease, with surgery reserved for medically refractory CD.[2,13] Historically conventional therapies in the form of azathioprine, methotrexate, 6-mercaptopurine, and corticosteroids were the treatment for CD.[2,13] These therapies however did little to change the natural history in many patients' refractory disease.

5-aminosalicylates

5-aminosalicylates (5-ASAs) have long been used in patients with IBD, especially ulcerative colitis. Efficacy was noted as early as the 1940s when this class of drugs showed improvement of gastrointestinal symptoms in patients with concurrent arthritis.[14] The exact mechanism of 5-ASAs remains unknown but they are thought to have anti-inflammatory effects, mostly topical at the level of the mucosa rather than systemic.[14] The active therapeutic compound in sulfasalazine is 5-aminosalicylate, with sulphapyridine acting as a transport molecule to deliver 5-ASAs to the colon.[14] Sulphapyridine is also responsible for most of the side effects of sulfasalazine which has led to the development of sulpha free 5-ASA preparations.[14]5- amino salicylates are used for the treatment of mild to moderate UC; the benefits in CD are however less clear.[15] A Cochrane review reported no significant benefit of 5-ASA over placebo for inducing response or remission.[7] Most guidelines recommend against the use of 5-ASAs in CD with a possible exception being colonic involvement.[7] Clinical studies suggest that sulfasalazine may be more effective for colonic disease versus other sites with a consensus group recommending it be limited to low-risk patients with colonic disease.[7]

Thiopurine therapy

Thiopurines are important immunomodulators in the management of CD. Thiopurine therapy consist of 6- mercaptopurine and its prodrug azathioprine which are purine analogues that directly interfere with nucleic acid synthesis and reduce cell proliferation and inhibit cell

growth thereby dampening the inflammatory process.[2,16] Thiopurines also inhibit the activation and proliferation of T-lymphocytes. [2,16]

These drugs are slow acting with a time of onset of three to four months and therefore should not be used as monotherapy for the induction of remission.[7,16] In a Cochrane review, thiopurine were shown to provide a modest benefit over placebo for induction of remission in active CD, however the evidence is of moderate quality.[16] Thiopurines are more effective than placebo for the maintenance of remission in CD, but the quality of evidence is poor. [17]

Thiopurines have many side effects such as bone marrow suppression, specifically leukopenia and thrombocytopenia.[2] Other toxic side effects include fever, arthralgias, liver injuries, gastrointestinal intolerance, and an increased risk of malignancy[16]. The increased risk of developing myelosuppression with thiopurines is associated with deficiency of the enzyme Thiopurine methyltransferase (TPMT). [16]

Methotrexate

Methotrexate, a folate antagonist, is another immunomodulator used to treat CD. Methotrexate can inhibit multiple enzymes related to DNA synthesis and downregulates an assortment of inflammatory cytokines, subsequently inhibiting T lymphocyte proliferation and the inflammatory response.[2] It is the principal alternative to thiopurine therapy.[18] The bioavailability and pharmacokinetics of intra-muscular methotrexate are very similar to subcutaneous methotrexate, the latter is thus the preferred method of administration and has been shown to be superior to oral administration.[1] Methotrexate is effective in inducing and maintaining clinical remission in patients with refractory CD, as well as allowing discontinuation of steroid therapy as suggested from evidence in a randomized controlled trial.[18] Conversely, oral methotrexate does not show any benefit over placebo or thiopurine therapy in the induction of remission in patents with Crohn's disease.[18] With regards to maintenance of remission in CD, there is moderate quality evidence indicating that intramuscular methotrexate of 15mg/week is superior to placebo.[19] Oral Methotrexate does not appear effective for maintenance of remission in patients with CD.[19] Methotrexate can cause fatigue, nausea, vomiting, atypical pneumonia, hypoalbuminemia[2], yet remains an important part of the arsenal in management of steroid refractory or steroid dependant CD.

Corticosteroids

Corticosteroids blunt the inflammatory response by down regulating the production of inflammatory cytokines such as Interleukin (IL)1, IL-6, and tumour necrosis factor alpha (TNF-α), as well as inhibiting protein synthesis.[20] Corticosteroids can be used effectively to induce symptomatic remission in patients with CD and remain a cornerstone in the management of IBD.[20] However, corticosteroids should not be used for maintaining remission in patients with CD, due to a high potential of serious side effects and lack of long-term efficacy.[2] A Cochrane review recommended against the routine use of steroids for maintaining remission, as well as in non-active CD as these agents did not show any significant reduction in disease recurrence over a 24 month period.[21] Common side effects include opportunistic infections, hypertension, osteoporosis, cataracts, and avascular bone necrosis.[7] Steroids should also be used with caution in specific populations like poorly controlled diabetes and patients with a history of steroid-induced psychosis.

Antibodies targeting Tumour Necrosis Factor alpha (TNF-α).

TNF-α is a pro-inflammatory cytokine that play an important role in the pathogenesis of CD, driving inflammation and reducing apoptosis and proliferation.[2] Overexpression of TNF-α can lead to chronic inflammation, autoimmune disease, and tissue damage.[2] Anti-TNF monoclonal antibodies exert their effect by inhibiting the inflammatory process associated with TNF-α. [2]

The advent of monoclonal antibodies targeting TNF-α towards the end of the last century was a breakthrough in the treatment of CD. In 1993 the first patient was successfully treated for CD with a chimeric monoclonal IgG1 anti-TNF antibody infliximab.[22] Subsequently several additional anti-TNFs have entered clinical practice. These newer anti-TNF agents are structurally different from infliximab, designed to improve side effect profile and prolong drug half-life.[22] Adalimumab and golimumab are IgG1 monoclonal antibodies targeting TNF-α, which are given subcutaneously. Certolizumab is IgG1 monoclonal antibody which is bound to polyethylene glycol and prevents the interaction with the Fc receptor.[22] Infliximab, adalimumab, and certolizumab are the only anti-TNFs approved in CD.[22]

Anti-TNF agents are proteins which are recognised as foreign by the human body leading to the formation of anti-drug antibodies (ADAs).[23] The formation of ADA is more problematic

with infliximab as it is more immunogenic than adalimumab due to the chimeric nature of its structure.[24,25] ADAs are predominantly directed against the mouse portions of the infliximab molecule and are thus sometimes referred to as human anti-chimeric antibodies (HACA).[26] The formation of ADAs can be circumvented by giving regular, scheduled maintenance therapy rather than episodic treatment as well as using them in combination with an IMM such as azathioprine which reduces immunogenicity.[27,28] It has been shown that the combination of an anti-TNFs with IMMs is associated with improved outcomes.[29]

Infliximab

Infliximab is a chimeric monoclonal anti-TNF antibody, composed of a human constant region of immunoglobulin G1 antibody coupled to a variable region of a mouse anti-TNF-alpha monoclonal antibody.[24] Infliximab has the ability to fix complement and lyse cells expressing TNF alpha, which encourages downregulation of inflammation in the gastrointestinal mucosa and is highly effective in inducing and maintaining remission in CD.[28] Infliximab is administered intravenously so that 100% is bioavailable and clearance is via catabolism with low volume of distribution due to its high molecular weight and hydrophilic properties; the median half-life is 14 days.[24]

