Annals of Gastroenterology (2024) **37,** 674-681

Infliximab trough levels among patients with inflammatory bowel disease in correlation with infliximab treatment escalation: a cross-sectional study from a Greek tertiary center

Afroditi Orfanidou[a], Konstantinos Katsanos[a], Theodoros Voulgaris[b], Aristeidis Kofinas[c], Maria Veatriki Christodoulou[d], Maria Konstandi[c], Dimitrios Christodoulou[a]

University General Hospital of Ioannina, University of Ioannina; Aretaieion University Hospital, Athens; University of Ioannina, Greece

Abstract

Background Infliximab monitoring correlates with improved outcomes in inflammatory bowel disease (IBD). We aimed to evaluate the association between serum infliximab trough levels (TLs) and therapeutic outcomes in Greek patients with Crohn's disease (CD) or ulcerative colitis (UC).

Methods This cross-sectional study included consecutive adult patients with IBD receiving intravenous infliximab maintenance therapy at a Greek tertiary center. Therapeutic outcomes assessed were clinical remission (CR), steroid-free clinical remission (SFCR), biochemical remission (BR: C-reactive protein <5 mg/L), and combined (steroid-free and biochemical) remission (SFCBR).

Results Seventy-seven patients participated (62.3% with CD, 16.8% on concomitant immunomodulators), with a mean infliximab infusion duration of 5.1±4.6 years. Forty-seven (61%) patients underwent treatment escalation. Infliximab mean TLs were 7.2±4.9 μg/mL, correlating only with treatment escalation (9.7 vs. 3.6 μg/mL, P<0.001). CR was achieved in 88.3% of patients, SFCR in 80.5%, BR in 62.3%, and SFCBR in 55.8%. In a subgroup analysis, for patients without treatment escalation, higher mean TLs were significantly associated with BR (4.2 vs. 0.8 μg/mL, P=0.020) and SFCBR (4.3 vs. 1.5 μg/mL, P=0.035). In receiver operating characteristic analysis, TLs predicted SFCBR (P=0.016) with good accuracy (area under the curve [AUC] 0.768, 95% confidence interval [CI] 0.584-0.952), with an optimal TL cutoff at 3.4 μg/mL. For patients with treatment escalation, TLs predicted SFCBR (P=0.018) with fair accuracy (AUC 0.653, 95%CI 0.527-0.755), with an optimal TL cutoff at 11 μg/mL.

Conclusions Infliximab TLs correlate with treatment escalation. Higher infliximab TLs may predict combined remission among patients with treatment escalation.

Keywords Inflammatory bowel disease, infliximab monitoring, infliximab trough levels, treatment intensification, treatment escalation

Ann Gastroenterol 2024; 37 (6): 674-681

Conflict of Interest: None

Funding: The diagnostic ELISA kits of infliximab and antibodies-to-infliximab were funded by the Institute of Internal Medicine and Hepatogastroenterology at the University of Ioannina

Correspondence to: Afroditi Orfanidou, MD, PhD candidate of University of Ioannina, Faculty of Medicine, University of Ioannina, Mpizani 455 00, Ioannina, Greece, e-mail: afroditi_orf@hotmail.com

Received 6 July 2024; accepted 8 October 2024; published online 31 October 2024

DOI: https://doi.org/10.20524/aog.2024.0926

Introduction

The introduction of biologic agents in the late 1990s transformed the management of inflammatory bowel disease (IBD), reshaping therapeutic strategies. Infliximab, a chimeric IgG1 monoclonal antibody targeting tumor necrosis factor-α (TNFα), was the first agent used, and to date it remains a cornerstone in providing effective induction and maintenance of disease remission in both Crohn's disease (CD) and ulcerative colitis (UC) [1]. However, a substantial number of patients experience difficulties with the infliximab treatment. Approximately 30% of patients present no clinical improvement following the induction phase (primary non-response, PNR), and an additional 20-40% may develop a secondary loss of response (SLR) over time, with an annual

risk estimated to be around 10-13% per patient year of treatment [2-4]. The SLR is commonly addressed through empirical approaches, such as dose or frequency escalation, the introduction of an immunomodulator, or transition to an alternative anti-TNF agent or to an agent targeting a different pathway of inflammation [5]. Pharmacokinetic and pharmacodynamic mechanisms both play a role in PNR and SLR to anti-TNFα therapy. Pharmacokinetic factors include low or undetectable drug concentrations, which may result from increased non-immune drug clearance, often related to a heightened inflammatory burden, or from immune-mediated clearance due to the development of immunogenicity. This is characterized by the production of antibodies to infliximab (ATI), also associated with immune-mediated reactions, such as infusion reactions. Pharmacodynamic factors are involved when therapeutic drug concentrations are achieved but the patient fails to respond, indicating an inflammatory pathway that is not primarily TNF-driven [6,7].

Several exposure-response relationship studies have shown a positive correlation between infliximab trough levels (TLs) and therapeutic outcomes, including clinical, biochemical, endoscopic and histological remission, in IBD, particularly during maintenance treatment, while certain IBD phenotypes, such as fistulizing CD, perianal CD and acute severe UC, may require even higher drug concentrations because of the increased inflammatory burden [8-14]. Moreover, infliximab TLs have been utilized to optimize treatment in patients with IBD [15-17]. Studies evaluating the drug concentration–effect relationship in IBD suggest a therapeutic window of infliximab at 3-7 μg/mL during maintenance therapy; however, others prefer higher infliximab trough concentrations at 5-10 μg/mL to reach better objective results, including mucosal healing, and to prevent suboptimal drug exposure and the development of ATI in the future [3,6,18,19]. Therapeutic drug monitoring, which involves the measurement of infliximab TLs and ATI, followed by dose titration to maintain levels within a therapeutic range, has become a valuable tool for diagnosing underexposure and guiding dose optimization [20].

