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Impact of thiopurine dose in anti-tumor necrosis factor combination therapy on outcomes in inflammatory bowel disease

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Abstract

Background Combination therapy with thiopurines and anti-tumor necrosis factor (TNF) is superior to monotherapy in Crohn's disease (CD) and ulcerative colitis (UC). The optimal dose of thiopurines in combination therapy remains unclear. We investigated the impact of thiopurine dose in combination therapy on outcomes in inflammatory bowel disease (IBD).

Methods This was a single-center, retrospective study of patients with IBD treated with thiopurine and anti-TNF combination therapy between 1/2012 and 11/2020. A therapeutic dose of thiopurines was defined as ≥1 mg/kg for 6-mercaptopurine and ≥2 mg/kg for azathioprine. The primary outcome was anti-drug antibody (ADA) formation in patients on a therapeutic thiopurine dose vs. a lower thiopurine dose group. Secondary outcomes included steroid-free clinical remission, endoscopic healing (absence of ulcers/erosions in CD and Mayo endoscopic score ≤1 for UC), and normal serum C-reactive protein (CRP) in patients who were on combination therapy.

Results A total of 108 patients were included (median age 31.5 years; 58.3% male). A therapeutic dose of thiopurine was used in 19%. In the therapeutic thiopurine dose group, 23.8% developed ADA vs. 29.9% (P=0.58) in the lower dose group. No significant differences were noted between the therapeutic and lower dose thiopurine groups in terms of steroid-free clinical remission (57.1% vs. 60.9%, P=0.75), endoscopic healing (55% vs. 60%, P=0.69), and normal CRP (52.4% vs. 52.9%, P=0.27).

Conclusion In our cohort of patients with IBD on anti-TNF combination therapy, thiopurine dose was not associated with significant differences in anti-TNF immunogenicity and clinical outcomes.

Keywords Inflammatory bowel disease, combination therapy, azathioprine, 6-mercaptopurine

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Introduction

Infliximab (IFX) was the first anti-tumor necrosis factor (TNF) agent approved in the United States for Crohn's disease (CD), in 1998 [1]. Since then, multiple studies have demonstrated the efficacy of anti-TNF agents in decreasing hospitalizations and surgeries, and improving quality of life for patients with inflammatory bowel disease (IBD) [2-5]. However, anti-TNF treatment failure is common. It is estimated that 10% to 40% of patients do not respond to induction therapy with IFX and adalimumab (ADL), and about 24-46% develop a secondary loss of response after one year of treatment [6-9].

Immunogenicity or the development of anti-drug antibodies (ADA) is a major contributor to the loss of treatment response to anti-TNF agents. In the Personalising Anti-TNF Therapy in Crohn's Disease (PANTS) trial, the overall rates of ADA associated with IFX and ADL were 62.8% and 28.5%, respectively [10]. The combination of thiopurines, such as azathioprine (AZA) and 6-mercaptopurine (6-MP), with anti-TNF agents has been shown to decrease immunogenicity and boost anti-TNF drug levels, and is associated with favorable clinical outcomes. The

Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease (SONIC) trial demonstrated higher rates of corticosteroid-free remission in the IFX-AZA combination group compared to either IFX or AZA monotherapy [11]. It also showed that combination therapy was associated with higher IFX serum concentrations and lower rates of immunogenicity. Similar to the SONIC trial in CD, the ulcerative colitis (UC) SUCCESS trial demonstrated that combination therapy with IFX and AZA was superior to monotherapy with either IFX or AZA [12].

The therapeutic effect of AZA and 6-MP, when used as monotherapy in the management of IBD, is dose-dependent. The recommended dose is 2-2.5 mg/kg for AZA and 1-1.5 mg/kg for 6-MP [13-15]. It is, however, unclear what is the ideal thiopurine dose in anti-TNF combination therapy. A few studies have demonstrated an association between thiopurine metabolite 6-thioguanine nucleotide (6-TGN) levels and improved anti-TNF pharmacokinetics in IBD, but have not necessarily identified an ideal thiopurine starting dose [16,17]. Our study aimed to determine whether a therapeutic dose versus a lower dose of thiopurines in combination with an anti-TNF agent leads to different anti-TNF pharmacokinetics and clinical outcomes.