The induction dose of infliximab for moderately to severely active Crohn disease, including fistulizing disease, is 5 mg/kg intravenously at 0,2, and 6 weeks.[24]

Patients who respond to induction therapy will require repeat infusions of 5 mg/kg eight weekly to maintain remission.[24]

Patients who have a disease flare while on maintenance dosing can be managed by escalating the dose or shortening the dosage interval. Individuals who achieve an incomplete response can be managed in a similar fashion. Dose escalation can be accomplished by either decreasing the dosing interval (from eight weeks to six weeks) or by increasing the dose (from 5 mg/kg to 10 mg/kg), the maximal dose of 10 mg/kg every four weeks.[30]

Adalimumab

Adalimumab is fully humanised IgG1 kappa monoclonal antibody targeting TNF-α.[25] Adalimumab is given subcutaneously. The bioavailability was shown to be 64% after subcutaneous injection in healthy volunteers, with a half-life of 2 weeks.[25] Antibodies against monoclonal antibodies are associated with infusion reaction and loss of efficacy.[25] Fully humanised human monoclonal antibodies tend to be less immunogenic compared to chimeric monoclonal antibodies like Infliximab.[25]

Induction therapy consists of 160 mg at week 0, followed by 80mg at week 2, and then 40mg every other week.[25] Patients who have a disease flare while on maintenance dosing can be managed by escalating the dose to weekly.[31]

Mechanism of action of anti-TNFs

TNF-α is a pleiotropic, highly pro-inflammatory cytokine synthesized as a transmembrane protein on the surface of the cell, of which the extracellular part is cleaved off by TNF-α converting enzyme (TACE) to release soluble TNF-α.[22,32] Both soluble TNF and its precursor transmembrane TNF are involved in the inflammatory response, with transmembrane TNF-α exerting its biological function in the cell's local environment, whereas soluble TNF-α can exert its effects at distant sites from where it was produced.[32] Soluble TNF-α has been implicated in TNF mediated inflammatory pathology in Crohn's disease and Rheumatoid Arthritis.[32] TNF-α induces inflammation by recruitment of immune cells and the advancement of tissue destruction.[33]

TNF exerts its effect on two receptors namely TNF receptor 1 (TNFR) and TNFR2 with distinct signalling pathways.[33] TNFR1 is activated by both soluble TNF and transmembrane TNF.[22] TNFR1 activates a capase-8 dependant cell death signalling pathway which results in the activation of nuclear factor kappa B transcription factor family which is an activating switch for the innate immune response that induces important anti-apoptotic response.[22] The expressions of TNFR2, usually activated by transmembrane TNF, is restricted to specific cells such as neurons, immune cells, and endothelial cells and does not activate the capase-8 dependant death signalling pathways; rather it mediates local homeostatic effects such as cell survival and tissue regeneration.[22,33]

The mechanism of action of anti TNF agents is largely through the binding and neutralisation of soluble TNF with distinct biological effects against transmembrane TNF, which in part may explain the difference in clinical efficacies.[32] New approaches to block TNF effective in Rheumatoid Arthritis have failed in Crohn's disease, which indicates that mechanisms other than neutralization of soluble TNF may be at play. [22]There are two mechanism of actions that have emerged in recent years which are the induction of lamina propria T cell apoptosis and Fc region-dependant induction of M2-type healing macrophages.[22]

New targets

Anti-TNFs are effective in inducing and maintaining clinical response and remission in patients with CD.[29]

However, resolution of clinical symptoms is no longer considered a sufficient target in CD, as these correlate poorly with more objective markers of inflammation, such as endoscopic, radiographic, and biochemical activity.[2] In recent years there has been a move to target these harder endpoints, notably biochemical remission (normalisation of C-reactive protein and faecal calprotectin), endoscopic remission (absence of mucosal ulceration), and radiographic response and remission.[34] Deep remission is defined as clinical, biochemical, and endoscopic remission, and this endpoint is associated with superior outcomes such as a reduction in hospitalisation and surgery and alters the disease course in a significant percentage of patients with IBD.[2,35]

According to the Stride II guideline updated in 2021 the most important long-term goals in the treatment of patients with CD are clinical remission, endoscopic healing, restoration of quality of life, and absence of disability.[36] Clinical response is considered an immediate target to achieve with an adequate response being 50% reduction of abdominal pain and stool frequency using a patient- reported outcome tool (PRO2) with a strong recommendation to change treatment if this is not achieved.[36] However clinical remission, indicated by a Harvey Bradshaw Index of less than 5, a PRO 2 abdominal score of less than or equal to 1, and a stool frequency score of less than or equal to 3 is considered an intermediate/medium term target with strong recommendation to change therapy if these are not achieved; clinical response and clinical remission are no longer considered sufficient to be long term targets.[36]

Biochemical markers, specifically C reactive protein (< than the upper limit of normal) are considered a short to intermediate/ medium term target, while a Faecal calprotectin (FC) value between 100-250mcg/g is considered an intermediate target.[36] The cut off value of FC is dependent on the targeted outcome, with lower thresholds (≤100mcg/g) proposed to echo deep endoscopic or histological healing, and higher thresholds less stringent results.[36] Both these markers are used frequently in CD; however, FC outperforms CRP.[36] In a recent meta-analysis FC showed 72% specificity and 82 % sensitivity in identifying patients with endoscopic disease activity and was noted to be a reliable marker to indicate endoscopic disease activity in patients with IBD.[36]

Endoscopic healing is associated with improved long-term outcomes and reduced damage to the bowel.[36] In patients in clinical remission, ongoing mucosal inflammation is associated with disease complications, Crohn's disease flares, and surgeries.[36] Endoscopic remission is a long-term target however endoscopic response should be achieved in a shorter duration.[36] For an adequate endoscopic response more than a 50 % reduction in the Simple Endoscopic Score in Crohn's disease (SES-CD) or the Crohn's disease endoscopic index of disease severity (CDEIS) is required, and for endoscopic remission the aim is a SES-CD of less than or equal to 2 or a CDEIS of less than 3 with absence of ulcerations.[36]

Histological or transmural healing are currently not considered treatment targets in patients with Crohn's disease, and although both outcomes are welcomed there is insufficient validated, reliable evidence to justify intensification of immunosuppressive therapy to achieve these goals.[36]