Despite extensive recent research in this area, the optimal range of therapeutic infliximab concentrations required to achieve positive therapeutic outcomes remains largely uncertain. Moreover, few therapeutic drug monitoring studies of infliximab have been performed in a Greek population. This study aimed to assess the correlation between serum infliximab TLs and positive therapeutic outcomes in Greek patients with CD or UC, following intravenous infliximab maintenance therapy. In addition, the relationship between the infusion reactions and the presence of ATI was investigated.

[a]Department of Gastroenterology, University General Hospital of Ioannina, University of Ioannina (Afroditi Orfanidou, Konstantinos Katsanos, Dimitrios Christodoulou); [b]Endoscopic Unit, 2nd Department of Surgery, Aretaieion University Hospital, Athens (Theodoros Voulgaris); [c]Department of Pharmacology, Faculty of Medicine, University of Ioannina, Ioannina (Aristeidis Kofinas, Maria Konstandi); [d]Department of Radiology, University General Hospital of Ioannina, University of Ioannina (Maria Veatriki Christodoulou), Greece

Patients and methods

Patients and study design

This observational cross-sectional study included patients with IBD (UC, CD and unclassified colitis) receiving infliximab maintenance therapy at the University Hospital of Ioannina (Ioannina, Greece). These patients were prospectively recruited at the gastroenterology infusion room from January 2019 until July 2019. The study's inclusion criteria included patients aged 18 or older, with a confirmed diagnosis of UC, CD or unclassified colitis that was determined by endoscopic, radiological and/or histological criteria, who had completed at least the induction phase with infliximab 5 mg/kg at weeks 0, 2 and 6 from the initiation of treatment. Patients who declined to participate in the study and those who had not undergone biological therapy for a duration of >9 weeks were not included.

Demographic and clinical data were obtained from each patient. Data gathered at baseline included sex, age, body mass index (BMI), smoking status, type of IBD, family history of IBD, age at diagnosis, disease duration, disease extension, disease behavior, extraintestinal manifestations and prior treatment history, including prior biologic failure and IBD related surgery. Data regarding infliximab treatment were obtained, including treatment duration, treatment escalation, reported adverse events (severe adverse events were considered those leading to hospitalization), concomitant treatment with 5-aminosalicylates, corticosteroids (oral or intravenous budesonide, prednisone or methylprednisolone at any dose taken within the last 8 weeks) or immunosuppressants (azathioprine or methotrexate), and history of combination with immunosuppressants since infliximab initiation. Clinical disease activity was determined using the partial Mayo score in patients with UC and the Harvey-Bradshaw Index (HBI) in patients with CD. Biomarkers of disease activity, including C-reactive protein (CRP) and albumin, were obtained. In addition, infliximab TLs and ATI, if indicated, were measured. Among the therapeutic outcomes explored, clinical remission (abdominal pain ≤1 and stool frequency ≤3 or HBI <5 for CD; rectal bleeding=0 and stool frequency=0 or partial Mayo <3 with no subscore >1 for UC) [21], steroid-free clinical remission (withdrawal of systemic corticosteroids for ≥12 weeks before assessment), biochemical remission (CRP <5 mg/L) and combined remission (steroid-free clinical remission and biomarker remission) were included. Therapeutic outcomes were assessed at inclusion.

The study protocol was approved by the institutional research board of the University Hospital of Ioannina (Protocol Number: 82/27-2-2019). Before joining the study, all participants signed an informed consent form.

Blood sampling and preservation

Blood samples were taken in serum tubes immediately before infliximab infusion from peripheral veins and were

then centrifuged at 3500 rpm for 10 min. Serum was stored in cryotubes at -20°C until analysis.

Determination of infliximab TLs and ATI

Serum levels of infliximab and ATI were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (apDia, Turnhout, Belgium), according to the protocols provided by the manufacturer. The infliximab ELISA test uses a highly specific monoclonal antibody (clone 6B7), developed at the KU Leuven, that detects infliximab originator, as well as infliximab biosimilars. Infliximab trough concentrations were measured in μg/mL. For the calibrator and the controls, the anti-infliximab ELISA test uses a highly specific monoclonal antibody (clone 10F9), developed at the KU Leuven, that only bridges infliximab. According to the manufacturer, it is not accurate in the presence of high infliximab concentrations (drug-sensitive assay), therefore, it is recommended to be used with subtherapeutic infliximab concentrations (<1 μg/mL). ATI concentrations were measured in ng/mL and recorded as positive or negative.

Statistical analysis

The statistical analysis was performed using SPSS V23 (SPSS software; SPSS Inc, Chicago, IL, USA). Data were expressed as frequencies, mean ± standard deviation, or median (interquartile range), as appropriate. Quantitative variables were compared between groups using Student's *t*-test or the Mann-Whitney test for normally distributed and non-normally distributed variables, respectively. Qualitative variables were compared using the chi-squared test or Fisher's exact test, as appropriate. The associations between quantitative variables were assessed using Spearman's correlation coefficient. The area under the receiving operating characteristic (AUROC) curves for trough levels predictability, as well as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The c-statistics of AUROC curves were provided with their 95% confidence intervals (CI). Diagnostic accuracy was considered poor when the c-statistic was <0.65. The optimal cutoff was selected from the AUROC curves as the point which provided the maximum sum of sensitivity and specificity.

Results

Participants, infliximab treatment characteristics, infliximab TLs and ATI

A total of 77 patients (62.3% male, median age 43 years) were enrolled in this study. Twenty-six patients (33.8%) had UC, 48 (62.3%) had CD, and 3 (3.9%) unclassified colitis. The median IBD duration was 10 years, with a median age at diagnosis at 28 years. Of the 77 patients, 61 (79.2%) were biologic-naive prior to infliximab initiation. Among the remaining 16 patients (20.8%), 12 (15.6%) had been exposed to 1 biologic agent, 3 (3.9%) to 2 agents, and 1 (1.3%) to 3 agents. All 16 patients had been previously treated with anti-TNFα (7.8% with infliximab, 9.1% with adalimumab and 7.8% with golimumab). The baseline characteristics of the patients who were included in this study are presented in Supplementary Table 1.

