BMJ Open Gastroenterology

Association between 6-thioguanine nucleotide levels and preventing production of antibodies to infliximab in patients with inflammatory bowel disease

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To cite: Phillips J, Leary S, Tyrrell-Price J. Association between 6-thioguanine nucleotide levels and preventing production of antibodies to infliximab in patients with inflammatory bowel disease. *BMJ Open Gastroenterol* 2023;**10**:e001149. doi:10.1136/bmjgast-2023-001149

Received 19 March 2023
Accepted 30 May 2023

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ABSTRACT

Objective Combination therapy with infliximab and a thiopurine has been shown to be more effective than monotherapy in patients with inflammatory bowel disease (IBD). The therapeutic efficacy of thiopurines is correlated with 6-thioguanine (6-TGN) levels between 235 and 450 pmol/8×10^8 erythrocytes. The primary aim of the study was to investigate the association between 6-TGN levels and inhibition prevention of the production of antibodies to infliximab (ATI).

Design We performed a retrospective review of the medical records of patients being treated with infliximab for IBD at University Hospitals Bristol NHS Foundation Trust. Demographic and biochemical data were extracted, alongside thiopurine metabolite levels, trough levels of infliximab and the presence of ATI. χ^2 tests were used to investigate the association between 6-TGN levels and prevention of ATI. Logistic regression was used to compare the odds of prevented ATI between those with a 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes, those with a 6-TGN level outside of this range, and the baseline group who were on infliximab monotherapy.

Results Data were extracted for 100 patients. Six of 32 patients with a 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes developed ATI (18.8%) compared with 14 out of 22 (63.6%) patients with a 6-TGN outside of this range and 32 out of 46 (69.6%) patients on monotherapy (p=0.001). The OR (95% CI) for prevented ATI in those with a 6-TGN between 235 and 450 pmol/8×10^8 erythrocytes compared with a 6-TGN outside of this range was 7.6 (2.2, 26.3) (p=0.001) and compared with monotherapy was 9.9 (3.3, 29.4) (p=0.001).

Conclusion 6-TGN levels between 235 and 450 pmol/8×10^8 erythrocytes prevented production of ATI. This supports therapeutic drug monitoring to help guide treatment and maximise the beneficial effects of combination therapy for patients with IBD.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The therapeutic efficacy of thiopurines is correlated with 6-thioguanine (6-TGN) levels between 235 and 450 pmol/8×10^8 erythrocytes. However, recent studies have shown that lower 6-TGN levels may be adequate to achieve therapeutic levels of infliximab and reduce antibody formation.

WHAT THIS STUDY ADDS

⇒ We found that 6-TGN levels between 235 and 450 pmol/8×10^8 erythrocytes prevented the production of antibodies to infliximab 7.6-fold compared with patients with a 6-TGN outside of this range.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings support therapeutic drug monitoring to help guide treatment and maximise the beneficial effect of combination therapy for patients with inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract (GI tract). There are two main clinical forms; Crohn's disease (CD), which can affect any part of the gastrointestinal tract and ulcerative colitis (UC), in which inflammation only affects the colonic mucosa. They are lifelong conditions, with periods of active disease alternating with periods of remission. Usually diagnosed between 10 and 30 years old, IBD causes significant disability, often restricting the ability to work normally. The symptoms vary, but commonly include diarrhoea, abdominal pain, weight loss and blood or mucus in the stool. Patients can also suffer joint, eye and skin problems.

Patients with IBD often require treatment with immunosuppressant or biological therapy. Thiopurines, including mercaptopurine (6MP) and its prodrug azathioprine, are among the most commonly prescribed immunosuppressant medications in IBD. Alongside these medications, the introduction

of anti-TNF biological therapy infliximab (IFX) in the late 1990s revolutionised the treatment of inflammatory bowel disease, dramatically improving outcomes for patients.[1]

Unfortunately, many patients do not respond to initial induction therapy with infliximab (primary non-response) or later lose response to treatment (secondary loss of response). It is estimated that 40% of patients with CD will lose response with the annual risk of loss of response being 13% per patient-year of treatment.[2] There are several reasons for secondary loss of response to biological therapies, perhaps the most important of which is immunogenicity. Antibodies to infliximab (ATI) are associated with lower serum levels of infliximab, a significantly higher risk of loss of clinical response and worse clinical outcomes for patients.[3–5]

Several studies have demonstrated the superiority of combination therapy (infliximab with azathioprine) over infliximab monotherapy.[6–8] This is likely due to the enhanced benefit of two effective drugs as well as the suppression of immunogenicity. Furthermore, the addition of an immunomodulator can reverse antibody formation and restore clinical response in patients with IBD treated with infliximab.[9] However, combination therapy has been associated with an increased risk of infections and lymphomas.[10 11] This increased risk has raised the question of whether using lower doses of thiopurines could provide the same benefit in reducing immunogenicity to biological therapy while keeping the immunosuppression required to a minimum.