Non-response

Despite the major benefits of anti-TNFs, up to 30% of patients with IBD are primary non-responders (PNR) and another 20%–50% of patients have a secondary loss of response (SLR) over time requiring dose-intensification or a switch to another biologic such as vedolizumab or ustekinumab. [23]The mechanisms involved in non-response can be explained by the pharmacokinetic and pharmacodynamic properties of the different agents; and can be a consequence of inadequate drug concentrations due to non-immune clearance of the drug, immune medicated pharmacokinetic non-response due to the development of ADAs, or a non-TNF driven inflammatory process (mechanistic failure).[23] Non-immune mediated pharmacokinetic non-response can be due to non-compliance to medication, excessive inflammation, rapid drug clearance, or a low serum albumin.[34] Anti-drug antibody mediated non-response usually presents as secondary non-response during the maintenance phase of treatment.[34] Mechanistic failure is due to an inflammatory process that is driven by cytokines other than TNF; and may present as primary non-response during the induction phase or secondary loss of response.[34]

Primary non-response (PNR)

There is no formal definition of primary non- response because of differences in response criteria such as clinical symptoms, biochemical parameters (CRP and FC), and endoscopic findings.[37] In addition, the timepoint at which to assess response differs among studies and clinical trials. This is further complicated by differences in disease phenotype, location, and treatment regimen.[37] Despite these shortcomings PNR is usually defined as a lack of improvement of objective markers of active inflammation on completion of induction therapy in patients with adequate drug concentrations and no ADAs.[37] The mechanisms behind PNR are not fully understood and are likely due to multiple factors including the pharmacokinetic and pharmacodynamic processes described above.[37]

Risk factors for PNR include a long duration of disease, smoking, extensive small bowel involvement, a normal CRP at the start of therapy, and previous exposure to a biologic agent. [29] PNR can be avoided by targeting therapeutic serum drug concentrations during the induction period to reduce immunogenicity; higher drug levels during the induction period are required due to the high burden of disease activity.[38]

Secondary loss of response (SLR)

SLR occurs when a patient loses the initial response to a biologic agent after successful induction therapy, usually during the maintenance phase.[27] There are several mechanisms that may be responsible for SLR, one of which is non-compliance which needs to be ruled out.[27]

Secondary loss of response can occur due to the formation of anti-drug antibodies (ADAs) which increase the clearance of anti-TNFs and are a consequence of either immunogenicity of the drug or alternatively inadequate serum concentrations. [27,29]

SLR can also be a consequence of low serum anti-TNF levels associated with disease activity, especially with a high burden of inflammation which increases drug clearance independent of ADAs leading to low serum drug levels and therefore further increases the formation of ADAs.[27,38] Other factors increasing clearance are male gender, obesity, and a low serum albumin.[39]

Therapeutic Drug Monitoring (TDM)

The adoption of therapeutic drug monitoring (TDM) in clinical practice has improved anti-TNFs therapy allowing more efficient use of these agents.[40] TDM involves the measurement

of serum drug trough levels (TLs) and ADAs and is used to guide therapeutic decision making.[34] TDM has been used successfully for many years to optimise the use of other medications such as antibiotics and immunosuppressants.

Since the first use of anti TNF agents in the treatment of CD in the late 1990's it was only a matter of time before TDM would be employed in IBD to optimize therapeutic efficacy of these drugs, given the high rates of PNR and SLR.[29] TDM is influenced by multiple variables such as individual pharmacokinetics and pharmacodynamics, variability in assay sensitivity to detect drug levels and ADAs, optimum drug concentration thresholds, and when to perform TDM.[40] The principle behind TDM is to individualize treatment, based on valuable information derived from the pharmacokinetics and pharmacodynamics profile of an individual patient to maximise the efficacy of biologic agents[34]. This is key as anti-TNFs are costly and finding a means to optimize dosing and minimize clinical sequelae of IBD will prove invaluable in a resource limited setting.

TDM involves the measurement of serum drug trough concentrations and ADAs. Loss of response in patients on anti-TNF agent may be attributed to failure to maintain adequate serum drug concentrations and from the formation of ADAs.[29] ADAs are associated with several adverse effects such as hypersensitivity reactions or serum sickness, as well as increase drug clearance.[41]

Older drug-sensitive assays such as the Bridging Enzyme Linked Immunosorbent Assay (ELISA) and Antibody Binding Test (ABT) were limited in their ability to detect ADAs in patients with detectable serum drug levels.[29] Drug interference occurs when the drug in the serum interacts with ADAs that get captured by the same drug initially coated on the ELISA plate.[41] These drug sensitive assays can only measure accurate ADAs in the absence of serum drug which limits their clinical utility.[41] This has led to the development of drug tolerant assays which can detect both free ADAs and bound ADA by dissociating the bound ADAs from the drug to give an accurate reading of the sample.[29] An example of a drug tolerant assay is Homogenous Mobility Shift Assay (HMSA), which in a study was shown to be significantly more sensitive in detecting ADAs even in the presence of high drug concentrations.[29,41]

There are 3 mechanism of drug failure (see Figure 3). The first is mechanistic failure which occurs despite adequate drug levels and is caused by an inflammatory process that is not

inhibited by the anti TNF; this scenario should prompt a swich to another class of biologic such as vedolizumab or ustekinumab, as a 2nd anti-TNF will not be effective. [42]

The second mechanism for drug failure is non-immune mediated pharmacokinetic failure in which patients have low serum drug concentrations and no ADAs. This can be due to non-compliance or due to rapid clearance of the drug.[42]

Finally poor response can be due to immune-mediated pharmacokinetic failure and is caused by the development of neutralizing ADAs resulting in low serum drug concentrations.[42]

The potential benefits of TDM include reduced rates of PNR and SLR, and fewer empiric switches out of class for presumed PNR.[38] TDM also allows more efficient use of biologics by increasing patients' retention on the first agent, reducing the need for unnecessary dose escalation, and allowing dose de-escalation.[38] TDM has been associated with improved quality of life, more rapid onset of remission, less need for combination therapy with immunomodulators and therefore less side effects, and a reduced need for corticosteroids.[38] TDM also allows rapid attainment of therapeutic drug levels resulting in a reduction in the formation of ADAs caused by subtherapeutic drug levels.[38]

There are two main TDM strategies, reactive and proactive TDM.[34]

Proactive TDM

Proactive TDM involves routine and repeated measurement of drug and ADA concentrations, usually at set intervals and regardless of disease activity, followed by adjustment of the treatment to target threshold drug concentrations.[43] The aim of proactive TDM is to prevent loss of response, specifically PNR, by optimising the drug concentration.[43] Higher drug concentrations of biologic agents during and after induction therapy has been shown to be associated with more favourable outcomes.[43] Proactive TDM can also guide de-escalation of anti TNF therapy or discontinuation of IMMs in patients that are in deep remission and have supra-therapeutic drug concentrations.[43]

Proactive TDM can be used in high-risk population groups which include children, patients with a high burden of disease, those with low serum albumin levels, and patient at high risk of immunogenicity receiving anti TNF agents without concomitant immunomodulators.[34] The rationale being that these patients are at risk of developing ADAs due to the high clearance of the drug, proactive TDM can avoid the transition from non-immune mediated to immune

mediated pharmacokinetic failure by optimizing the drug levels to prevent the development of ADAs.[34]

