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Real-World Use of Azathioprine Metabolites Changes Clinical Management of Inflammatory Bowel Disease

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Abstract

Background: Thiopurines such as 6-mercaptopurine and azathioprine have complex metabolism, resulting in significant inter-individual differences in clinical efficacy and risk of drug toxicity, making conventional weight-based dosing inaccurate and potentially unsafe. Therapeutic drug monitoring (TDM) of thiopurine metabolites improves clinical outcomes through dose optimization and toxicity monitoring. Despite evidence for TDM, use is limited, due in part to test availability and awareness. The objectives of this study were twofold: (1) to investigate how thiopurine TDM impacts clinical management of IBD patients and (2) to evaluate proportion of patients outside therapeutic 6TGN levels or exhibiting signs of toxicity

Methods: Patients who received thiopurine TDM as part of routine care underwent chart review of demographics, disease activity, medication dosing, metabolite levels, and adverse events. Changes in clinical management following TDM were measured. Additionally, we conducted a retrospective review of clinical decision making blinded and unblinded to TDM result.

Results: A total of 92 IBD patients were included. Levels of 6TGN were therapeutic in 29% of patients. 6TGN levels correlated weakly with weight-based dosing ($r^2 = 0.057$, $P = 0.02$). Adverse reactions were observed in 6.5%. TDM informed clinical management in 64%. Significantly more changes to clinical management occurred in those with active disease than in remission (73% versus 48%; $P = 0.02$) and in those on mono- versus combination therapy (48% versus 27.5%; $P = 0.03$).

Conclusions: TDM informs clinical decision making in over two-thirds of patients. The demonstrated poor efficacy of weight-based dosing and impact of TDM on clinical management contributes to the evidence supporting the need for greater availability and uptake of thiopurine TDM.

Keywords: *Azathioprine metabolites; Crohn's disease; Therapeutic drug monitoring*

Introduction

The thiopurines, 6-mercaptopurine (6MP) and its pro-drug azathioprine (AZA) are important immunomodulatory agents in the treatment of inflammatory bowel disease (IBD) [1,2].

After being absorbed from the gastrointestinal tract, the pro-drug AZA is converted to 6MP nonenzymatically by reduced glutathione, and enzymatically by the action of glutathione-S-transferase (Figure 1). 6MP is then subject to multiple competing enzymatic pathways, each with a large number of intermediaries [2,3]. Key metabolites include 6-thiouric acid (6TU), 6-methylmercaptopurine (6MMP) and 6-thioguanine nucleotide (6TGN) in various phosphorylated forms [2–4] are incorporated at nucleic acid sites and consequently interfere with the function of DNA processing

AZA

↓ Enzymatic or non-enzymatic

6MP

Multiple enzymes incl. TPMT →

6TGN and related metabolites

Therapeutic effect and possible myelosupression at high levels

TPMT →

6MMP and related metabolites

Possible hepatotoxicity at high levels

Multiple enzymes incl. TPMT and XO →

6TU and other inactive metabolites
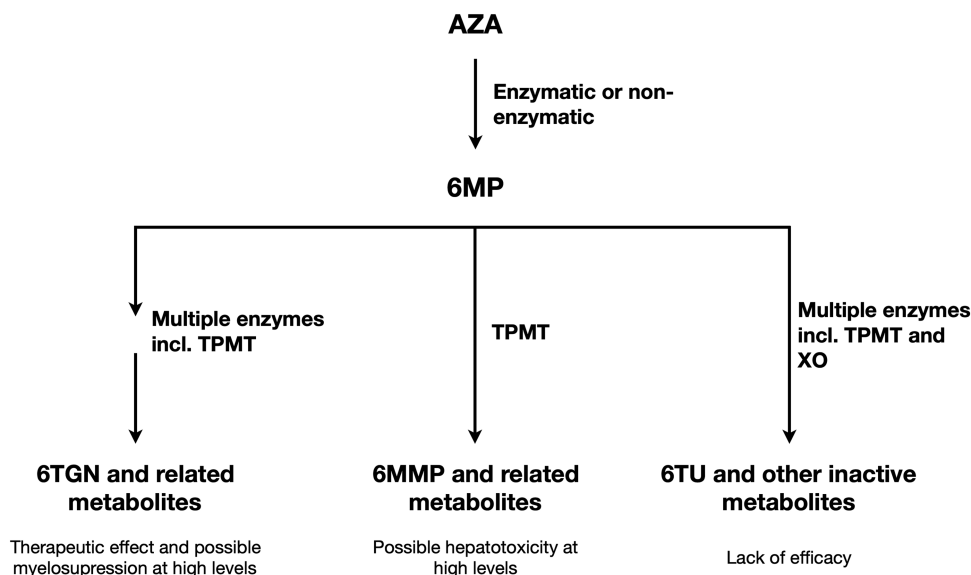
Lack of efficacy