The median duration of infliximab treatment until the measurement of infliximab TLs and ATI was 2.9 years. Infliximab treatment was escalated according to the physician's decision in 47 patients (61%). All of them had a decrease in dose intervals; 35 (45.5%) had an infusion every 4 weeks and 12 (15.6%) every 6 weeks. Seven patients (9.1%) had a further dose escalation, among whom 6 (7.8%) received infliximab 7.5 mg/kg and 1 (1.3%) infliximab 10 mg/kg. Concomitant therapy combined with infliximab at the time of TLs and ATI sampling was given to 13 patients (16.8%); 4 patients (5.2%) received azathioprine as an immunosuppressant, while 9 patients (11.7%) received corticosteroid therapy (Table 1).

Table 1 Characteristics of infliximab treatment in patients with inflammatory bowel disease (n=77)

Characteristics	Value
Infliximab treatment duration, median (range, years)	2.9 (0.2-20)
Infliximab escalated treatment, n (%)	47 (61)
Infliximab dose	
5 mg/kg, n (%)	70 (90.9)
7.5 mg/kg, n (%)	6 (7.8)
10 mg/kg, n (%)	1 (1.3)
Infliximab intervals	
8 weeks, n (%)	30 (39)
6 weeks, n (%)	12 (15.6)
4 weeks, n (%)	35 (45.5)
Adverse events, n (%)	21 (27.3)
Severe adverse events, n (%)	3 (3.9)
Type of adverse events	
Infusion reaction, n (%)	8 (10.4)
Respiratory tract infection, n (%)	7 (9.1)
Influenza-like illness, n (%)	4 (5.2)
Headache, n (%)	4 (5.2)
Atopic dermatitis relapse, n (%)	1 (1.3)
Oral candidiasis, n (%)	1 (1.3)
Leptospirosis, n (%)	1 (1.3)
Herpes zoster, n (%)	1 (1.3)
Pneumonic embolism, n (%)	1 (1.3)
Concomitant treatment	
Azathioprine, n (%)	4 (5.2)
Corticosteroids, n (%)	9 (11.7)
5-Aminosalicylates, n (%)	26 (33.8)
Combo therapy since infliximab initiation, n (%)	18 (23.4)
Azathioprine, n (%)	11 (14.3)
Methotrexate, n (%)	6 (7.8)
Both, n (%)	1 (1.3)
Combo therapy duration, median (range, years)	2 (0.1-9)

Infliximab mean TLs were 7.2±4.9 μg/mL. Mean TLs were only correlated with treatment escalation (9.7 vs. 3.6 μg/mL, P<0.001), but not with sex, BMI, smoking status, IBD type (UC vs. CD), CRP levels, albumin levels or the combined treatment with azathioprine or corticosteroids (Supplementary Table 2). Twelve patients (15.6%) had undetectable infliximab TLs, 9 (11.7%) had TLs that were detectable but <3 μg/mL, 14 (18.2%) had TLs in the range of 3-7 μg/mL and 42 patients (54.5%) had levels >7 μg/mL.

Of the 20 patients with infliximab TLs <1 μg/mL, only 10 patients were positive for ATI.

Correlation of the therapeutic outcomes with TLs and ATI

Sixty-eight patients (88.3%) out of the 77 tested in this study had clinical remission, while 62 (80.5%) had steroid-free clinical remission. Biochemical remission was observed in 48 patients (62.3%). Combined remission was noted in 43 patients (55.8%).

When categorizing patients into groups of TLs ≥3 or <3 μg/mL, no difference was observed in their rates of clinical remission (75% vs. 55.6%, P=0.199), steroid-free clinical remission (74.2% vs. 66.6%, P=0.385), biochemical remission (77.1% vs. 65.5%, P=0.200) or combined remission (79.1% vs. 64.7%, P=0.126), although the rate of TLs ≥3 μg/mL was numerically greater among patients with remission for all therapeutic outcomes.

Among patients with TLs <1 μg/mL, 9 showed combined remission, 4 of whom had ATI detected. Of the 11 patients with no combined remission and TLs <1 μg/mL, 6 (54.5%) had positive ATI.

Correlation of TLs with remission in patients with or without treatment escalation

A subgroup analysis indicated that, among the 30 patients without infliximab treatment escalation, 27 (90%) had clinical remission, while steroid-free clinical remission was observed in 25 of them (83.3%). Biochemical remission was observed in 22/30 patients (73.3%). Combined remission was observed in 19/30 patients (63.3%). Mean infliximab TLs were significantly greater among patients with biochemical (4.2 vs. 0.8 μg/mL, P=0.020) and combined (4.3 vs. 1.5 μg/mL, P=0.035) remission (Table 2, Fig. 1). According to the ROC curve analysis, TLs could accurately predict (AUC 0.768, 95%Cl 0.584-0.952, P=0.016) those patients with combined remission. The optimal TL cutoff was set at 3.4 μg/mL (sensitivity 63.2%, specificity 91%, PPV 92.3%, NPV 58.8%; Fig. 2).

Among 47 patients with infliximab treatment escalation, clinical remission was observed in 41 of them (87.2%), while steroid-free clinical remission was observed in 37 patients (78.7%). Biochemical remission was observed in 26/47 patients (55.3%) and combined remission was observed in 24/47 patients (51.1%). Mean TLs for each therapeutic outcome are shown in Table 2 and Fig. 3. According to the ROC curve analysis, TLs could predict with fair accuracy (AUC 0.653, 95%CI 0.527-0.755, P=0.018) patients with combined remission. The optimal TL cutoff was set at 11 μg/mL (sensitivity 66.7%, specificity 69.6%, PPV 69.6%, NPV 66.7%; Fig. 4).