This form of TDM has gained a lot of traction over the past few years although consensus has not been achieved, and the lack of agreement in recommendations has been motivated by a scarcity of high quality of evidence.[44] In a recent systematic review and meta-analysis, there was no benefit of proactive TDM when compared to conventional treatment strategies for achieving or maintaining clinical remission in patients with IBD.[44] This was stable across subgroup analyses including CD, UC, and type of anti-TNF. There was also no benefit when comparing proactive TDM to reactive TDM.[44]

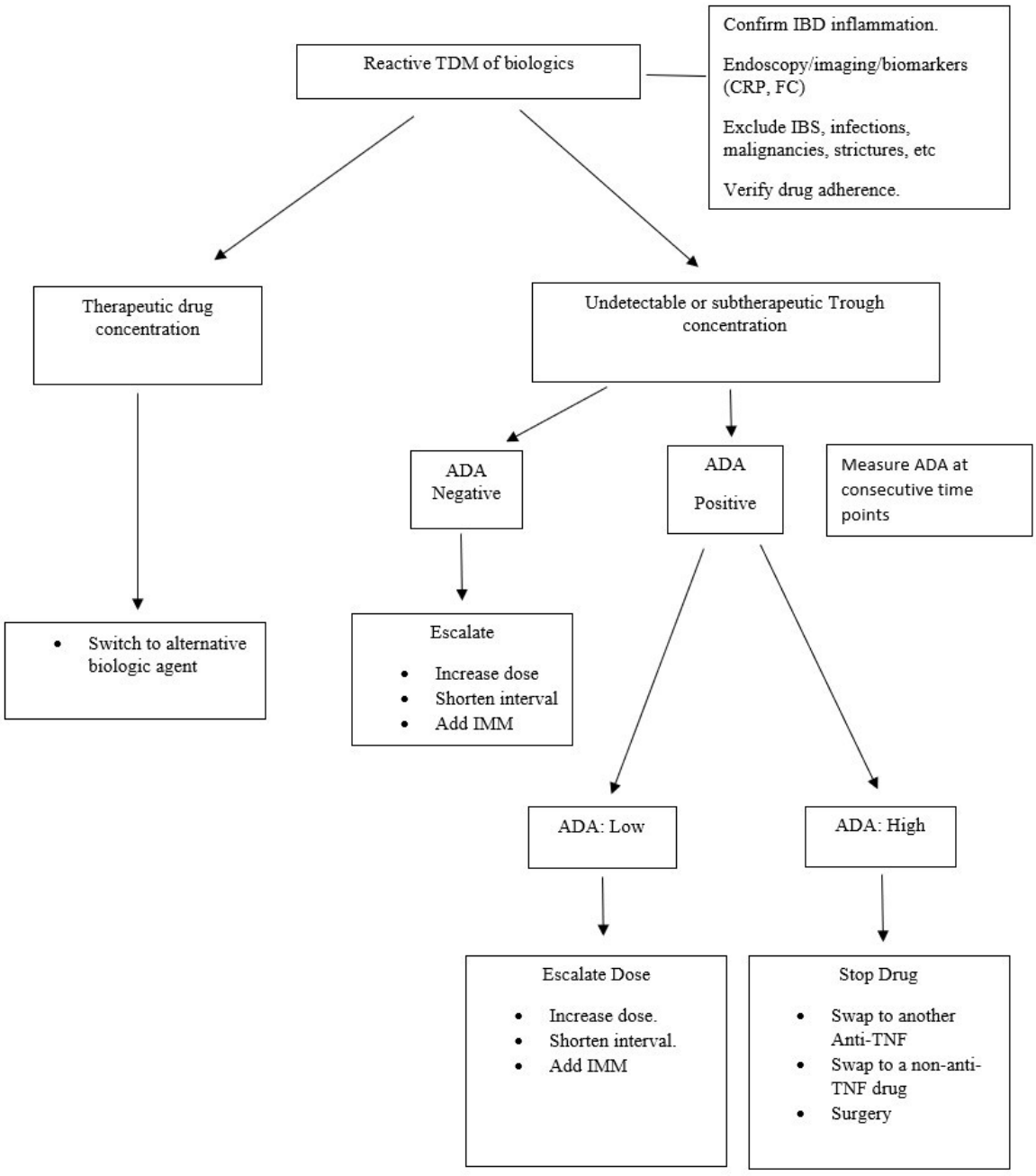
Reactive TDM

In contrast to proactive TDM, reactive TDM has been widely adopted in clinical practice and is considered standard of care in patients with IBD who have either PNR or SLR.[29] Reactive TDM has shown clinical benefits, is cost effective, and has been endorsed by international consensus guidelines.[38] Prior to reactive TDM patients would have empiric dose escalation which now is considered suboptimal and may lead to additional cost.[45] Reactive TDM should be performed on patients with active CD who have PNR or SLR to identify the mechanism behind the loss of response.[46] Reactive TDM also gives an opportunity to optimise the first biologic agent before switching agents; it has long been recognised that the 1st biologic used is associated with higher rates of efficacy when compared to subsequent biologic agents.[46]

With reactive TDM there are four scenarios that can be encountered (see Figure 3), with almost 50 % of patients having undetectable or subtherapeutic drug levels and undetectable ADAs. This situation usually indicates rapid drug clearance or non-immune mediated pharmacokinetics and is resolved by increasing the dosage of drug or shortening the interval between administration.[47] The second and third scenarios are when the patient has undetectable or subtherapeutic drug levels with positive ADAs, which can occur in up to 20% of patients.[47] In these patients some may have a low titre of ADAs antibodies, in this case the options are to increase the dose, shorten the interval, or add an immunomodulator to recapture response.[47] Should the patient have undetectable or subtherapeutic drug levels with high ADAs, which is an immune mediated pharmacokinetic failure, the offending drug should be discontinued, and the patient switched to an alternative anti-TNF as the antibodies are not cross reactive.[47] The last scenario is the patient with therapeutic drug levels; here

inflammation is not driven by TNF, and this should prompt a switch to another drug classer. This situation, termed mechanistic failure, can occur in up to 25% of patients undergoing reactive TDM. [47]

Figure 3. Diagram showing algorithm for reactive TDM.[23,29]



Threshold targets may vary depending on the disease phenotype and outcomes wished to attain, as well as whether TDM is performed during induction or maintenance therapy.[42] Recommended threshold targets for reactive TDM (see figure 4) vary between societies. For example, the American Gastroenterology association recommends trough levels for infliximab ≥5 mcg/ml and ≥7.5mcg/ml for adalimumab for patients with active inflammatory bowel disease during maintenance therapy.[42] These thresholds are likely inadequate during induction therapy as higher levels are likely to be required.[42]

Treatment options for IBD remains limited, therefore optimizing the first biologic usually yields higher rates of efficacy when compared to subsequent biologic treatments and an expert consensus on TDM suggest that biologic discontinuation should not be considered until a trough of at least 10-15 mcg/ml for both infliximab and adalimumab.[46]