Figure 1. Overview of thioprine metabolism. AZA, azathioprine; MP, mercaptopurine; MMP, methylmercaptopurine; TGN, thioguanine nucleotide; TU, thiouric acid; TPMT, thiopurine Smethyltransferase; XO, xanthine oxidase.

enzymes, while related 6TGN-methylated metabolites provide some inhibition of de novo purine synthesis (4).

6TGN is the therapeutically active thiopurine metabolite, and response to treatment has been shown to increase with 6TGN concentration to the upper limit of approximately 450 pmol/8×10^8 RBC (2–4). Meta-analyses have shown a pooled odds ratio of 3.3 for remission with 6TGN concentrations greater than 230 to 260 pmol/8×10^8 RBC (4). However, at 6TGN concentrations, over 450 pmol/8×10^8 RBC, there is an increased risk of myelotoxicity proportional to 6TGN concentration (2,3,5).

Metabolism of 6MP and multiple downstream molecules via thiopurine-S-methyl-transferase (TPMT) results in formation of multiple metabolites, including 6MMP. 6MMP is not associated with therapeutic efficacy (2,6) and there is a threefold increased risk of hepatotoxicity above concentrations of 5700 pmol/8×10^8 RBC (2–5,7).

Not only is thiopurine metabolism complex due to the nature of multiple and incompletely characterized interdependent biochemical pathways involving various methylation groups, phosphorylation groups and enzymes producing substantial numbers of active and inactive metabolites, but there are also substantial inter-individual differences (7–9). Genetic differences have only been partially characterized, limiting clinical utility of genetic pretreatment testing and functional studies of enzymatic activity are similarly sparse. Therefore, applied indiscriminately, thiopurine use results in heterogeneous metabolism and subsequent large differences in active and inactive metabolite formation, yielding inconsistent response to treatment and risk of drug toxicity that is hard to predict (6,9–11).

Despite this, the most commonly used method of thiopurine dosing is based on patient weight at 1.0 to 1.5 mg/kg/day for 6MP and 2.0 to 2.5 mg/kg/day for AZA (4,5).

Initial studies supporting thiopurine weight-based dosing were performed at a time when thiopurine metabolite testing was not available. Since then, numerous studies have reported poor correlation between weight-based dose and the measured 6TGN concentration, suggesting that individual variability in drug metabolism is more important than dose (5,6).

Furthermore, without understanding an individual's metabolism 'phenotype', some patients can have complete absence of therapeutic effect and be at risk of harm. A subset of patients have preferential metabolism to 6MMP while 6TGN levels remain below therapeutic range (6,9). Patients with this skewed metabolic profile, classified as 'shunters', are at significantly greater risk of therapeutic inefficacy and adverse events (6,9). A 6MMP:6TGN ratio of greater than 20 is generally accepted as a reasonable definition of shunting, but this cut-point is strictly numerical rather than biological, and is dose dependent (12). While shunting is a common occurrence in thiopurine metabolism, with an estimated incidence of 15% in a large single centre series of over 7000 patients (13), the cause is not well understood and may relate to polymorphisms in enzymes in the thiopurine metabolic pathway (13,14). Allopurinol co-prescription with concomitant thiopurine dose reduction has been shown to remedy shunting and restore 6TGN preferential metabolism, mediated by TPMT inhibition (15).

The characterization of key metabolites in the major pathways of thiopurine metabolism has enabled the development of thresholds by which metabolite concentration has been correlated with clinical efficacy. This results in five patterns of

Figure 2. Patterns of thiopurine metabolism and action to optimize thiopurine. MMP, methylmercaptopurine; TGN, thioguanine nucleotide.

thiopurine metabolism, with each informing clinical care in a different way (Figure 2). For a given dose, different patients may have (1) subtherapeutic 6TGN (with low or normal 6MMP), (2) isolated excess production of 6GTN, (3) normal 6TGN and 6MMP, (4) shunting with low or normal 6TGN and high 6MMP or (5) super-therapeutic 6TGN and 6MMP (3,16).