Infliximab-related adverse events and immunogenicity

Of the 77 patients on infliximab, 21 (27.3%) reported adverse events, with 3 (3.9%) experiencing severe adverse events (leptospirosis, herpes zoster and pneumonic embolism in 1 patient each); however, none of these led to discontinuation of infliximab, as managed by the physician. Eight patients (10.4%) recorded a reaction attributed to infliximab infusion. Infusion reactions showed a tendency to correlate with the presence of ATI. Among the 5 patients who both had ATI measured and reported an infusion reaction, 4 were ATI positive. Conversely, of the 10 patients with positive ATI, 4 experienced an infusion reaction, while only 1 of the 10 patients with negative ATI reported an infusion reaction

Table 2 Subgroup analysis of correlations between infliximab trough levels and clinical remission, steroid-free clinical remission, biochemical remission and combined (steroid-free and biochemical) remission in patients with or without infliximab treatment escalation

Remission	Patients without treatment escalation		Patients with treatment escalation	
	Trough levels (μg/mL)	P-value	Trough levels (μg/mL)	P-value
Clinical remission		0.094		0.142
Yes	3.7±3.6		9.6±3.9	
No	0.0±.0.1		8.2±4.1	
Steroid-free clinical remission		0.375		0.576
Yes	3.6±3.4		9.8±4.0	
No	2.0±4.4		9.3±3.6	
Biochemical remission		0.020		0.314
Yes	4.2±3.7		9.3±4.0	
No	0.8±1.3		10.1±3.8	
Combined remission		0.035		0.114
Yes	4.3±3.5		10.4.1±3.8	
No	1.5±3.0		9.1±3.9	

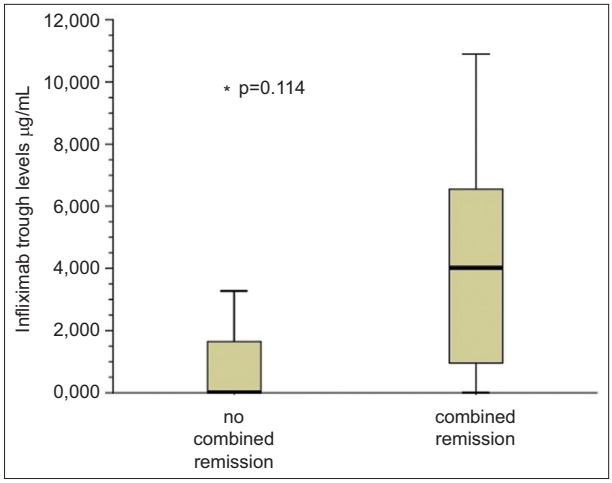

Figure 1 Comparison of infliximab trough levels (µg/mL) among patients without treatment escalation with or without combined remission

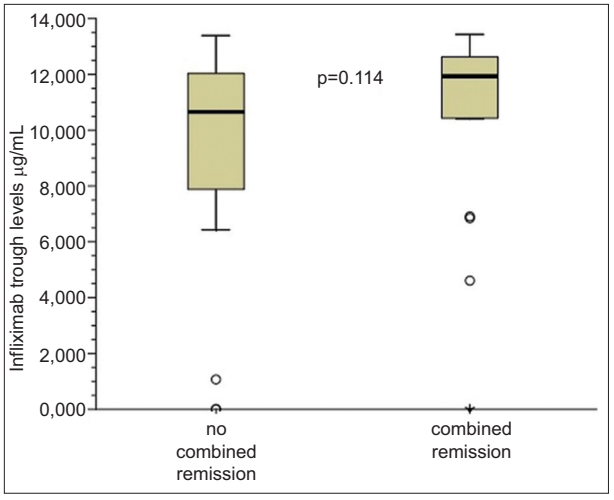

Figure 3 Comparison of infliximab trough levels (µg/mL) among patients with treatment escalation with or without combined remission

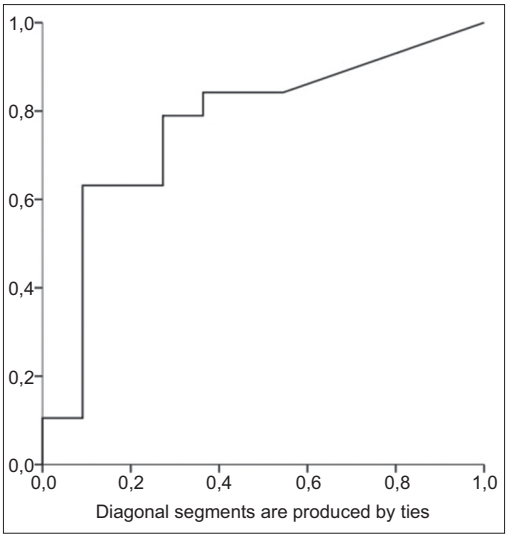

Figure 2 Receiver operating characteristic curve analysis for infliximab trough levels among patients without treatment escalation with combined remission

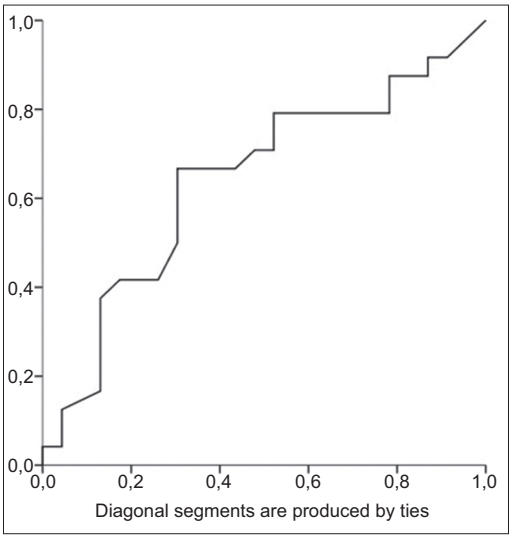

Figure 4 Receiver operating characteristic curve analysis for infliximab trough levels among patients with treatment escalation with combined remission

(P=0.152). It is important to note that these data are limited by the small study groups, as ATI measurements were only conducted in patients with infliximab TLs below 1 µg/mL, as specified by the assay protocol.