The active immunosuppressive metabolites of thiopurines are 6-thioguanine nucleotides (6-TGN). The therapeutic efficacy of thiopurines is correlated with 6-TGN levels between 235 and 450 pmol/8×10^8 erythrocytes.[12 13] However, recent studies have shown that lower 6-TGN levels may be adequate to achieve therapeutic levels of infliximab and reduce antibody formation.[14–16]

The primary aim of the study was to investigate the association between 6-TGN levels and preventing the production of ATI in patients with inflammatory bowel disease on combination therapy, using a monotherapy group as a baseline.

MATERIALS AND METHODS

We performed a retrospective review of the medical records of patients being treated with infliximab for IBD at University Hospitals Bristol NHS Foundation Trust. Patients were identified from a database of patients with IBD on biological therapy under our care. We included patients with a confirmed diagnosis of CD, UC or IBD unclassified established by clinical, endoscopic, histological or radiological criteria who were receiving maintenance therapy with infliximab and azathioprine or 6-mercaptopurine (6MP). A group of patients on infliximab monotherapy was used as a baseline. The sample size of 100 patients was guided by similar studies in the literature.[14 16]

Infliximab maintenance therapy was defined as at least 6 infusions in a 12-month period with no interval longer than 8 weeks between infusions. All patients had their infliximab levels and ATI measured at each infusion, as is standard care in our hospital. The assay used is the ImmunDiagnostik ELISA. The antibody assay is a drug tolerant, total antibody assay with a measuring range of 10 AU/mL to 400 AU/mL. The outcome measure was development of ATI at any time in the treatment cycle. For patients on a thiopurine, where thiopurine metabolite levels were taken as part of clinical management, these were recorded.

Variables extracted included demographic details (age, gender and weight), disease phenotype (including duration and extent of disease and presence of fistulating disease in Crohn's) and smoking history. The duration of treatment was recorded and for each infusion, IFX trough concentrations, ATI, C reactive protein and albumin were recorded.

The baseline characteristics of the study population were described by frequency (percentage) or median (IQR) as appropriate. χ^2 tests were performed to investigate the association between 6-TGN levels and ATI. Logistic regression was used to calculate ORs, comparing the odds of prevented ATI between those with a 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes and those with a 6-TGN level outside of this range and those on monotherapy. Unadjusted ORs, and ORs adjusted for age and gender, then additionally weight, smoking status, diagnosis, duration of disease and duration of infliximab treatment were calculated.

RESULTS

Data were extracted for 100 patients being treated with infliximab for IBD. Fifty-four patients were on combination treatment with infliximab and a thiopurine, four

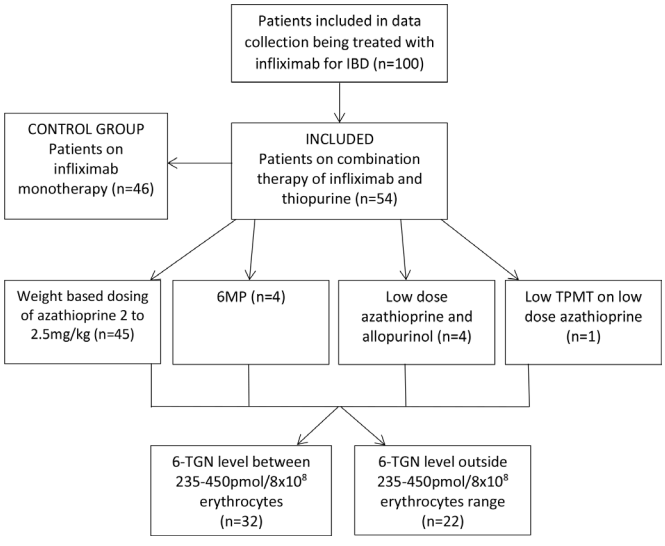

Figure 1 Flow diagram of the sample's treatment groups. IBD, inflammatory bowel disease; 6MP, 6-mercaptopurine; 6-TGN, 6-thioguanine; TPMT, thiopurine methyltransferase.