Patients and methods

This study was a single-center, retrospective review of all IBD patients managed on combination thiopurine and anti-TNF therapy from January 1, 2012, to November 1, 2020. The study protocol was approved by the Yale University Institutional Review Board (IRB#2000030551).

We included patients who had a confirmed diagnosis of IBD and were treated with an anti-TNF in combination with a thiopurine. We excluded patients who had missing information on anti-TNF and thiopurine dosing. Data were collected retrospectively from the electronic medical record. The baseline characteristics and disease-related variables, including age, sex, ethnicity, baseline body mass index, smoking status, IBD disease duration, IBD subtype, disease location, disease phenotype, thiopurine S-methyltransferase (TPMT) activity, and presence of documented extraintestinal manifestations, were extracted from each participant's medical record to form the data set. Treatment-related variables included prior medical and surgical therapies for IBD, dates of initiation of combination therapy, dose and frequency of anti-TNF and thiopurine medication, time of initiation of combination therapy, and use of corticosteroids at the time of initiation of combination therapy.

For the measurement of anti-TNF levels and antibodies, enzyme-linked immunosorbent assay was performed in 63.8% patients followed by chemiluminescence immunoassay in 20.3%, high-performance liquid chromatography in 7.4%, and homogenous mobility shift assay in 3.7 % of patients. We did not have data regarding the methodology used for measurement of anti-TNF levels and antibodies in 4.6% of the patients. Reactive therapeutic drug monitoring based on disease activity (clinical, biochemical, endoscopic) was used to guide dose escalation for this study population. If TPMT activity was normal, the thiopurine starting dose was determined at the discretion of the treating provider. If TPMT activity was intermediate, then thiopurines were started at half of the recommended therapeutic dose or lower.

Variables related to disease outcomes included ADA formation, anti-TNF trough levels, data on symptoms (i.e., clinical remission measured at 3, 6-12, and >12 months), laboratory markers (albumin, C-reactive protein [CRP]) within 30 days before starting combination therapy and after use, and endoscopic findings were extracted for time points after 3, 6-12, and >12 months of combination therapy.

Patients were stratified by thiopurine dose into 2 groups. Group 1: therapeutic dose of thiopurines, defined as ≥1 mg/kg for 6-MP and ≥2 mg/kg for AZA; and Group 2: lower thiopurine dose, defined as <1 mg/kg for 6-MP and <2 mg/kg for AZA.

The primary outcomes of the study were the rates of anti-TNF antibody formation and anti-TNF trough levels in patients on a therapeutic thiopurine dose vs. those receiving a lower dose. Secondary outcomes included achievement of steroid-free clinical remission, as described by the treating physicians' global assessment, endoscopic healing (defined as the absence of ulcers or erosions in CD and Mayo endoscopic subscore of ≤1 for UC), and normal serum CRP (defined as ≤5 mg/L).

Statistical analysis

JMP® (SAS Institute Inc., Cary, North Carolina, United States) statistical software was used for data analysis. Continuous variables were analyzed using an unpaired Student's *t*-test. Categorical variables were analyzed using a Pearson's chi-square test. A P-value <0.05 was considered statistically significant. A multivariate analysis was performed to evaluate factors associated with anti-TNF ADA.

Results

A total of 108 patients were included in the study (Table 1). The median age was 31.5 (range 13-69) years, and 58.3% of patients were male. With respect to the IBD subtype, 66.6% of patients had CD (16.6% with perianal involvement), 29.6% had UC, 2.7% had indeterminant colitis and 0.9% had pouchitis. Of the total population, 28 (25.9%) had a history of prior bowel resection. A total of 51 (47.2%) patients were on corticosteroids at the time of initiation of combination therapy, and 28 (25.9%) had an extra-intestinal manifestation before the start of combination therapy. A total of 53 patients (49.0%) were exposed to biologics previously (of those, 37.0% were exposed to one biologic and 12.0% were exposed to more than one biologic). Concomitant mesalamine use was noted in 12 patients. None of the patients in the cohort were on allopurinol therapy.