Discussion

Our study investigated the correlation between infliximab TLs and positive therapeutic outcomes (clinical, steroid-free, biochemical and combined remission) in Greek patients with IBD. We found a significant correlation between infliximab TLs and treatment escalation (P<0.001). Subgroup analysis revealed that patients without treatment escalation had significantly higher TLs associated with biochemical and combined remission (P=0.020 and P=0.035, respectively). Furthermore, ROC curve analysis showed that TLs could significantly predict the combined remission for both non-escalated and escalated patients (P=0.016 and P=0.018, respectively). The optimal infliximab TL cutoff for achieving combined remission was 3.4 µg/mL for non-escalated patients (sensitivity 63.2%, specificity 91%, PPV 92.3%, NPV 58.8%) and 11 µg/mL for escalated patients (sensitivity 66.7%, specificity 69.6%, PPV 69.6%, NPV 66.7%). These findings show that higher infliximab TLs were associated with clinical and biochemical remission, particularly in patients requiring treatment escalation.

Numerous studies have shown that higher infliximab TLs result in better therapeutic outcomes in terms of clinical, biochemical, endoscopic and histological remission, in patients

with IBD [8-14]. It has also been suggested that patients with specific IBD phenotypes, such as fistulizing or perianal CD, extensive disease or acute severe UC, often require higher drug serum concentrations to attain remission, because of the greater burden of inflammation [10,13,14,22,23].

Interestingly, when we divided patients into 2 groups, based on treatment escalation or not, we found a markedly significant difference in the TLs of patients on a standard regimen with biochemical and combined remission. It should also be noted that CRP as a biomarker, although not specific or highly sensitive for intestinal inflammation, has a positive correlation with clinical and endoscopic activity and therefore, it could predict a relapse of the disease [24,25]. In addition, normalization of CRP between 8 and 14 weeks post-treatment has been shown to predict a sustained long-term response to anti-TNF agents [26-28]. According to STRIDE-II, achieving clinical remission and normalizing CRP levels should be considered as intermediate (medium-term) treatment goals in both CD and UC [21]. Regarding the relationship between infliximab TLs and CRP, there are studies suggesting that CRP levels may serve as an indicator of serum infliximab TLs, in terms of predicting a loss of response. In fact, it was shown that a decline in infliximab TLs <1 μg/mL occurred before the loss of response to the drug, which could also be easily identified by detecting an elevation in CRP [29]. In a study of patients with worsening symptoms while receiving infliximab, CRP levels above 12 mg/L were highly specific in identifying patients with infliximab TLs <3 μg/mL [30]. Roblin *et al* [31], demonstrated that a loss of response to infliximab can be accurately predicted by considering a combination of CRP, infliximab TLs and stable ATI. A previous Greek study showed a decrease in infliximab TLs over time, which was associated with a concurrent increase in CRP levels [32].

To our knowledge, no previous studies have demonstrated that higher infliximab TLs can predict positive therapeutic outcomes specifically in patients requiring treatment escalation, compared to those on standard dosing. In our cohort, patients who had previously undergone treatment escalation empirically due to SLR showed an initial favorable response and remained in infliximab treatment. This suggests that pharmacokinetic, rather than pharmacodynamic factors, may be contributing to the current cases of non-remission that are more frequently associated with SLR [33,34]. This observation likely reflects a higher inflammatory burden in patients who achieved and maintained clinical and biochemical remission after dose escalation. Recent studies advocate for targeting a broader therapeutic range for infliximab (3-10 μg/mL) [3,20,35-37]. This wider range could encompass both patients who have undergone treatment escalation and those on a standard dose regimen.

Several previous studies have explored the association between infliximab TLs and treatment escalation after SLR [38-42]. It has been previously shown that responders exhibited a greater increase in serum infliximab TLs compared to non-responders during treatment intensification (8.8 vs. 3.0 μg/mL, P=0.035 using a reporter gene assay; 9.9 vs. 4.7 μg/mL, P=0.04 using a homogeneous mobility shift binding assay), indicating inadequate drug levels in a subgroup of patients [38]. Furthermore, infliximab TLs ≥1 μg/mL, low circulating interleukin 6 and adequate albumin levels before infliximab dose escalation in patients with loss of response, were significantly correlated with remission 40 weeks after escalation [39]. However, an earlier study found no difference in infliximab TLs at baseline between patients, who responded to treatment intensification and those who did not, suggesting that clinical improvement might occur after infliximab intensification, regardless of TLs [40]. In a retrospective study, infliximab TLs over 3.8 μg/mL identified with 90% specificity non-responders to either dose increase or switch to another anti-TNF agent [41]. Another study of patients with infliximab TLs >3 μg/mL and negative ATI found that TLs were significantly lower among patients in clinical remission compared to those with active symptoms, at both 6 and 12 months following treatment escalation, with an optimal cutoff at 4.8 μg/mL [42]. Nevertheless, Papamichael *et al* [20], suggested that, in cases of active disease during maintenance therapy, discontinuation of the infliximab treatment should be typically avoided unless drug concentrations exceed 10 μg/mL. In our study, there were no available data on infliximab concentrations prior to treatment escalation for comparison. Furthermore, the present study was not conducted under conditions of SLR but presented data only from patients routinely followed in our hospital center.