Thiopurine therapeutic drug monitoring (TDM) has been demonstrated to be effective when used in a prospective manner to optimize dose, as well as on an ad hoc basis to identify the underlying cause of suboptimal clinical response and adverse events (7,10,16–18). There is also some evidence that thiopurine metabolites may change over time, developing a late onset rise in 6MMP (13).

Despite this evidence, there continues to be a significant disparity in the use of thiopurine TDM in North America, likely due in part to low test knowledge, limited test availability and lack of high-quality prospective randomized studies (9,10,18). Additionally, there are no studies to our knowledge that examine the effect of thiopurine TDM on clinical decision making.

The aims of this study were twofold: (1) to investigate the relationship between thiopurine dose, metabolite levels and the prevalence of adverse events and (2) to understand the impact of thiopurine TDM on the clinical management of patients undergoing thiopurine therapy for IBD, in particular, to determine in which clinical situations and patient groups TDM might lead to a change in management.

MATERIALS AND METHODS

Participant Characteristics

This study took place in the Inflammatory Bowel Disease Research and Consultation Clinic in Victoria, Canada, and investigated IBD patients in an outpatient setting from December 2016 to December 2017. Patients who underwent AZA or 6MP TDM as part of routine clinical care for IBD were identified through either retrospective chart review or prospective capture at the time of TDM request. Our pattern of practice was to routinely obtain at least one TDM for patients on thiopurine. Eligibility required that participants were being treated with AZA or 6MP for IBD, and had TDM performed while on a stable medication dose.

Study Design

At the time of study initiation, thiopurine metabolites were not available locally, so whole blood samples were collected and shipped unfrozen in EDTA tubes to either the Hospital for Sick Children (SickKids) in Toronto, ON or Prometheus Laboratories Inc. in San Diego, CA. The 6TGN and 6MMP metabolite concentrations were determined by high-performance liquid chromatography method at SickKids, (reference range: 6TGN 400 to 750 pmol/8×10^8 RBC and 6MMP <6600 pmol/8×10^8 RBC) and liquid chromatography tandem mass spectrometry (LC-MS/MS) at Prometheus (reference range: 6TGN 230 to 400 pmol/8×10^8 RBC, and 6MMP <5700 pmol/8×10^8 RBC). Using these data sets, a novel LC-MS/MS method was developed and validated locally at Victoria General Hospital. This method has been previously reported (19), with adjusted reference ranges of 280 to 500 pmol/8×10^8 RBC for 6TGN and <5700 pmol/8×10^8 RBC for 6MMP. In those patients who required allopurinol due to shunting, allopurinol was instituted at 50 mg daily (20).

Low allopurinol doses are sufficient to optimize azathioprine therapy in IBD patients with inadequate thiopurine metabolite concentrations, and a second round of metabolite levels were

recorded and included in the study for comparison. If abhorrent metabolism was not fixed with this dose, allopurinol was increased to 100 mg daily.

Adverse events associated with azathioprine therapy were monitored and recorded. Adverse events were defined as hepatotoxicity if alanine aminotransferase (ALT) > 2.0 times upper limit of normal and/or myelosuppression if leukocytes < 3.0 × 10^9. Drug-induced nausea was determined by treating physician if nausea occurred with medication initiation, resolved with drug discontinuation and there was not a more likely alternative etiology. Similarly drug-induced pancreatitis was determined according to standard clinical criteria (two of symptoms, imaging and lipase elevation) and lack of an alternative etiology.

The majority of the patients in the present analysis were accepted into clinical practice on azathioprine, and therefore, TPMT results were not available.

An observational analysis of change in clinical management following TDM was performed. This analysis explored the quantity and quality of real-world clinical changes by physicians after TDM results were reviewed. The direct impact of thiopurine metabolite levels on clinical decision making was estimated through a secondary retrospective review of all charts. This analysis was carried out by a blinded physician without knowledge of the thiopurine metabolite levels initially reviewing the clinical scenario (demographics, active or inactive disease, mono or combo therapy, thiopurine dose, routine laboratory values including complete blood count, liver enzymes and C-reactive protein). This blinded physician then provided their assessment of what action they would perform: no change; thiopurine dose decrease or withdrawal; thiopurine dose increase; addition of allopurinol or initiation or optimization of biologic. Physicians were then unblinded to thiopurine metabolites and asked to re-evaluate their initial treatment recommendation. In both scenarios, overall changes to management were analyzed as well as the relative differences in changes to management between the following patient types: CD versus UC; active disease versus remission; monotherapy versus combination therapy; active disease on monotherapy versus active disease on combination therapy.