A total of 81 patients (75%) were on IFX, while 27 (25%) of patients were on ADL. In the whole cohort, anti-TNF antibody formation was observed in 31 patients. Of the patients who were on IFX, 26 (32.1%) had antibodies, while 5 patients (18.5%) on ADL developed antibodies. The mean anti-TNF

Table 1 Baseline characteristics

Variable	Value
Age (years), median (range)	31.5 (13-69)
Male, n (%)	63 (58.3)
BMI, median (range)	26.6 (14.2-81.7)
Ethnicity, n (%)	
Caucasian	75 (69.4)
Hispanic	7 (6.5)
African American	15(13.9)
Asian	7 (6.5)
Others	4 (3.7)
IBD subtype	
Crohn's disease, n (%)	72 (66.6)
L1	15 (20.5)
L2	8 (10.9)
L3	46 (63.0)
L4	5 (6.8)
B1	22(30.1)
B2	17 (23.3)
B3	34 (46.6)
Perianal disease, n (%)	18 (16.6)
Ulcerative colitis, n (%)*	32 (29.6)
Proctitis	2 (5.7)
Left-sided colitis	8 (22.9)
Pancolitis	23(65.7)
Indeterminate colitis, n (%)	3 (2.7)
Pouchitis, n (%)	1 (0.9)
Type of thiopurine therapy, n (%)	
AZA	25 (23.1)
6-MP	83 (76.8)
Type of anti-TNF, n (%)	
IFX	81 (75)
ADL	27 (25)
Dose of IFX, n (%)	
5 mg/kg	30 (27.7)
7.5 mg/kg	8 (7.4)
10 mg/kg	43 (39.8)
Frequency of IFX, n (%)	
Every 8 weeks	28 (25.9)
Every 6 weeks	33 (30.5)
Every 5 weeks	5 (4.6)
Every 4 weeks	14 (12.9)
Dose of ADL, n (%)	
40 mg	22 (20.3)
80 mg	5 (4.6)
Frequency of ADL, n (%)	
Every 2 weeks	8 (7.4)
Every week	19 (17.5)

*Two patients had missing information regarding ulcerative colitis disease location

BMI, body mass index; IBD, inflammatory bowel disease; AZA, azathioprine; 6-MP, 6-mercaptopurine; IFX, infliximab; ADL, adalimumab

drug level was 18.9±16.6 μg/mL in patients treated with IFX and 13.2±8.4 μg/mL in the patients treated with ADL. Most of the patients (94.4%) had drug levels measured during the maintenance phase. Anti-TNF levels and antibodies were measured within 6 months of starting combination therapy in 21.3%, while 72.7% had anti-TNF levels and antibodies measured after 6 months of initiating combination therapy. A total of 6 patients had 0 drug levels and all these patients had high antibodies to anti-TNF (≥100 μg/mL).

There were a total of 21 patients (19%) in the thiopurine therapeutic dose group. There was no significant difference in baseline and disease characteristics between the therapeutic and lower thiopurine dose groups, as shown in Table 2. Information on TPMT enzyme activity was available for only 56 patients (7 in the thiopurine therapeutic dose group and 49 in the lower dose group). Normal TPMT activity was noted in the 7 patients in the thiopurine therapeutic dose group vs. 40 in the lower dose group (P=0.10). The mean follow-up time was 42.8±29.4 months in the thiopurine therapeutic dose group compared to 38.3 ± 28.9 months in the lower dose group (P=0.52).

In the therapeutic thiopurine dose group, 23.8% developed ADA vs. 29.9% in the lower dose group (P=0.58) (Fig. 1). The mean anti-TNF drug level was numerically higher in the therapeutic thiopurine dose group (19.8±13.8 μg/mL) compared to the lower dose group (16.9±15.5 μg/mL), but the difference was not statistically significant (P=0.44) (Fig. 2). Restricting the analysis to patients treated with IFX (excluding ADL), 28.6% (n=4) of patients in the thiopurine therapeutic dose group developed anti-TNF antibodies, compared to 32.8% (n=22) of patients in the lower dose group (P=0.76). The mean IFX drug level was 25.3±13.3 μg/mL in the thiopurine therapeutic dose group, compared to 17.7±16.9 μg/mL in the lower dose group (P=0.08).

