

Azathioprine dosing and metabolite measurement in pediatric inflammatory bowel disease: does one size fit all?

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Abstract

Background Azathioprine is widely used for the maintenance of remission in children with inflammatory bowel disease (IBD). Measuring thiopurine metabolites 6-thioguanine (6-TGN) and 6-methyl-mercaptopurine (6-MMP) can aid in optimizing treatment and preventing toxicity. We report a proactive approach combining early metabolite measurements with IBD activity index to achieve optimal azathioprine dosing.

Methods The reporting of azathioprine dosing, IBD activity indexes and thiopurine metabolites was evaluated retrospectively in 40 children with IBD. Additional treatments and the effect of azathioprine on blood counts were also examined.

Results Forty children (40% female) with IBD (26 Crohn's disease, 12 ulcerative colitis, and 2 unclassified IBD), mean age 12.2 ± 3.4 years, were included in the study. The mean azathioprine dose was 1.3 ± 0.4 mg/kg; mean 6-TGN level was 280 ± 151 pmol/ 8×10^8 red blood cells (RBC) and mean 6-MMP level 1022 ± 1007 pmol/ 8×10^8 RBC. Disease activity index (Crohn's and ulcerative colitis, pediatric specific) at the time of metabolite measurement was 6.5 ± 8 . Twenty-eight children did not require azathioprine dose adjustment, while it was increased in 12. Data from children with azathioprine monotherapy were analyzed separately and the results were similar.

Conclusion Timely measurement of thiopurine metabolites and clinical assessment can provide a powerful tool to optimize azathioprine dosing and reduce serious adverse effects in children with IBD.

Keywords Pediatric inflammatory bowel disease, 6-thioguanine, 6-methyl-mercaptopurine, thiopurine toxicity, activity index

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Introduction

Inflammatory bowel disease (IBD) is a chronic, immune-regulated inflammatory condition of the digestive system. It is thought to have a complex etiology involving genetic, immunological and environmental factors [1]. The worldwide incidence and prevalence of IBD in children is increasing: the current incidence of Crohn's disease (CD) in children ranges between 2.5 and 11.4/100,000 [2]. Pediatric IBD is associated

with more extensive and aggressive disease that has higher rates of surgical management [3,4]. The main treatment goals in IBD management include timely and effective induction of remission followed by maintenance, with crucial focus on optimizing nutrition, monitoring growth and quality of life, and limiting drug-related side effects [5].

IBD disease activity may be assessed using the activity indexes (AI) Pediatric Ulcerative Colitis Activity Index (PUCAI) and Pediatric Crohn's Disease Activity Index (PCDAI), which allow clinical symptoms, physical signs and biochemical markers to be converted into an activity score. These are reliable indicators of disease severity and remission status and are helpful in evaluating the response to treatment changes. An AI score of <10 accurately reflects clinical remission [6,7].

Thiopurines, such as azathioprine and mercaptopurine, are immunomodulatory agents recommended for the maintenance of steroid-free remission in children with IBD [4]. They are initiated soon after diagnosis and can take up to 14 weeks to be fully effective. The recommended starting doses are currently 2-2.5 mg/kg for azathioprine and 1-1.5 mg/kg for mercaptopurine [4,8].

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Conflict of Interest: None

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Thiopurine use is associated with dose-dependent adverse drug reactions (ADRs), often leading to dose reduction or drug discontinuation [9]. Hematological toxicity in the form of myelosuppression, secondary to either thiopurine or elevated levels of its metabolite 6-thioguanine (6-TGN), is the most serious ADR. A review of 66 trials including 8302 patients reported a cumulative incidence of myelosuppression of 7%, predominantly occurring within the initial few months of treatment [10,14].

Although the use of thiopurine in the management of pediatric IBD is increasing and the measurement of the thiopurine metabolites 6-TGN and 6-methyl-mercaptopurine (6-MMP) can be helpful in determining compliance and optimizing therapy, these metabolites are not routinely monitored [11]. The cost and availability of the tests have been cited as reasons for this [10].

Thiopurine metabolite measurement is available at Evelina London Children's Hospital and is currently routinely used as a tool for monitoring children receiving thiopurine therapy. In this paper we describe a proactive approach, combining clinical data and thiopurine metabolite measurement, to guide azathioprine dosing in children with IBD.

Patients and methods

Pediatric IBD patients on azathioprine therapy were identified through a retrospective review of medical records between April 2015 and June 2017. Patients' demographics, disease phenotype, treatments and clinical data to establish disease AI were extracted from electronic medical records. Thiopurine methyltransferase (TPMT) status, 6-TGN and 6-MMP levels, blood count and inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) were retrieved from the hospital's electronic records. The relevant disease-specific AIs were used (PCDAI and PUCAI).

Clinical remission was defined as a PCDAI or PUCAI <10 [12,13]. Children with deficient or intermediate TPMT enzyme activity (normal range 26-50 pmol/h/mgHb) were excluded, as this affects azathioprine dosing. Children with very low (<150) 6-TGN levels due to lack of compliance with treatment were also excluded, although continued support was provided to develop strategies to improve adherence to medication.

The study was registered with and approved by the hospital review board; a full ethical review was not deemed to be necessary.