Statistical Analyses

Data were recorded and analyzed in Microsoft Excel 2017 and IBM SPSS. Data were expressed descriptively as percentages and either mean and standard deviation or median and range according to the data distribution. All 6MP doses were converted to AZA doses using a conversion factor of 2.08 in order to make them comparable (15,16). Proportions were compared using a Fisher's exact test. Continuous variables were compared using Student's *t*-test and Pearson's correlation was used to measure linear association between variables. A *P*-value of 0.05 or less was considered statistically significant.

Ethical Considerations

Ethical approval was granted by the Clinical Research Ethics Board of the Vancouver Island Health Authority (VIHA). Institutional approval was granted by VIHA's Research and Capacity Building department. Informed consent was not obtained as participant participation in metabolite testing was standard of care and no identifiable information was collected.

RESULTS

Patient Characteristics

Ninety-two patients with a history of at least one episode of thiopurine metabolite testing were included in this study (Table 1). Of these, 64% had active disease and 63% had Crohn's disease (CD). Of the patients with CD, the median age was 36 years old (17 to 73 years old), 59% were female and median BMI was 25 (16.8 to 42.5). Demographics were similar for patients with ulcerative colitis (UC). The median duration of disease at inclusion was 9 years for both CD (1 to 44 years) and UC (1 to 34 years).

Treatment Characteristics

At the time of thiopurine TDM, 93% of patients were being treated with azathioprine with a mean dose of 1.9 mg/kg (± 0.5 mg/kg) and 7% were treated with 6MP with a median dose of 0.8 mg/kg (± 0.2 mg/kg). Fifty-seven per cent of the samples were obtained from patients on monotherapy, while 43% were on combination therapy with a biologic (Table 1).

Thiopurine Metabolite Levels Following Weight-Based Dosing

Thiopurine TDM revealed that, overall, 87% of patients had at least one metabolite (6TGN or 6MMP) concentration outside of therapeutic ranges. Overall, patterns of thiopurine metabolites based on total analyzed assays were as follows: (1) subtherapeutic 6GTN (with low or normal 6MMP), 48%; (2) isolated excess production of 6GTN, 11%; (3) normal 6GTN and 6MMP, 28%; (4) shunting with low or normal 6TGN and high 6MMP, 11%; (5) super-therapeutic 6GTN and 6MMP, 1%.

6TGN

The concentrations of 6TGN varied widely according to weight-based dosing (Figure 3), with Prometheus results showing a weak correlation (Pearson *r* = 0.285, *P* = 0.041), and SickKids showing no correlation (Pearson *r* = 0.098, *P* = 0.574; outlier removed Pearson *r* = 0.154, *P* = 0.382). 6TGN concentration was within therapeutic range in 29% of patients, while 53% of patients had subtherapeutic levels, and 14% had levels above acceptable upper limits. There was no significant difference in mean 6TGN concentrations between

Table 1. Patient and medication characteristics

	Crohn's disease	Ulcerative colitis
Patients	58 (63)	34(37)
Age, mean years (range)	36 (17–73)	39 (25–69)
Female	34 (59)	18 (53)
Body mass index, mean (range)	25 (16.8–42.5)	27.1 (19.4–53.2)
Disease Duration, mean years (range)	13.6 (1–44)	10.6 (1–34)
Disease Subtype - Montreal Classification		
Crohn's Disease		
Ileal	20 (34)	
Colonic	22 (38)	
Ileocolonic	15 (26)	
Upper Disease	1(2)	
Ulcerative Colitis		
Proctitis		6 (18)
Left-sided		13 (38)
Pancolitis		15 (44)
Medication		
AZA	55 (95)	31(91)
6-MP (n)	3 (5)	3(9)
AZA Dose, mean mg (range)	135.5 (25–250)	134.7 (75–200)
6 -MP Dose, mean mg (range)	58.3(12.5–75)	66.7 (50–75)
Monotherapy	33 (57)	19 (56)
Combo therapy with biologic	25 (43)	15 (44)
Allopurinol	5 (9)	0

Data presented in n (%) unless otherwise indicated. All P values > 0.05.

those with active disease and those in remission (312.7 versus 294.2 pmol/8×10^8 RBC; $P = 0.66$).

6MMP

Fourteen per cent of the patients were found to have 6MMP concentrations above the acceptable upper limit (Figure 4). Of these, 30% experienced symptoms likely the result of an adverse drug reaction, including two cases of elevated liver enzymes. No correlation was found between thiopurine dose and 6MMP concentration following weight-based dosing (Prometheus: Pearson $r = 0.0174$, $P = 0.9$; Sick Kids: Pearson $r = 0.0355$ $P = 0.8$).