In our study, ATI were measured in 20 patients using a drug-sensitive assay, capable of identifying ATI only at low infliximab concentrations (<1 μg/mL). Consequently, the presence of ATI may have been underestimated. Recent advances in drug-tolerant assays have addressed this limitation by enabling ATI measurement even in the presence of infliximab, to a certain extent [43]. Nevertheless, the clinical benefit of employing a drug-tolerant assay in the presence of adequate infliximab TLs remains a subject of debate [20,43,44]. Among the 20 patients, 11 did not achieve combined remission, with 6 testing positive for ATI. This suggests a potential correlation between treatment failure and the development of immunogenicity, as indicated by the presence of ATI. However, because of the lack of serial ATI and infliximab TL measurements in our study, we cannot definitively establish this correlation. Intriguingly, among the remaining 9 patients, who achieved combined remission, 4 tested positive for ATI. This occurrence could be linked to a transient appearance of ATI.

A total of 8 patients reported reactions attributed to infliximab infusion. Notably, there was a tendency indicating a correlation between infusion reactions and the presence of ATI. Specifically, among the 5 patients who reported infusion reactions and had ATI measured, 4 tested positive for ATI. Similarly, 4 of 10 patients with positive ATI reported infusion reactions, in contrast to 1 patient with negative antibody status. While the limited sample size prevents definitive conclusions, these findings align with previous research that linked ATI development to a higher risk of infusion reactions

to infliximab [45]. Immunogenicity against infliximab can be prevented and suppressed by the concomitant use of an immunomodulator [46].

Our study demonstrates a pioneering effort in identifying varying infliximab concentrations in relation to treatment escalation. Additionally, all patients were consistently followed at the same hospital center throughout the study period, where clinicians adhered to similar empirical methods. Moreover, all samples were stored under the same conditions and tested concurrently for infliximab TLs and ATI using the same ELISA plates in the same laboratory setting. It is important, though, that we also acknowledge certain limitations of our study. Firstly, the patient sample size was relatively modest, comprising solely individuals on infliximab maintenance therapy at a single hospital center. Moreover, our study lacked data on fecal calprotectin as a biomarker and endoscopic findings. Furthermore, as this was a cross-sectional study, each patient underwent only a single measurement of infliximab TLs, which could fluctuate over time, along with ATI, which might be transient if detected as positive. Another limitation is the utilization of a drug-sensitive assay, even though the debate over the clinical advantage of employing a drug-tolerant assay in the presence of adequate infliximab TLs remains ongoing.

In conclusion, our study demonstrated that infliximab TLs are associated with treatment escalation. Higher infliximab TLs are significantly linked to achieving biochemical and combined remission in patients without treatment escalation, highlighting the importance of maintaining adequate TLs for optimal therapeutic outcomes. Based on our results, targeting higher infliximab TLs and adopting a broader therapeutic range, as previous research has suggested, could include both patients undergoing treatment escalation and those on a standard dose regimen to achieve combined remission. These findings indicate that personalized infliximab dosing strategies, based on regular monitoring of TLs, could improve clinical and biochemical remission rates in IBD patients, particularly those requiring treatment adjustments. Large prospective studies are needed to confirm these associations and determine the optimal infliximab TL thresholds for patients with treatment escalation.

Summary Box

What is already known:

- Infliximab trough levels (TLs) have a positive correlation with therapeutic outcomes in inflammatory bowel disease
- The optimal therapeutic range of infliximab TLs necessary to achieve positive outcomes remains largely uncertain
- Studies on infliximab monitoring within a Greek population are limited

What the new findings are:

- Infliximab TLs correlated with treatment escalation
- Patients without treatment escalation with biochemical and combined (steroid-free clinical and biochemical) remission had significantly higher infliximab TLs
- Higher infliximab TLs may be predictive of combined remission in patients undergoing treatment escalation

References

1. D'Haens GR, van Deventer S. 25 years of anti-TNF treatment for inflammatory bowel disease: lessons from the past and a look to the future. *Gut* 2021;**70**:1396-1405.
2. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009;**104**:760-767.
3. Cheifetz AS, Abreu MT, Afif W, et al. A comprehensive literature review and expert consensus statement on therapeutic drug monitoring of biologics in inflammatory bowel disease. *Am J Gastroenterol* 2021;**116**:2014-2025.
4. Savelkoul EHJ, Thomas PWA, Derikx LAAP, den Broeder N, Römkens TEH, Hoentjen F. Systematic review and meta-analysis: loss of response and need for dose escalation of infliximab and adalimumab in ulcerative colitis. *Inflamm Bowel Dis* 2023;**29**:1633-1647.
5. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease - algorithm for practical management. *Aliment Pharmacol Ther* 2016;**43**:30-51.
6. Papamichael K, Cheifetz AS. Use of anti-TNF drug levels to optimise patient management. *Frontline Gastroenterol* 2016;**7**:289-300.
7. Marsal J, Barreiro-de Acosta M, Blumenstein I, Cappello M, Bazin T, Sebastian S. Management of non-response and loss of response to anti-tumor necrosis factor therapy in inflammatory bowel disease. *Front Med (Lausanne)* 2022;**9**:897936.
8. Papamichael K, Rakowsky S, Rivera C, Cheifetz AS, Osterman MT. Association between serum infliximab trough concentrations during maintenance therapy and biochemical, endoscopic, and histologic remission in Crohn's disease. *Inflamm Bowel Dis* 2018;**24**:2266-2271.
9. Morita Y, Bamba S, Takahashi K, et al. Prediction of clinical and endoscopic responses to anti-tumor necrosis factor-α antibodies in ulcerative colitis. *Scand J Gastroenterol* 2016;**51**:934-941.
10. Yarur AJ, Kanagala V, Stein DJ, et al. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn's disease. *Aliment Pharmacol Ther* 2017;**45**:933-940.
11. Papamichael K, Rakowsky S, Rivera C, Cheifetz AS, Osterman MT. Infliximab trough concentrations during maintenance therapy are associated with endoscopic and histologic healing in ulcerative colitis. *Aliment Pharmacol Ther* 2018;**47**:478-484.
12. van Hoeve K, Dreesen E, Hoffman I, et al. Higher infliximab trough levels are associated with better outcome in paediatric patients with inflammatory bowel disease. *J Crohns Colitis* 2018;**12**:1316-1325.
13. Gordon BL, Battat R. Therapeutic drug monitoring of infliximab in acute severe ulcerative colitis. *J Clin Med* 2023;**12**:3378.
14. El-Matary W, Walters TD, Huynh HQ, et al. Higher postinduction infliximab serum trough levels are associated with healing of fistulizing perianal Crohn's disease in children. *Inflamm Bowel Dis* 2019;**25**:150-155.
15. Ben-Horin S, Mao R, Chen M. Optimizing biologic treatment in