Figure 4. Suggested Trough concentrations for reactive TDM [42,46,48]

	Drug	Suggested Trough concentration mcg/mL
AGA- American gastroenterology association (during maintenance therapy)	Infliximab	≥5
	Adalimumab	≥7.5
CATAG - Council of Australian therapeutic Advisory Groups (during maintenance therapy)	Infliximab	≥3
	Adalimumab	≥5
ACG- American college of Gastroenterology	Infliximab	At week 2: >20-25
		Week 6: >15-20
		week 14: 7-10
		Maintenance: 5 to 10
	Adalimumab	At week 4: 8-12
		Maintenance: 8-12

Therapeutic Drug Monitoring of anti-TNF biologics in patients with Crohn's disease at Groote Schuur Hospital, Cape Town, South Africa

Study aim

To date there is no data on reactive TDM in a South African setting. As such the aim of this study was to explore the utility and efficacy of TDM since it was first implemented in our practice and the impact on clinical outcomes in patients with CD in a resource limited setting

Methods and design

We conducted a retrospective cohort study of patients with CD attending the IBD clinic at Groote Schuur Hospital, Cape Town, South Africa, a tertiary academic hospital affiliated with the University of Cape Town. All patients with Crohn's disease treated with an anti-TNF biologic who subsequently underwent reactive TDM for either PNR or SLR between July 2018 and March 2023 were included. Patients that were excluded are patients less the 18 years of age, diagnosed with ulcerative colitis or IBD unclassified and those treated with biologic agents other than Infliximab or adalimumab. A drug tolerant ELISA test performed at a referral laboratory was used in all cases.

Definitions

Based on guidelines from the American Gastroenterology Association, TLs were considered sub-therapeutic for infliximab and adalimumab if less than 5ug/ml and 7.5ug/ml respectively.[42]

The presence of any ADAs was considered positive regardless of titre.

Clinical remission was defined as a Harvey Bradshaw Index (HBI) ≤4

Conventional therapies included azathioprine, 6-mercaptopurine, methotrexate, or mycophenolate mofetil.

Primary non-response was defined as lack of improvement of symptoms or signs, or endoscopic evidence of active inflammation after induction therapy with anti-TNF therapy.

Secondary loss of response was defined when a patient who initially responded to an anti-TNF loses response over time.

Ethical Approval

The study was approved by the Human Research Ethics Committee (HREC) of the University of Cape Town.

Data collection

Patients with CD treated with either infliximab or adalimumab who had undergone TDM were identified from an existing database, as well as a search of NHLS records. Data was collected through a manual review of patient notes.

The following demographic variables were recorded: age at diagnosis, age at initiation of 1st anti-TNF, gender, disease behaviour and location (as per the Montreal classification- Figure 1), smoking status, medication use prior to initiating anti-TNFs, surgery prior to anti -TNF therapy and six months post TDM.

Disease activity was defined by the Harvey Bradshaw Index (Figure 2) and calculated at diagnosis, at the time of first initiating anti-TNF therapy, and 3 and 6 months after performing TDM.

The following data pertaining to TDM was recorded: TLs, ADAs, duration of anti-TNF therapy before undergoing TDM, and action taken based on TDM. Outcomes following the use of TDM included clinical response and remission at 3- and 6-months post TDM, as well as biochemical, radiographic, and endoscopic response.

Statistical analysis

Normally distributed continuous variables are expressed as means ± (standard deviations SDs). Continuous variables that were not normally distributed are expressed as medians and interquartile ranges (IQRs). Continuous variables were compared using univariate analysis. Categorical variables were compared using the $\chi 2$ test, or Fisher's exact test when appropriate. This was performed using STATA version 11 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP). *P* values less than 0.05 were considered statistically significant.

Results

Seventy-four patients with Inflammatory bowel disease treated with an anti-TNF who attended the IBD clinic at Groote Schuur Hospital; 69 (93.3%) had CD, 4 (5.3%) UC, and 1(1.3 %) IBD-unclassified. For the purposes of this study, we limited further analysis to patients with CD who represented most of our cohort.

The median age at diagnosis was 26 years (IQR 19-36 years), and 37(53.6%) were male. Twenty-eight patients (40%) were current smokers. Twenty-one patients had perianal CD,

while 60.3% had ileocolonic involvement (L3), and 50.7% penetrating disease behaviour (B3).

Prior to initiating therapy with an anti TNF, 64 (93%) of patients were receiving treatment with an IMM and/or corticosteroids, the median HBI score was 7 (IQR 5-9), with most patients having a penetrating phenotype (72%) and ileocolonic involvement (66.6%). Forty eight percent had perianal CD, and more than half of the patients had a history of IBD related surgery. (Table 2). The median CRP was 18 (IQR6-58), and the median faecal calprotectin was 888(91-1267). The median albumin was 40 (IQR 35-44). We had 69 patients that were started on Anti TNF therapy, of which 32 patients received infliximab as their 1st anti-TNF and 37 patients received adalimumab.

Fifty-three patients (76.8%) in the cohort underwent TDM for loss of response; no patients had proactive TDM. The median time between initiation of the 1st anti-TNF and first TDM was 9.5 months (IQR). Sixty seven percent of patients treated with either infliximab or adalimumab had subtherapeutic TLs, the median infliximab TL was 1.49 (IQR 0.88-3.97) and the median adalimumab TL was 2.8 (IQR 0.5-10.8). Only 7 (13% of the cohort had positive ADA titres. Sub-therapeutic trough levels were not associated with disease behaviour or location, nor with any biochemical markers of disease activity (Table 4,5). There was also no association between sub-therapeutic trough levels at first TDM and clinical remission at 3 or 6 months (HBI≤4) table 5.

In 54.7% of the cohort no change was made to their anti-TNF therapy. In the sub-group of patients in whom the results of TDM prompted a change in treatment strategy, 87.5% had either an increase in anti-TNF dose or a decrease in the dosing interval. Only 2 patients were switched to a second biologic therapy.

In patients with sub-therapeutic TLs and no ADAs increasing the dose, decreasing the dosing interval, or switching to another biologic did not impact clinical remission rates at either 3 month (OR 0.7; 0.14-3.4, p =0.66) or 6 months (0.67;0.07-6.11, p =0.72) of follow up, when compared to those in whom no action was taken.

At 6 months 75% of the overall cohort had a clinical response as deemed by the attending clinician, 10 patients required IBD-related hospitalization, while none required surgical intervention. Hospitalisation rates and the need for steroids at 6 months was no different in patients in whom TDM prompted a change in therapeutic strategy, when compared to those in whom treatment was unchanged.

Sixty seven percent of patients that underwent a second TDM, after an average of 20 months (IQR 13-48), were found to have sub-therapeutic TLs and 10.8% had positive ADAs. The median trough level for infliximab was 3.66ug/ml (IQR 0.52-4.97) and adalimumab 6.52ug/ml (IQR 4.1-10.39). As seen after the 1st TDM there was no association between 6-month clinical remission or response rates when comparing action versus no action (p=0.47 and p=1.00).