Shunters

Thiopurine TDM identified 12% of patients as thiopurine shunters. Follow-up TDM results were available for 12% of the identified shunters, providing information on metabolite levels pre- and post-allopurinol initiation. Mean 6MMP concentration decreased from 9180 ($\pm$2095) to 500 pmol/8×10^8 RBC ($\pm$306, $P < 0.01$), and 6TGN concentration increased from 175 ($\pm$61) to 226 pmol/8×10^8 RBC ($\pm$71, $P = 0.1$) despite an average thiopurine dose decrease of 105 mg on initiation of low dose allopurinol (Figure 5).

Adverse Events

Probable adverse drug reactions at the time of TDM were observed in 6.5% of participants. Of these probable adverse events, 50% experienced mildly increased liver enzymes (>2.0× upper limit of normal ALT), 33% had mild myelosuppression (leukocytes < 3.0× 10^9), and 17% drug-induced nausea. No cases of drug-induced pancreatitis were observed.

Clinical Management

Observed Impact of Thiopurine TDM on Clinical Management

Overall, changes to clinical management were observed in 64% of patients, with some patients receiving more than one change to their management (Figure 6). In 28% of patients, thiopurine dosing was decreased; in 12%, dosing increased and in 5%, discontinued altogether. A biologic was added to the drug regimen in 15% of patients. Eight per cent of the patients were classified as shunters, and were prescribed allopurinol with a concomitant decrease in thiopurine dose. Some patients on combination therapy had alterations to their biologic, with dose optimization in 7%, and change of biologic in 5% (Figure 6).

Significantly more changes to clinical management were seen in those with active disease than in those in remission (73% versus
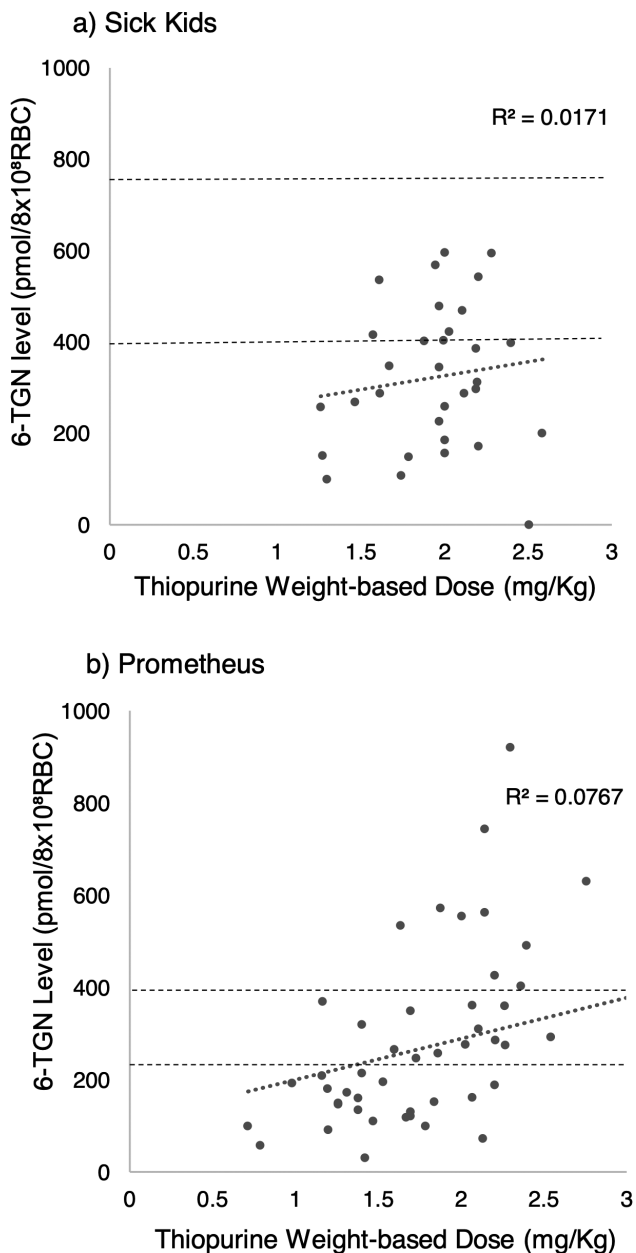
Figure 3. 6-TGN Levels from (a) Sick Kids Lab: HPLC, ref range: 400–750 pmol/8 × 10^8RBC and (b) HPLC, ref range: 230–400 pmol/8 × 10^8RBC. Reference ranges indicated by dashed lines. HPLC, high-performance liquid chromatography.