IBD: objective measures, but when, how and how often? *BMC Gastroenterol* 2015;**15**:178.
16. Moss AC. Optimizing the use of biological therapy in patients with inflammatory bowel disease. *Gastroenterol Rep (Oxf)* 2015;**3**:63-68.
17. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treatment failure in Crohn's disease: post hoc analysis of a randomized controlled trial. *Am J Gastroenterol* 2014;**109**:1055-1064.
18. Ungar B, Levy I, Yavne Y, et al. Optimizing anti-TNF-α therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2016;**14**:550-557.
19. Gibson DJ, Ward MG, Rentsch C, et al. Review article: determination of the therapeutic range for therapeutic drug monitoring of adalimumab and infliximab in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2020;**51**:612-628.
20. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2019;**17**:1655-1668.
21. Turner D, Ricciuto A, Lewis A, et al; International Organization for the Study of IBD. 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;**160**:1570-1583.
22. Strik AS, Löwenberg M, Buskens CJ, et al. Higher anti-TNF serum levels are associated with perianal fistula closure in Crohn's disease patients. *Scand J Gastroenterol* 2019;**54**:453-458.
23. Papamichael K, Vande Casteele N, Jeyarajah J, Jairath V, Osterman MT, Cheifetz AS. Higher postinduction infliximab concentrations are associated with improved clinical outcomes in fistulizing Crohn's disease: an ACCENT-II *post hoc* analysis. *Am J Gastroenterol* 2021;**116**:1007-1014.
24. Mosli MH, Zou G, Garg SK, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol* 2015;**110**:802-819.
25. Plevris N, Lees CW. Disease monitoring in inflammatory bowel disease: evolving principles and possibilities. *Gastroenterology* 2022;**162**:1456-1475.
26. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014;**63**:1721-1727.
27. Echarri A, Ollero V, Barreiro-de Acosta M, et al; EIGA Group. Clinical, biological, and endoscopic responses to adalimumab in antitumor necrosis factor-naive Crohn's disease: predictors of efficacy in clinical practice. *Eur J Gastroenterol Hepatol* 2015;**27**:430-435.
28. Chang S, Malter L, Hudesman D. Disease monitoring in inflammatory bowel disease. *World J Gastroenterol* 2015;**21**:11246-11259.
29. Hibi T, Sakuraba A, Watanabe M, et al. C-reactive protein is an indicator of serum infliximab level in predicting loss of response in patients with Crohn's disease. *J Gastroenterol* 2014;**49**:254-262.
30. Boyle MP, Moss AC, O'Toole AM, Vaughn B, Cheifetz AS. C-reactive protein as a predictor of low trough infliximab concentrations in patients who lose response to infliximab. *J Dig Dis* 2017;**18**:678-683.
31. Roblin X, Marotte H, Leclerc M, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. *J Crohns Colitis* 2015;**9**:525-531.
32. Orfanoudaki E, Gazouli M, Foteinogiannopoulou K, et al. Infliximab trough levels are decreasing over time in patients with inflammatory bowel disease on maintenance treatment with infliximab. *Eur J Gastroenterol Hepatol* 2019;**31**:187-191.
33. Vande Casteele N, Herfarth H, Katz J, Falck-Ytter Y, Singh S. American Gastroenterological Association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology* 2017;**153**:835-857.
34. Fine S, Papamichael K, Cheifetz AS. Etiology and management of lack or loss of response to anti-tumor necrosis factor therapy in patients with inflammatory bowel disease. *Gastroenterol Hepatol (N Y)* 2019;**15**:656-665.
35. Sánchez-Hernández JG, Rebollo N, Martin-Suarez A, Calvo MV, Muñoz F. A 3-year prospective study of a multidisciplinary early proactive therapeutic drug monitoring programme of infliximab treatments in inflammatory bowel disease. *Br J Clin Pharmacol* 2020;**86**:1165-1175.
36. Fernandes SR, Bernardo S, Simões C, et al. Proactive infliximab drug monitoring is superior to conventional management in inflammatory bowel disease. *Inflamm Bowel Dis* 2020;**26**:263-270.
37. Fernandes SR, Serrazina J, Rodrigues IC, et al. Proactive therapeutic drug monitoring is more effective than conventional management in inducing fecal calprotectin remission in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2021;**33**:1539-1546.
38. Steenholdt C, Bendtzen K, Brynskov J, et al. Changes in serum trough levels of infliximab during treatment intensification but not in anti-infliximab antibody detection are associated with clinical outcomes after therapeutic failure in Crohn's disease. *J Crohns Colitis* 2015;**9**:238-245.
39. Suzuki Y, Matsui T, Ito H, et al. Circulating interleukin 6 and albumin, and infliximab levels are good predictors of recovering efficacy after dose escalation infliximab therapy in patients with loss of response to treatment for Crohn's disease: a prospective clinical trial. *Inflamm Bowel Dis* 2015;**21**:2114-2122.
40. Pariente B, Pineton de Chambrun G, Krzysiek R, et al. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012;**18**:1199-1206.
41. Yanai H, Lichtenstein L, Assa A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin Gastroenterol Hepatol* 2015;**13**:522-530.e2.
42. Ungar B, Ben-Shatach Z, Ben-Haim G, et al. Infliximab therapy intensification upon loss of response: Is there an optimal trough level? *Dig Liver Dis* 2019;**51**:1106-1111.
43. Atiqi S, Hooijberg F, Loeff FC, Rispens T, Wolbink GJ. Immunogenicity of TNF-inhibitors. *Front Immunol* 2020;**11**:312.
44. Van Stappen T, Vande Casteele N, Van Assche G, Ferrante M, Vermeire S, Gils A. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. *Gut* 2018;**67**:818-826.
45. O'Meara S, Nanda KS, Moss AC. Antibodies to infliximab and risk of infusion reactions in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2014;**20**:1-6.
46. Fousekis FS, Papamichael K, Kourtis G, et al. The efficacy of immunomodulators in the prevention and suppression of anti-drug antibodies to anti-tumor necrosis factor therapy in inflammatory bowel disease. *Ann Gastroenterol* 2022;**35**:1-7.