Discussion

We have presented a retrospective review of adult patients known with IBD who have been treated with anti-TNF biologics, specifically infliximab and adalimumab, and who underwent TDM at Groote Schuur Hospital over a 5-year period. In this review we focused on patients with CD which made up more than 90% of the IBD population in our setting. The study describes the demographic profile, clinical features, use of anti TNF agents, and TDM in patients attending the Gastroenterology clinic.

Inflammatory bowel disease patients are started on immunomodulators and/or corticosteroids depending on disease extent, behaviour, perianal disease, and disease activity and severity, with a stepwise escalation of therapy prior to starting biologic therapy.[49] The combination of immune modulators and anti-TNFs is often the therapy of choice in patients with CD who have failed or intolerant of conventional therapies.[50] The SONIC study showed that combination therapy of azathioprine and infliximab was more effective than infliximab monotherapy and azathioprine monotherapy to induce and maintain steroid free clinical remission.[51] Anti TNF therapy, infliximab, and adalimumab, is restricted by budgetary constraints at our facility, and these agents are reserved for patients that has have a poor or inadequate response to maximal conventional therapy. In our study most patients had moderate disease activity prior to starting anti TNFs, with 93% of patients already on IMMs and/or corticosteroids. In addition, most patients had a complicated penetrating disease phenotype and extensive ileocolonic involvement, with more than half of the patients having a history of IBD related surgery; likely representing a group of patients with severe disease that requires escalation of therapy.

In our cohort all patients that had TDM underwent reactive TDM. The most common indication for requesting TDM was SLR, which accounted for 76.8% of patients. In our

practice we do not perform routine TDM after starting anti TNF therapy and therefore no proactive TDM was done.

In our cohort two thirds of patients had subtherapeutic trough levels at the time of the 1st TDM test, while 87% had undetectable ADAs. Subtherapeutic levels in the absence of ADAs is seen in almost 90% of patients undergoing reactive TDM. The far larger numbers with subtherapeutic TLs in our series, compared with the literature, could reflect a higher burden of disease resulting in protein loss through the inflamed mucosa. Given that ours is a large referral centre, most of the complicated CD patients in the Western Cape are under our care; these patients have a high burden of disease which could explain this finding.

Only 13% of our patients had detectable ADA. The small number differs from that shown in other series where detectable antibodies are far more common. This may reflect the high rates of combination therapy in our patients; IMMs are known to reduce to reduce anti-TNF immunogenicity.
Several studies have shown better outcomes in patients with higher drug concentration, including the TAILORIX trial in a post-hoc analysis.[29] In general higher infliximab concentrations during and early after induction phase are associated with favourable short- and long-term outcomes, whereas low or undetectable drug concentrations are associated with PNR, SLR, and treatment discontinuation.[23] Mechanisms underlining SLR include low serum TLs with or without the formation of ADA, or mechanistic failure where TLs are therapeutic and inflammation is driven by cytokines other than TNF.[23] Besides immunogenicity and a high burden of disease, several other factors can contribute to low TLs, such as under dosing, long intervals between doses, and compliance to medication. Our study showed no association between TLs and rates of clinical remission; there was also no association with disease behaviour, location, or perianal disease.

Implementing TDM into clinical practice is plagued with challenges including its availability, cost and lag times for results. A survey suggested that if these challenges were removed, almost all clinicians would use TDM at least reactively.[52] Addressing cost and availability is limited by resources of the region. For TDM to be applied effectively laboratories require access to a drug tolerant assay, and ideally point of care testing to reduce lag times. Point of care blood drug concentration measurement which can be acted on

immediately would facilitate personalised treatment for every patient, much like a glucometer which has transformed treatment for patients with diabetes.

In our study only 45% of all patients undergoing TDM had their treatment adjusted based on test results, through dose escalation, shortening of intervals between doses, switching to a second anti-TNF, or switching to another biologic class. In all other patients no actions were taken. This may indicate poor adherence to TDM guided decision making after the first TDM, which improved after the second TDM. Part of the issue is that there are no universally accepted cutoffs for optimal serum drug concentrations, and physicians may be reluctant to act on results. A study showed increasing infliximab dose based on a combination of symptoms, biomarkers, and serum drug concentration does not lead to corticosteroid free clinical remission in a larger proportion of patients than increasing the dose based on symptoms alone.[50]
There was also no association with remission whether TDM was acted on or not, at either 3 months or 6 months of follow up. This may be explained by the small number of patients in our sample, and it is possible that TDM may have a more meaningful impact if patient numbers were increased.

TDM has increasingly been adopted into clinical practice and is a useful tool in optimising anti-TNF therapy in patients with IBD. However, it can be challenging to implement TDM in real-world clinical settings; in order to address this deficiency clinicians need to be educated, availability increased, costs reduced, lag time minimised, and protocols standarised.[52,53]

Limitations of the study

There are several study limitations. Firstly, all patients were seen in a single tertiary unit which deals with very complicated CD; this will likely bias outcomes. Secondly, given that data was collected retrospectively, there were many variables that were missing which can influence results. In addition, the sample size of the study was very small.
Due to the Covid 19 pandemic and the de-escalation of non-essential services, many patients' routine follow-up visits were cancelled, elective endoscopy and radiographic cross-sectional images studies delayed, as well as impacting negatively on medication access. These factors could have influenced outcomes but were not assessed.

Conclusions

We found that 2 thirds of patients that underwent TDM had subtherapeutic trough levels and that there was no association with low trough levels and remission or disease profile. Significant predictors of subtherapeutic trough levels were not found.

In our study we noted that reactive TDM was not associated with an increase in response or remission rates in patients where TDM was used to adjust therapy. Given the small sample size the true benefit of TDM in a local setting may have been underestimated; this would require a larger study.

Tables

Table 1. At the Time of diagnosis

		Median	*Interquartile range*
Age		26	19-36
Harvey Bradshaw Index		7	5.75-9
		n	***Percentage***
Gender	**Male**	37	53.6%
	Female	32	46.4%
Disease Location	**L1**	9	13.2%
	L2	18	26.5%
	L3	41	60.3%
	L4	0	
Disease behaviour	**B1**	18	26.9%
	B2	15	22.4%
	B3	34	50.7%
Peri anal disease	**Yes**	21	30.9%
	No	47	69.1%
Smoking	**Yes**	26	37.7%
	No	28	40.6%
	Ex smoker	15	21.7%