48%, respectively; $P = 0.02$). No significant differences were found in the relative numbers of changes among other patient types.

Clinical Decision Making Pre- and Post-thiopurine Metabolite Unblinding

Changes to management

Significant changes to physician clinical management before and after metabolite revealing were found. While there was no significant difference in the total number of changes to management, there were significant differences to the relative proportions of the types of changes being made.
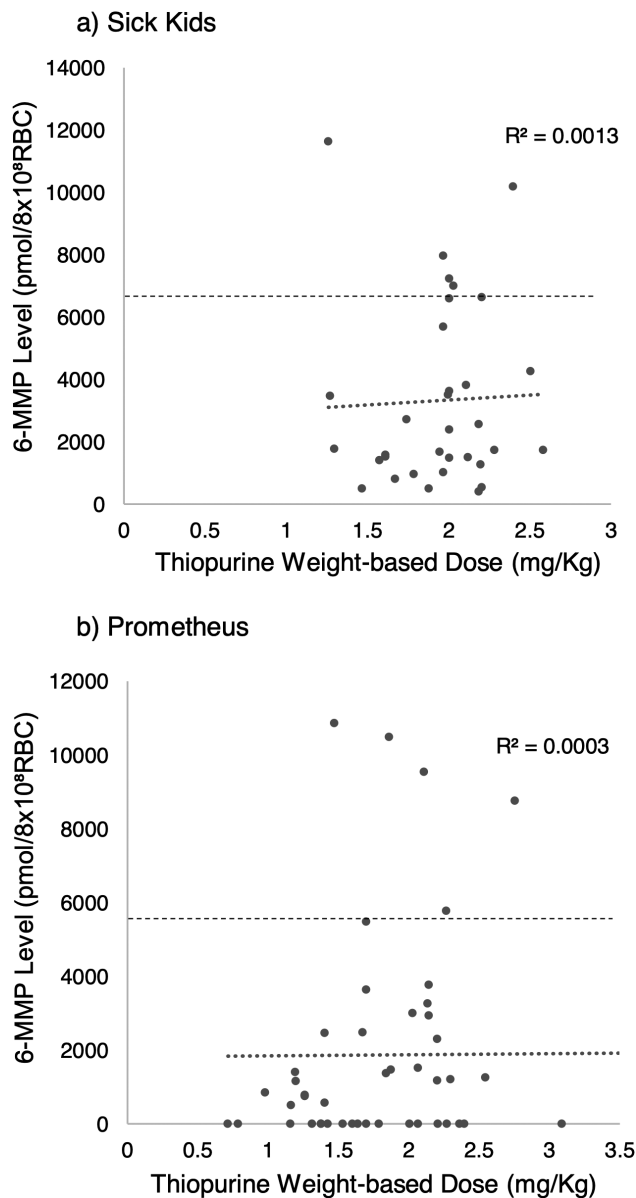

Figure 4. 6-MMP levels from (a) Sick Kids Lab: HPLC, upper limit: 6600 pmol/8 × 10^8RBC and (b) Prometheus Labs; HPLC, upper limit: 5700 pmol/8 × 10^8RBCLabs; Upper limits indicated by dashed lines. HPLC, high-performance liquid chromatography.

Before unblinding, a thiopurine dose reduction was recommended in 26% of the patients, compared with 43% of cases after unblinding ($P < 0.05$). Prior to the revealing of metabolite levels, allopurinol was not included in any patient's drug regimens; however, after metabolite levels indicated shunting, physicians added allopurinol to 9% of drug regimens ($P < 0.05$).