Table 1 Baseline characteristics of the sample (n=100)

	Monotherapy group (n=46)	6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes (n=32)	6-TGN level outside 235–450 pmol/8×10^8 erythrocytes (n=22)	Whole sample (n=100)
Male sex, n (%)	22 (47.8)	19 (59.4)	12 (54.6)	53 (53.0)
Age, years, median (IQR)	38 (29, 53)	34 (28, 41)	32 (27, 37)	34 (28, 46)
Weight, kg, median (IQR)	79 (68, 95)	72 (60, 80)	73 (65, 82)	74 (65, 88)
Diagnosis of Crohn's (vs UC and IBD-U), n (%)	33 (71.7)	24 (75.0)	19 (86.4)	76 (76.0)
Disease extent				
Crohn's (%)	33 (71.7)	24 (75)	19 (86.4)	76 (76)
Colonic	10 (21.7)	5 (15.6)	7 (31.8)	22 (22)
Ileocolonic	16 (34.8)	17 (53.1)	9 (40.9)	42 (42)
Ileal	6 (13.0)	2 (6.3)	2 (9.1)	10 (10)
Upper	0 (0)	0 (0)	1 (4.6)	1 (1)
Pouchitis postcolectomy	1 (2.2)	0 (0)	0 (0)	1 (1)
Perianal disease	5 (10.9)	6 (18.8)	6 (27.3)	17 (23)
Fistulating disease	4 (8.7)	13 (40.6)	9 (40.1)	26 (34)
UC or IBD-U (%)	13 (28.3)	8 (25)	3 (13.6)	24 (24)
Left-sided	8 (17.4)	6 (18.8)	2 (9.1)	16 (16)
Pancolonic	3 (6.5)	2 (6.2)	1 (4.5)	6 (6)
Pouchitis postcolectomy	2 (4.4)	0 (0)	0	2 (2)
Active smoker, n (%)	9 (19.6)	6 (18.8)	2 (9.1)	17 (17.0)
Duration of disease, years, median (IQR)	12 (7, 17)	11 (6, 17)	10 (6, 17)	11 (6, 17)
Time since first IFX, years, median (IQR)	4 (2, 12)	6 (1, 14)	5 (2, 11)	4 (2, 12)
Average drug level AU/mL, median (IQR)	3.5 (2.0, 5.1)	2.6 (1.9, 4.1)	2.3 (1.7, 3.4)	2.7 (1.8, 4.5)

IBD-U, inflammatory bowel disease unclassified; 6-TGN, 6-thioguanine; UC, ulcerative colitis.

of these were on 6MP, four were on azathioprine and allopurinol and one patient was on azathioprine dosed to TGN level because of a low thiopurine methyltransferase (figure 1). The remaining 45 patients had their azathioprine dosed to weight at 2–2.5 mg/kg. All patients on combination therapy were either already on a thiopurine prior to starting infliximab, or the thiopurine was commenced at the time of starting infliximab. Forty-six patients on infliximab monotherapy were included as a control group. This was to ascertain the proportion of patients on monotherapy who developed ATI.

The baseline characteristics of the study group are shown in table 1. The groups were similar in terms of baseline characteristics, despite a slightly higher percentage of patients with CD and a lower percentage of active smokers in the group with a 6-TGN level outside 235–450 pmol/8×10^{8}erythrocytes.

All patients with 6-TGN levels outside of range had a 6-TGN level below 235 8×10^8 erythrocytes. The prevention of ATI was compared between patients with a 6-TGN level between 235–450 pmol/8×10^8 erythrocytes, those with a 6-TGN level below this range and those on infliximab monotherapy (table 2). Of 32 patients with a 6-TGN between 235 and 450 pmol/8×10^8 erythrocytes, 6 developed ATI (18.8%) compared with 14 out of 22 (63.6%) patients with a 6-TGN outside of this range and 32 out of 46 (69.6%) patients on monotherapy (p=0.001).

There was a strong association between prevention of ATI in those patients with a 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes compared with monotherapy (OR 9.9) and a 6-TGN level between 235 and 450 pmol/8×10^8 erythrocytes compared with 6-TGN level outside of this range (OR 7.6). However, there was no evidence of an association between prevention of ATI in those patients with a 6-TGN level outside of range compared with monotherapy. After adjusting for potential confounders, the strength of association increased with full adjustment, and additional adjustment to account for infliximab drug levels increasing the prevention of ATI in the group with a 6-TGN level between 235 and 450 pmol/8×108 erythrocytes to 12.9-fold and 12.2-fold, respectively, compared with the monotherapy group.