A total of 57.1% of patients achieved steroid-free clinical remission in the thiopurine therapeutic dose group, compared to 60.9% in the lower dose group (P=0.75). Endoscopic healing was achieved in 55% vs. 60% (P=0.69), respectively (13 patients did not have endoscopic data: 1 in the thiopurine therapeutic dose group and 12 in the lower dose group). Discontinuation of anti-TNF therapy was noted in 38.1% vs. 37.9% (P=0.99) in the thiopurine therapeutic dose group vs. the lower dose group, respectively. In the thiopurine therapeutic dose group, normal CRP was observed in 52.4% of the patients compared to 52.9% (P=0.44) in the lower dose group (Fig. 3). Restricting the analysis to patients receiving IFX (excluding ADL), there were no significant differences between the therapeutic and lower thiopurine dose groups in terms of steroid-free clinical remission (50% vs. 59.7%; P=0.50), endoscopic healing (42.9% vs. 53.7%; P=0.38), normal CRP (57.1% vs. 52.2%; P=0.20) and rate of anti-TNF discontinuation (42.9% vs. 31.3%; P=0.41).

A multivariate model analysis was performed incorporating factors that have been associated with anti-TNF antibody formation in the previous literature. The model included type of anti-TNF (IFX vs. ADL), need for anti-TNF dose escalation, anti-TNF drug level, and thiopurine dose. Anti-TNF drug level was the only factor independently associated with anti-TNF antibody formation (P=0.002) (Supplementary Table 1).

Discussion

In this single-center retrospective study of patients with IBD on thiopurine and anti-TNF combination therapy, we found no

Table 2 Comparison of characteristics between therapeutic vs. lower thiopurine dose

Patient characteristics	Therapeutic dose (n=21)	Lower dose (n=87)	P-value
Baseline characteristics			
Age (years), mean (SD)	29.8 (11.6)	35.1 (12.7)	0.08
Female sex, n (%)	10 (47.8)	35 (40.2)	0.54
Smoking, n (%)	2 (9.5)	6 (6.9)	0.68
Follow-up (months), mean (SD)	42.8 (29.4)	38.3 (28.9)	0.52
Disease characteristics			
Disease type, n (%)			
Crohn's disease	13 (61.9)	59 (67.8)	0.64
Ulcerative colitis	8 (38.1)	24 (27.6)	
Indeterminate colitis	0	3 (3.5)	
Pouchitis	0	1 (1.1)	
Prior bowel resection, n (%)	3 (14.3)	25 (28.7)	0.18
Presence of extra intestinal manifestations, n (%)	5 (23.8)	23 (26.4)	0.81
Medication factors			
Infliximab, n (%)	14 (66.7)	67 (77)	0.33
Escalated anti-TNF dose, n (%)	2 (9.5)	20 (23)	0.17
Labs (immediately pre-combo therapy)			
CRP*, mean (SD)	15.8 (28.7)	10.9 (17.5)	0.49
Albumin**, mean (SD)	3.96 (0.59)	3.98 (0.59)	0.94

*Data missing for 60 patients

**Data missing for 36 patients

TNF, tumor necrosis factor; CRP, C-reactive protein; SD, standard deviation

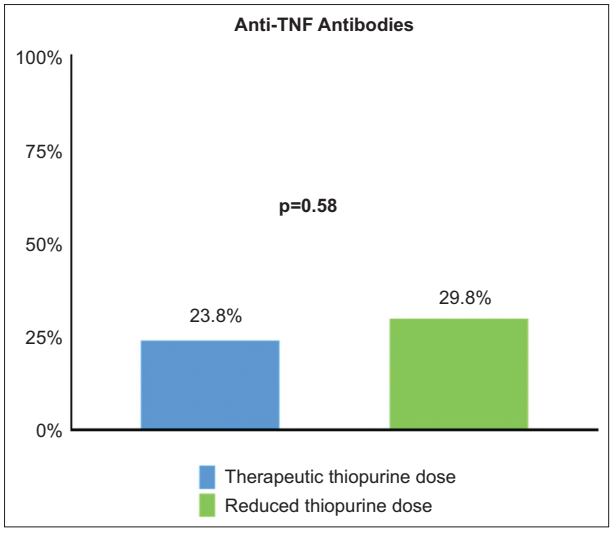

Figure 1 Rates of anti-TNF antibody formation in the therapeutic thiopurine dose vs. lower thiopurine dose groups
TNF, tumor necrosis factor