Changes by patient type

After TDM results were revealed to physicians, changes in management were significantly more frequent in patients with active disease than in those in remission (47% versus 24%; $P < 0.05$).
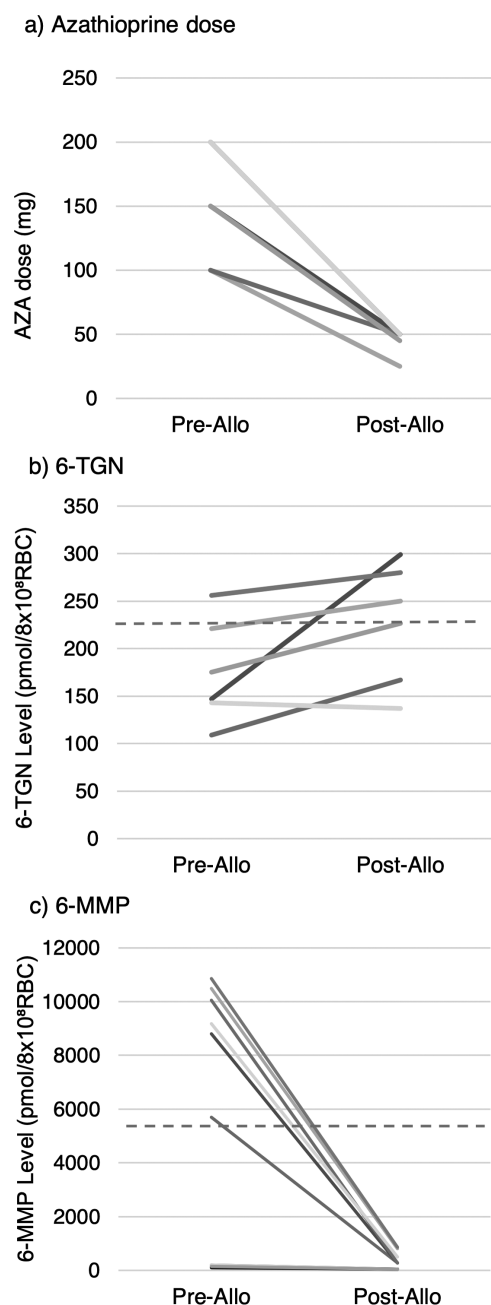
Figure 5. Azathioprine dose and metabolite levels pre and post induction of allopurinol in thiopurine shunters. Therapeutic threshold (6-TGN) and upper limit (6-MMP) indicated by dashed lines.

Likewise, patients on thiopurine monotherapy had significantly more changes made to their management than those on combination therapy with a biologic (48% versus 27.5%; $P < 0.05$). There was no significant difference in the number of changes made among those with CD versus UC (43% versus 32%).

Among patients in remission, significantly more changes were seen in those on monotherapy versus combination therapy (41.2%, 6.3%; $P < 0.05$). No significant difference was seen among patients with active disease on monotherapy versus combination therapy (51.4%, 37.5%).

Discussion

Despite a shifting landscape of drug therapy in IBD with an increasing number of available biologic and nonbiologic therapies, a significant number of patients do not achieve clinical or endoscopic remission rates, even in recent clinical trials (21–23). For the time-being, azathioprine continues to be required by health care payers in many countries prior to approval of more advanced therapy. Additionally, a substantial percentage of refractory patients are continued on the combination therapy of a thiopurine and biologic to achieve or maintain mucosal healing. However, complex inter-individual differences in thiopurine metabolism result in substantial variability of clinical efficacy and drug toxicity (3,5,11). This exposure–response variability limits the adequacy of conventional weight-based dosing. Only 29% of patients in this analysis had 6TGN levels in the therapeutic range, despite 'conventionally-accepted' weight-based dosing. Additionally, 14% of the participants had a supratherapeutic 6MMP level, which improved with either dose reduction, addition of allopurinol, or cessation of thiopurine. Numerous other studies have shown that TDM of thiopurine metabolites improve clinical outcomes through dose optimization and monitoring for potential toxicity (7,9,10,16,24). Our results add to this evidence base by demonstrating that thiopurine TDM leads to changes to clinical management and decision making in a real-world context, and examines which clinical scenarios were most likely to benefit from use of TDM.

Thiopurine TDM promotes early identification of shunters, with 9% of study participants being classified as such following TDM, but only a quarter of which had abnormal liver biochemistry and none of whom were identified until unblinding in the sequential blinded analysis. This is in line with available evidence, showing that the use of TDM as a prognostic tool to identify shunters promotes early thiopurine dose adjustment and initiation of allopurinol, decreasing the likelihood of adverse events and increasing the chance of remission (6,16,25). Additionally, this study showed that low-dose allopurinol was effective in suppressing 6MMP for the majority of patients. This low-dose regimen has now become the starting dose for allopurinol in our clinic.

In our sequential blinded analysis of the impact of thiopurine TDM on clinical decision making, we also found that thiopurine TDM had an especially large impact on the management of patients with active disease. Dose adjustment was the most common change to clinical management, followed by the addition of a biologic or allopurinol. This is in line with previous studies showing high rates of dose adjustment following TDM and subsequent increases in remission rates (7,10,18).