Table 2. Time of first biologic

		n	*Percentage*
Disease Location	**L1**	12	17.39
	L2	10	14.49
	L3	46	66.67
	L4	1	1.45
Disease behaviour	**B1**	7	10.29
	B2	12	17.65
	B3	49	72.06
Peri anal disease	**Yes**	33	47.83
	No	36	52.17
Treatment Before initiating first Anti TNF	**No treatment**	5	7.25
	Immunomodulators and/or steroid	64	92.75
Smoking	**none**	23	46.94
	Smoker	14	28.57
	ex-smoker	12	24.49
Surgery before first anti TNF	**No**	31	46.38
	Yes	38	53.62
Type of Anti TNF started	**Infliximab**	32	46.38
	Adalimumab	37	53.62
		Median	***Interquartile range***
Harvey Bradshaw index		7	(5-9)
Haemoglobin		12.15	(10.8-13.5)
White cell count		8.83	(6.27-10.84)
Albumin		40	(35-44)
C reactive protein		18	(6-58)
Faecal calprotectin		888	(91-1267)
Platelets		446	(297-522)

Table 3. At the time of first TDM

	First TDM	
Biologic measured	**n**	**Percentage**
Infliximab	23	43.40%
Adalimumab	30	56.60%
Trough level (Infliximab and Adalimumab combined)		
Therapeutic	17	36.69%
Subtherapeutic	35	67.31%
Anti-drug antibodies (infliximab and adalimumab combined)		
No	48	92.31%
Yes	4	7.69%
Action based on TDM		
None (unchanged)	29	54.70%
Action taken	24	45.30%
	Months	IQR
Duration of Anti TNF before TDM (months)	9.5	5-30.5

Table 4. Univariate analysis at first TDM

Association with subtherapeutic trough levels	
	P value
Albumin	0.133
Haemoglobin	0.5
White cell count	0.05
Platelets	0.49
Creatinine	0.14
C reactive Protein	0.09
Faecal calprotectin	0.61
Previous Surgery	0.49
Gender	0.2
Crohn's Disease phenotype	0.38
Peri anal disease	0.21
Crohn disease extent	0.52
Harvey Bradshaw index	0.49
Type of Anti TNF	0.28
Medication (immunomodulators/steroids)	0.52
Number of anti TNF doses	0.28
Smoking	0.435

Table 5. Subtherapeutic Trough Levels at first TDM

			P Value
Subtherapeutic Trough Levels at first TDM, no association with:	behaviour		0.384
	location		0.52
	perianal CD		0.21
HBI remission at 6 months			
HBI remission at 6 months, no association with:	Behaviour		0.5
	Location		0.12
	Perianal CD		0.96
No association at 3 months either			

Table 6. 6 months after first Therapeutic drug monitoring

Response		**n**	**Percentage**
Clinical response		24	75%
No response		8	25%
Hospitalization	**No**	38	79.19%
	Yes	10	20.83%
Surgery	**None**		
		Median	**IQR**
Harvey Bradshaw index		4	(2.5-5)
White cell count		8.19	(7.3-9.7)
Haemoglobin		13.5	(11.9-14.4)
Platelets		328	(285-406)
C reactive protein		10.5	(3.5-19.5)
Biochemical response at 6 months			P value
Haemoglobin			0.44
White cell count			0.71
Platelets			0.317
Creatinine			0.66
Albumin			0.15
Faecal calprotectin			1

11. References

1. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. Vol. 68, Gut. NLM (Medline); 2019. p. s1–106.

2. Cai Z, Wang S, Li J. Treatment of Inflammatory Bowel Disease: A Comprehensive Review. Vol. 8, Frontiers in Medicine. Frontiers Media S.A.; 2021.

3. Michetti P, Juillerat P, Mottet C, Pittet V, Gonvers JJ, Vader JP, et al. Mild-to-moderate active luminal Crohn's disease. Vol. 76, Digestion. 2008. p. 92–8.

4. Cosnes J, Gowerrousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140(6):1785-1794.e4.

5. Spekhorst LM, Visschedijk MC, Alberts R, Festen EA, Van Der Wouden EJ, Dijkstra G, et al. Performance of the Montreal classification for inflammatory bowel diseases. World J Gastroenterol. 2014 Nov 7;20(41):15374–81.

6. Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation Between the Crohn's Disease Activity and Harvey-Bradshaw Indices in Assessing Crohn's Disease Severity. Clinical Gastroenterology and Hepatology. 2010 Apr;8(4):357–63.

7. Panaccione R, Steinhart AH, Bressler B, Khanna R, Marshall JK, Targownik L, et al. Canadian Association of Gastroenterology Clinical Practice Guideline for the Management of Luminal Crohn's Disease. Clinical Gastroenterology and Hepatology. 2019 Aug 1;17(9):1680–713.

8. Scharl M, Rogler G, Biedermann L. Fistulizing Crohn's Disease. Vol. 8, Clinical and Translational Gastroenterology. Nature Publishing Group; 2017.

9. Bataille F, Klebl F, Rümmele P, Schroeder J, Farkas S, Wild PJ, et al. Morphological characterisation of Crohn's disease fistulae. Gut. 2004;53(9):1314–21.

10. Gecse KB, Sebastian S, de Hertogh G, Yassin NA, Kotze PG, Reinisch W, et al. Results of the fifth scientific workshop of the ecco [ii]: Clinical aspects of perianal fistulising crohn's disease-the unmet needs. J Crohns Colitis. 2016 Jul 1;10(7):758–65.

11. Schwartz DA, Loftus E V., Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, et al. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. Gastroenterology. 2002;122(4):875–80.

12. AGA Technical Review on Perianal Crohn's Disease. 2003;

13. Burger D, Travis S. Conventional medical management of inflammatory bowel disease. Gastroenterology. 2011;140(6):1827-1837.e2.

14. Akobeng AK, Zhang D, Gordon M, Macdonald JK. Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's disease. Vol. 2016, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2016.

15. Lim WC, Wang Y, Macdonald JK, Hanauer S. Aminosalicylates for induction of remission or response in Crohn's disease. Vol. 2016, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2016.

16. Chande N, Townsend CM, Parker CE, Macdonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. Vol. 2016, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2016.

17. Chande N, Patton PH, Tsoulis DJ, Thomas BS, Macdonald JK. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2015.

18. Mcdonald JWD, Wang Y, Tsoulis DJ, Macdonald JK, Feagan BG. Methotrexate for induction of remission in refractory Crohn's disease. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2014.

19. Patel V, Wang Y, Macdonald JK, Mcdonald JWD, Chande N. Methotrexate for maintenance of remission in Crohn's disease. Vol. 2014, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2014.

20. Benchimol EI, Seow CH, Steinhart AH, Griffiths AM. Traditional corticosteroids for induction of remission in Crohn's disease. Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2008.

21. Steinhart AH, Ewe K, Griffiths AM, Modigliani R, Thomsen OO. Corticosteroids for maintenance of remission in Crohn's disease. Cochrane Database of Systematic Reviews. 2003 Oct 20;

22. Levin AD, Wildenberg ME, van den Brink GR. Mechanism of action of anti-TNF therapy in inflammatory bowel disease. Vol. 10, Journal of Crohn's and Colitis. Oxford University Press; 2016. p. 989–97.

23. Papamichael K, Cheifetz AS. Use of anti-TNF drug levels to optimise patient management. Vol. 7, Frontline Gastroenterology. BMJ Publishing Group; 2016. p. 289–300.