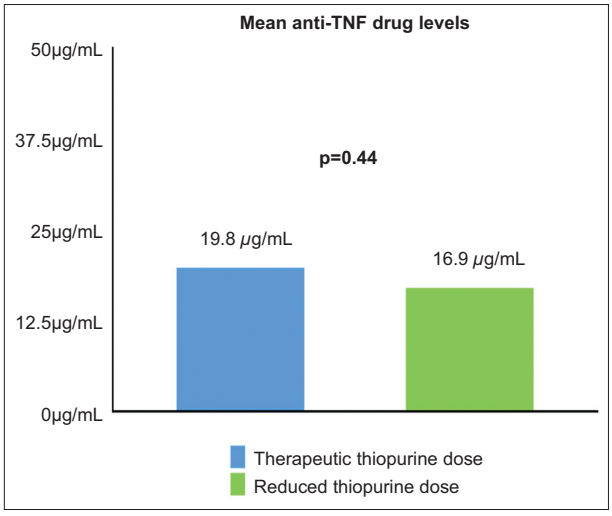

Figure 2 The mean anti-TNF trough concentration in the therapeutic thiopurine dose vs. lower thiopurine dose groups
TNF, tumor necrosis factor

differences in outcomes between patients receiving therapeutic vs. a lower thiopurine dose. In our cohort, a therapeutic dose of thiopurines in combination therapy was not associated with lower rates of anti-TNF antibody formation compared to a lower thiopurine dose. We also found that there was no significant difference in relevant clinical outcomes, including steroid-free clinical remission, endoscopic healing and normal CRP, between the group of patients receiving a therapeutic vs. a lower dose of thiopurines. The rate of discontinuation of anti-TNF also did not differ significantly between the groups.

There are minimal data on the optimal dose of thiopurines in anti-TNF combination therapy in IBD. The landmark randomized clinical trials such as SONIC and SUCCESS used a therapeutic dose of AZA at 2.5 mg/kg [11,12]. In a prospective, randomized clinical trial of patients with IBD in remission on combination therapy, a reduced AZA dose (1.25 mg/kg), but not the withdrawal of AZA, was as effective as the full-dose

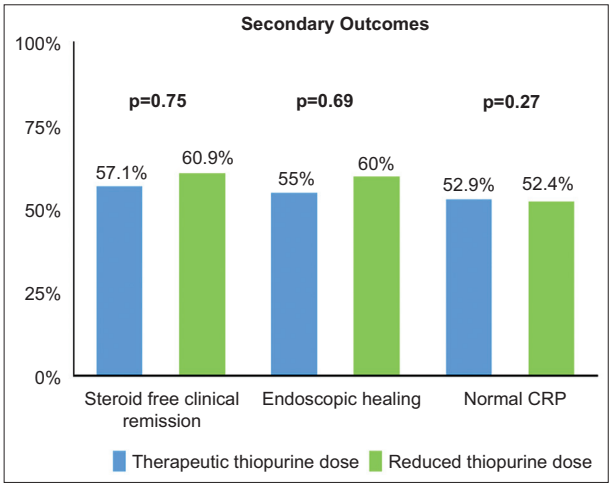

Figure 3 Rates of steroid-free clinical remission, endoscopic healing, and normal CRP in the therapeutic thiopurine dose vs. lower thiopurine dose groups
CRP, C-reactive protein

AZA (2.5 mg/kg) [18]. This observation is similar to the findings of our study, but our study differed in that we describe outcomes associated with a lower starting dose of thiopurines for the duration of use in combination therapy rather than thiopurine dose reduction after achieving remission.