While treatment strategies for IBD are improving, there remains an ongoing 'treatment gap' in the induction and maintenance of mucosal healing for patients with IBD. TDM is one potential avenue to optimize current therapies. Additionally,
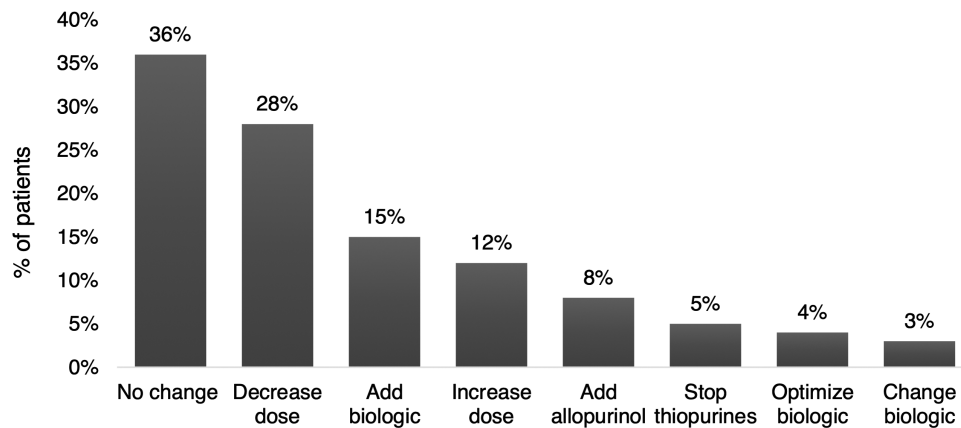
Figure 6. Changes to management following therapeutic drug monitoring.

it can allow for improved understanding of the underlying etiology behind non- or loss-of-response. There is little literature indicating the extent to which thiopurine TDM is utilized in North America; however, given the limited availability of testing facilities, it is likely that the practice is not as widespread as may be supported by the evidence. Until 2017, there was only one testing facility in Canada, located at SickKids Hospital in Toronto, Ontario. In 2015, access to thiopurine testing in British Colombia was initially provided by the Ministry of Health which enabled the development of the training and validation data set. In 2017, we developed a testing facility in Victoria, British Columbia (19). Since the development of an in-province assay, a laboratory fee code was submitted that became available through the provincial medical services plan. Given that most provinces have such mechanisms in place for tests which may not be available locally, and that several widely available methods are able to reliably test thiopurine metabolites, it is conceivable that the majority of IBD patients in Canada would be able to access metabolite testing.

There are several other labs tests which may also increase the safety of thiopurine use. TPMT variant testing predicts reduced enzymatic function and subsequent thiopurine dose reduction is associated with a reduced risk of myleosupression (26). More recently, a single-nucleotide polymorphism (rs2647087) has been identified that confers an increased risk of pancreatitis in homozygous patients (27).

Our study has several limitations, most notably its partial retrospective analysis, cross-sectional design, and scope. We limited our analysis to clinical indices and changes in management rather than long-term outcome measures. Due to low anticipated effect size and relatively slow time course of clinical changes in IBD, the measurement of changes to long-term outcomes following thiopurine TDM-guided medication adjustment would need to be highly powered and require years of follow-up. Future prospective studies exploring thiopurine TDM over time and across the natural history of IBD are needed. Thiopurine metabolism can be affected by other medications such as aminosalicylates (5ASA), and therefore, by design, confounding effects cannot be excluded (28). We included the blinded/unblinded chart review to provide an estimation of the impact of TDM on management. Our study found no association between 6TGN concentrations and disease status. While this lack of relationship has been reported in other small studies (5,10,20), other studies have shown a positive association between 6TGN concentration and response to treatment, and so, it is unclear if this discrepancy is due to our sample size or heterogeneous population characteristics of mono- versus combination therapy. Lastly, many patients in the present analysis had TDM performed after a prior duration of azathioprine use. This is both a strength and a weakness as on one hand it reflects the real-world impact of instituting thiopurine TDM in an established gastroenterology practice, but it is also likely that this particular group of patients has self-selected, potentially introducing a thiopurine survival bias. If this was a controlled trial of patients newly initiated on thiopurine, it is likely that the rate of aberrant metabolism would be even higher.

Overall, our study shows that weight-based dosing is poorly correlated with 6TGN level, is associated with high interindividual variability in thiopurine metabolism profiles, and shows a significant impact on physician decision making, especially in those not responding to treatment.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding publication of this article. D.L. has received consulting and speaking fees from AbbVie, Allergan, Ferring, Janssen, Merck, Pfizer, Shire and Takeda.

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