24. Hemperly A, Vande Casteele N. Clinical Pharmacokinetics and Pharmacodynamics of Infliximab in the Treatment of Inflammatory Bowel Disease. Vol. 57, Clinical Pharmacokinetics. Springer International Publishing; 2018. p. 929–42.

25. Plosker GL, Lyseng-Williamson KA. ADIS DRUG PROFILE Adalimumab In Crohn's Disease. Vol. 21, Biodrugs. 2007.

26. Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: A critical systematic review. Vol. 15, Inflammatory Bowel Diseases. 2009. p. 1264–75.

27. Roda G, Jharap B, Neeraj N, Colombel JF. Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. Vol. 7, Clinical and Translational Gastroenterology. Nature Publishing Group; 2016.

28. Adegbola SO, Sahnan K, Warusavitarne J, Hart A, Tozer P. Anti-TNF therapy in Crohn's disease. Vol. 19, International Journal of Molecular Sciences. MDPI AG; 2018.

29. Lodhia N, Rao S. Updates in therapeutic drug monitoring in inflammatory bowel disease. Vol. 28, World Journal of Gastroenterology. Baishideng Publishing Group Inc; 2022. p. 2282–90.

30. Sandborn WJ, Hanauer SB. Infliximab in the Treatment of Crohn's Disease: A User's Guide for Clinicians. 2002.

31. Assche G Van, Vermeire S, Rutgeerts P. Adalimumab in Crohn's disease. Biologics: Targets & Therapy. 2007.

32. Mitoma H, Horiuchi T, Tsukamoto H, Ueda N. Molecular mechanisms of action of anti-TNF-α agents – Comparison among therapeutic TNF-α antagonists. Cytokine. 2018 Jan 1;101:56–63.

33. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol. 2016 Jan 1;12(1):49–62.

34. Wu JF. Therapeutic Drug Monitoring of Biologics for Patients with Inflammatory Bowel Diseases: How, When, and for Whom? Vol. 16, Gut and Liver. Editorial Office of Gut and Liver; 2022. p. 515–24.

35. Papamichael K, Cheifetz AS. Therapeutic drug monitoring in inflammatory bowel disease: For every patient and every drug? Vol. 35, Current Opinion in Gastroenterology. Lippincott Williams and Wilkins; 2019. p. 302–10.

36. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. Gastroenterology. 2021 Apr 1;160(5):1570–83.

37. Papamichael K, Gils A, Rutgeerts P, Levesque BG, Vermeire S, Sandborn WJ, et al. Role for therapeutic drug monitoring during induction therapy with TNF antagonists in IBD: Evolution in the definition and management of primary nonresponse. Vol. 21, Inflammatory Bowel Diseases. Lippincott Williams and Wilkins; 2015. p. 182–97.

38. Sparrow MP, Papamichael K, Ward MG, Riviere P, Laharie D, Paul S, et al. Therapeutic Drug Monitoring of Biologics during Induction to Prevent Primary Non-Response. Vol. 14, Journal of Crohn's and Colitis. Oxford University Press; 2020. p. 543–56.

39. Cheifetz AS, Abreu MT, Afif W, Cross RK, Dubinsky MC, Loftus E V., et al. A Comprehensive Literature Review and Expert Consensus Statement on Therapeutic Drug Monitoring of Biologics in Inflammatory Bowel Disease. Vol. 116, The American journal of gastroenterology. NLM (Medline); 2021. p. 2014–25.

40. Truta B. Therapeutic drug monitoring in inflammatory bowel disease: At the right time in the right place. World J Gastroenterol. 2022 Apr 7;28(13):1380–3.

41. Wang SL, Ohrmund L, Hauenstein S, Salbato J, Reddy R, Monk P, et al. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. J Immunol Methods. 2012 Aug 31;382(1–2):177–88.

42. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S, Gerson L, et al. American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease. Gastroenterology. 2017 Sep 1;153(3):827–34.

43. Papamichael K, Stocco G, Ruiz Del Agua A. Challenges in Therapeutic Drug Monitoring: Optimizing Biological Treatments in Patients With Inflammatory Bowel Disease and Other Immune-Mediated Inflammatory Diseases. 2023.

44. Nguyen NH, Solitano V, Vuyyuru SK, MacDonald JK, Syversen SW, Jørgensen KK, et al. Proactive Therapeutic Drug Monitoring Versus Conventional Management for Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis. Gastroenterology. 2022;

45. Albader F, Golovics PA, Gonczi L, Bessissow T, Afif W, Lakatos PL. Therapeutic drug monitoring in inflammatory bowel disease: The dawn of reactive monitoring. Vol. 27, World Journal of Gastroenterology. Baishideng Publishing Group Inc; 2021. p. 6231–47.

46. Martins C de A, Garcia KS, Queiroz NSF. Multi-utility of therapeutic drug monitoring in inflammatory bowel diseases. Vol. 9, Frontiers in Medicine. Frontiers Media S.A.; 2022.

47. Shmais M, Regueiro M, Hashash JG. Proactive versus Reactive Therapeutic Drug Monitoring: Why, When, and How? Vol. 7, Inflammatory Intestinal Diseases. S. Karger AG; 2022. p. 50–8.

48. of Australian Therapeutic Advisory Groups C. Facilitating the translation of evidence into best practice: Therapeutic drug monitoring in inflammatory bowel disease [Internet]. Available from: www.catag.org.au

49. Ooi CJ, Hilmi I, Banerjee R, Chuah SW, Ng SC, Wei SC, et al. Best practices on immunomodulators and biologic agents for ulcerative colitis and Crohn's disease in Asia. Vol. 34, Journal of Gastroenterology and Hepatology (Australia). Blackwell Publishing; 2019. p. 1296–315.

50. D'Haens G, Vermeire S, Lambrecht G, Baert F, Bossuyt P, Pariente B, et al. Increasing Infliximab Dose Based on Symptoms, Biomarkers, and Serum Drug Concentrations Does Not Increase Clinical, Endoscopic, and Corticosteroid-Free Remission in Patients With Active Luminal Crohn's Disease. Gastroenterology. 2018 Apr 1;154(5):1343-1351.e1.

51. Frédéric Colombel J, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, Azathioprine, or Combination Therapy for Crohn's Disease A bs tr ac t. 2010.

52. Patel RN, Nigam GB, Jatale RG, Desai D, Makharia G, Ahuja V, et al. An Indian national survey of therapeutic drug monitoring with anti-tumor necrosis (TNF) medications in inflammatory bowel disease. Indian Journal of Gastroenterology. 2020 Apr 1;39(2):176–85.

53. Sethi S, Dias S, Kumar A, Blackwell J, Brookes MJ, Segal JP. Meta-analysis: The efficacy of therapeutic drug monitoring of anti-TNF-therapy in inflammatory bowel disease. Aliment Pharmacol Ther. 2023 Jun 1;57(12):1362–74.