A cross-sectional study by Polakovicova *et al*, in which IBD patients in remission on combination therapy (IFX and AZA) were stratified into 4 groups based on AZA dose (no AZA, AZA<1 mg/kg, AZA 1-2 mg/kg, and AZA>2 mg/kg), showed that AZA dose had a positive significant dose-dependent effect on IFX trough levels [19]. In our study, we showed that mean IFX levels were numerically higher in the thiopurine therapeutic dose group compared to the lower dose group, but this difference did not reach statistical significance. The study by Polakovicova *et al* only included patients in remission, which can significantly impact IFX levels and hence can account for the differences in results. As in our study, a single-center, retrospective study of 99 patients on combination therapy for IBD found no relationship between the AZA dose in combination therapy and anti-TNF antibodies, anti-TNF trough levels or inflammatory markers [20]. Most recently, a retrospective study by Lucas Ramos *et al* also showed that lower dose AZA (<2 mg/kg) had no difference in clinical efficacy (response and remission), anti-TNF levels, and anti-TNF antibodies compared to standard dose AZA (>2 mg/kg) [21]. Our study found similar results, but also showed that objective measures of inflammation, such as endoscopic healing, are also unaffected by the thiopurine dose.

Determining the optimal thiopurine dose in combination therapy carries a significant clinical impact. Thiopurines have many dose-dependent side-effects that may be avoided if a lower dose is found to be as effective when used in combination therapy. For example, gastrointestinal distress/nausea is one of the most common adverse effects of thiopurines, and is a major reason for non-adherence or discontinuation [22]; the effect is generally reversible with dosage reduction [23]. Bone marrow suppression and hepatotoxicity are also dose-related side effects of thiopurines [24-26]. Combination therapy is also associated with an increased risk of opportunistic infections compared to monotherapy [27,28], and decreasing the thiopurine dose may lead to a lower risk of opportunistic infections. Some observational studies suggest that there may possibly also be a dose-dependent risk of certain malignancies with AZA [29,30].

Overall, this study not only provides pharmacokinetic results demonstrating the effectiveness of lower dose thiopurine compared to a therapeutic dose, but also shows similar clinical outcomes between the groups. There are some limitations to our study, including the inherent limitations of a single-center retrospective study, which limits the generalizability of the results. Given the nature of retrospective reviews, some patients were lost to follow up, with missing data including TPMT enzyme activity and thiopurine metabolite levels. The lack of data on thiopurine metabolite levels is of particular significance, as previous cohorts have demonstrated favorable pharmacokinetic outcomes with higher 6-TGN levels [16,17]. For example, in a cross-sectional study of 72 patients, Yarur *et al* identified a 6-TGN level cutoff of 125 pmol/8×10^8 red blood cells to be associated with higher IFX drug levels [16,17].

In conclusion, our study found that a lower dose of thiopurines in anti-TNF combination therapy was not associated with an increased risk of ADA formation or lower anti-TNF trough levels compared to a therapeutic thiopurine dose. It also showed that clinical outcomes such as steroid-free clinical and endoscopic remission are not impacted by the thiopurine dose. Large, multicenter, prospective studies are needed to confirm these findings, which may lead to a better safety and tolerability profile of combination therapy for IBD patients.

Summary Box

What is already known:

- Development of anti-drug antibodies (ADAs) is a major contributor to loss of treatment response to anti-tumor necrosis factor (TNF) agents
- Combination therapy with thiopurines and anti-TNFs is superior to monotherapy in Crohn's disease and ulcerative colitis
- The therapeutic effect of azathioprine (AZA) and 6-mercaptopurine (6-MP), when used as monotherapy in the management of inflammatory bowel disease (IBD), is dose-dependent

What the new findings are:

- A lower dose of thiopurine in anti-TNF combination therapy was not associated with an increase in ADA formation or lower anti-TNF trough levels compared to a therapeutic thiopurine dose
- Clinical outcomes such as steroid-free clinical and endoscopic remission were not affected by the thiopurine dose in the combination therapy
- The therapeutic effect of AZA and 6-MP, when used as a combination therapy along with anti-TNFs in the management of IBD, was not dose-dependent

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Supplementary material

Supplementary Table 1 Multivariate regression analysis of factors associated with antibodies to anti-TNF

Variables	Adjusted coefficient	95%CI	P-value
Infliximab vs. adalimumab	-0.513	-1.13-0.03	0.079
Anti-TNF dose escalation	0.194	-0.35-0.79	0.501
Anti-TNF drug level	0.079	0.03-0.13	0.002
Therapeutic thiopurine dose	-0.031	-0.62-0.61	0.921

CI, confidence interval; TNF, tumor necrosis factor