Supplementary material

Supplementary Table 1 Baseline characteristics of patients with inflammatory bowel disease on infliximab (n=77)

Characteristics	Value
Male, n (%)	48 (62.3)
Age (years), median (range)	43 (18-86)
BMI (kg/m^2), median (range)	25 (17.51-37.87)
Smoking	
Non smoker, n (%)	47 (61)
Active smoker, n (%)	16 (20.8)
Former smoker, n (%)	14 (18.2)
Type of IBD	
UC, n (%)	26 (33.8)
CD, n (%)	48 (62.3)
Unclassified colitis, n (%)	3 (3.9)
Family history of IBD, n (%)	17 (22.1)
Age of diagnosis (years), median (range)	28 (10-72)
Disease duration (years), median (range)	10 (1-35)
Disease extension	
UC proctitis, n (%)	0 (0)
UC left-side colitis, n (%)	10 (40)
UC pancolitis, n (%)	15 (60)
CD ileitis, n (%)	17 (35.4)
CD ileocolitis, n (%)	11 (22.9)
CD colitis, n (%)	23 (47.9)
CD upper GI involvement, n (%)	5 (10.4)
Disease behavior	
Stricturing disease, n (%)	23 (47.9)
Penetrating disease, n (%)	16 (30.3)
Perianal disease, n (%)	21 (43.7)
Extraintestinal manifestations	
Musculoskeletal, n (%)	32 (41.6)
Peripheral arthritis, n (%)	22 (28.6)
Axonal arthritis, n (%)	3 (3.9)
Both, n (%)	7 (9.1)
Cutaneous, n (%)	14 (18.2)
Erythema nodosum, n (%)	2 (2.6)
Pyoderma gangrenosum, n (%)	3 (3.9)
Psoriasis, n (%)	5 (6.6)
Hidradenitis suppurativa, n (%)	1 (1.3)
Other, n (%)	2 (2.6)
Hepatobiliary, n (%)	5 (6.5)
Primary sclerosing cholangitis, n (%)	2 (2.6)
Cholelithiasis, n (%)	3 (3.9)
Other	
Iridocyclitis, n (%)	1 (1.3)
Pericarditis, n (%)	1 (1.3)
Osteoporosis, n (%)	5 (6.5)
Nephrolithiasis, n (%)	3 (3.9)
IgA nephropathy, n (%)	1 (1.3)
Deep vein thrombosis, n (%)	1 (1.3)
Pulmonary embolism, n (%)	1 (1.3)
Prior treatment	
Steroids, n (%)	70 (90.9)
5-Aminosalicylates, n (%)	61 (79.2)
Azathioprine, n (%)	42 (54.5)
Methotrexate, n (%)	16 (20.8)
Biologics, n (%)	16 (20.8)
1 previous biologic, n (%)	12 (15.6)
2 previous biologics, n (%)	3 (3.9)
3 previous biologics, n (%)	1 (1.3)
Infliximab, n (%)	6 (7.8)
Adalimumab, n (%)	7 (9.1)
Golimumab, n (%)	6 (7.8)
Vedolizumab, n (%)	3 (3.9)
IBD-related surgery, n (%)	20 (26)
Enterectomy, n (%)	6 (7.8)
Right colectomy, n (%)	4 (5.2)
Perianal abscess/fistula, n (%)	11 (14.2)
Enterocutaneous fistula, n (%)	1 (1.3)

(*Contd...*)

Supplementary Table 1 (*Continued*)

IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; GI, gastrointestinal

Supplementary Table 2 Univariate analysis of serum infliximab trough levels with sex, smoking status, age, body mass index (BMI), C-reactive protein (CRP) levels, albumin levels, IBD type and concomitant combination treatment in patients with inflammatory bowel disease

Parameters	Trough levels (µg/mL)	P-value (relative risk when applicable) univariate
Sex		
Male	6.6±5.0	0.172
Female	8.2±4.7	
Smoking status		
Yes	6.3±5.2	0.231
No	8.0±4.7	
Age		0.540 (0.071)
BMI		0.918 (-0.012)
CRP		0.434 (-0.094)
Albumin		0.227 (0.161)
IBD type		
UC	6.8±4.8	0.383
CD	7.9±5.2	
Concomitant combination treatment (AZA or steroids)		
Yes	9.2±3.7	0.131
No	6.9±5.1	
Treatment escalation		
Yes	9.7±3.9	<0.001
No	3.6±3.6	

BMI, body mass index; CRP, C-reactive protein; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; AZA, azathioprine