DISCUSSION

In this study, we found that patients with inflammatory bowel disease on combination therapy with

Table 2 Associations between TGN levels and inhibition of the production of ATI (N=100)

Adjustment	Exposure categories	OR (95% CI)	P value
None	6-TGN 235–450 pmol/8×10^8 erythrocytes vs monotherapy	9.9 (3.3 to 29.4)	<0.001
	6-TGN outside of range vs monotherapy	1.3 (0.4 to 3.8)	0.6
	6-TGN 235–450 pmol/8×10^8 erythrocytes vs 6-TGN outside of range	7.6 (2.2 to 26.3)	0.001
Minimally adjusted*	6-TGN 235–450 pmol/8×10^8 erythrocytes vs monotherapy	11.2 (3.6 to 34.9)	<0.001
	6-TGN outside of range vs monotherapy	1.5 (0.5 to 4.5)	0.5
	6-TGN 235–450 pmol/8×10^8 erythrocytes vs 6-TGN outside of range	8.0 (2.2 to 29.7)	0.002
Fully adjusted†	6-TGN 235–450 pmol/8×10^8 erythrocytes vs monotherapy	12.9 (3.8 to 43.0)	<0.001
	6-TGN outside of range vs monotherapy	1.7 (0.5 to 5.3)	0.4
Additionally adjusted average drug level AU/mL‡	6-TGN 235–450 pmol/8×10^8 erythrocytes vs monotherapy	12.2 (3.5 to 42.1)	<0.001
	6-TGN outside of range vs monotherapy	1.5 (0.5 to 5.0)	0.5
	6-TGN 235–450 pmol/8×10^8 erythrocytes vs 6-TGN outside of range	11.4 (2.2 to 59.2)	0.004

*Adjusted for gender and age only.
†Adjusted for gender, age, weight, diagnosis, smoking status, duration of disease and time on infliximab.
‡Adjusted for gender, age, weight, diagnosis, smoking status, duration of disease and time on infliximab and average infliximab drug level.
TGN, thioguanine.

infliximab and a thiopurine, 6-TGN levels between 235 and 450 pmol/8×10^8 erythrocytes prevented the production of ATI 7.6-fold compared with patients with a 6-TGN outside of this range and 9.9-fold compared with those on infliximab monotherapy.

Several studies have shown a superiority in outcomes when comparing combination therapy of infliximab and thiopurines with monotherapy. Thiopurines have been shown to reduce immunogenicity in a dose dependant manner with the lowest immunogenicity observed in patients with higher thiopurine doses.[17] However, when looking specifically at 6-TGN levels, some data suggest that levels below the usual recommended therapeutic range for monotherapy is sufficient for reducing development of ATI.[14 16]

Our findings are important because even though thiopurines are considered safe, their use is associated with an increased risk of infection and lymphomas and many of their adverse effects are dose dependant.[18] This study therefore supports therapeutic drug monitoring of thiopurines to help guide treatment and maximise the beneficial effects of combination therapy for patients with IBD.

This study has several important limitations. The retrospective nature of the study may have introduced bias into the results. Our findings show an association between 6-TGN levels and prevention of ATI but the study design means we cannot be sure of causation. We have considered confounding factors and although allowing for these does not alter the association found significantly, we cannot fully explain the slightly strengthened association. Furthermore, although the sample size was similar to previously published works, we did not undertake a power calculation and the CI is wide (2.2 to 26.3), likely due to the limited number of patients included.[14 16] All patients were on maintenance therapy which may have excluded those with more acute or active disease. This is important as the pharmacokinetics of infliximab may vary in patients with active inflammation.[19]

Further research is needed in order to determine the optimal treatment strategy for patients with inflammatory bowel disease on combination therapy. However, our study shows that 6-TGN levels between 235 and 450 pmol/8×10^8 erythrocytes prevents production of ATI and therefore targeting 6-TGN levels may optimise treatment for patients with IBD on combination therapy.

Contributors JP and JT-P conceptualised the work. JP, JT-P and SL designed the work. SL carried out the statistical analysis. JP completed data collection and drafted the original manuscript. All authors critically reviewed and revised the manuscript draft and approved the final version for submission. JP is the guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data collected and used in the analysis for this paper are available on reasonable request to the corresponding author.

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