Statistical analysis

Statistical analysis was performed using IBM SPSS® Statistics Version 22. Data were expressed as mean ± standard deviation (SD). Pearson's correlation coefficient was used to investigate the relationship between the variables. All statistical tests were 2-tailed and a P-value <0.05 was indicative of statistical significance.

Results

A total of 41 patients were identified, of whom 16 (40%) were female and 25 (60%) male. One patient was excluded as the initial TPMT value was <26. Of these identified patients, 12 had a diagnosis of ulcerative colitis (30%), 26 with Crohn's Disease (65%), and 2 with unclassified IBD (5%). The mean age at diagnosis was 12.2±3.4 years. Mean TPMT value was 41.8±11 pmol/h/mgHb. The azathioprine dose was 1.3±0.4 mg/kg.

To achieve the initial induction of remission, prednisolone was used in 13 patients, exclusive enteral nutrition in 19 patients, and aminosalicylates in 4. Four patients were treated in different institutions and their data were unavailable.

AI at time of starting therapy was 23±16, reducing to 6 after 65 days (interquartile range [IQR] 29-85). Mean 6-TGN value was 280±151 pmol/8 × 10⁸ red blood cells (RBC) and mean 6-MMP value 1023±1008 pmol/8 × 10⁸ RBC. The mean lymphocyte count was 1.7±0.8 × 10⁹/L. Twenty-eight children were in clinical remission with therapeutic 6-TGN, 12 had sub-therapeutic 6-TGN for which the azathioprine dose was increased. Twelve children were on 5-aminosalicylate and 5 were on biologic therapy (4 on infliximab and 1 on adalimumab) (Table 1). Mean age was 12.4±3.4 years, azathioprine dose 1.4±0.5 mg/kg, and AI at the start of azathioprine 23±14.

After 61 (IQR 27-74) days, levels of 6-TGN were 265±150 pmol/8 × 10⁸ RBC, 6-MMP 1112.3±1073.4 pmol/8 × 10⁸ RBC, white blood cells (WBC) 8.2±3.4 × 10⁹/L, lymphocytes 1.8±0.9 × 10⁹/L, neutrophils 5.1±3.2 × 10⁹/L and the AI at the time of measurement was 5±5 (Table 2 and Fig. 1). At the time of metabolite measurement, all children were on maintenance therapy having completed standard induction.

Table 1 Background information

Characteristic	Value
Total patients	40
Females (percent)	16 (40%)
Age (years±SD)	12.2±3.4
Ulcerative colitis	12 (30%)
Crohn's disease	26 (65%)
Unclassified inflammatory bowel disease;	2 (5%)
Azathioprine dose mg/kg (mean±SD)	1.3±0.4
Time metabolites measured (days±SD)	65±45
White blood cells×10 ⁹ (mean±SD)	8.1±3.1
Lymphocytes×10 ⁹ (mean±SD)	1.7±0.8
Neutrophils×10 ⁹ (mean±SD)	5.1±3.1
Children on infliximab	4
Children on adalimumab	1

SD, standard deviation

Discussion

Thiopurines such as azathioprine and mercaptopurine are immunomodulatory agents recommended for the maintenance of steroid-free remission in children with IBD [4]. Once absorbed, azathioprine is converted into its active metabolites, 6-MMP and 6-TGN, via an enzymatic pathway involving TPMT [9,8].

A therapeutic 6-TGN range is defined as 230-450 pmol/ 8×10^8 RBC. Interpretation of the range as low (<230) or high (>450) depends on the clinical features. In cases of active disease, a low or absent 6-TGN level may indicate non-adherence to medication or under-dosing, in which case patient education or a dose increase is indicated [14]. A low or normal 6-TGN level and a high 6-MMP level may indicate thiopurine intolerance, and the dose should be split: e.g. twice daily or allopurinol added as an adjunct to low-dose thiopurine [11]. A high 6-TGN level with active disease suggests thiopurine refractory disease, and alternative treatment should be sought [10,4]. This study evaluated a cohort of pediatric patients with IBD who achieved therapeutic thiopurine metabolites with lower than the target recommended azathioprine dose. Blood tests were performed according to current guidelines and azathioprine metabolites were measured 6 weeks after the initiation of therapy. The azathioprine dose was adjusted to achieve therapeutic metabolite levels.

To maintain therapeutic 6-TGN levels, the effective azathioprine dose for the whole group in this study was 1.3 ± 0.4 mg/kg, as there is emerging evidence to suggest a lower than standard azathioprine dose in patients receiving combination therapy with biologics [15]. Excluding the children on combination therapy, the effective azathioprine dose in children was 1.4 ± 0.5 mg/kg (Table 2). Similarly to previous reports, we were able to achieve clinical remission, as measured by disease AI (PUCAI and PCDAI), in children with

a mean 6-TGN of 260 ± 151 pmol/ 8×10^8 RBC (in the whole cohort) under azathioprine monotherapy. None of the children developed azathioprine toxicity as defined by myelosuppression or abnormal liver function tests. The azathioprine dose was not changed in 28 children who were in clinical remission and had therapeutic 6-TGN levels, while in 12 children 6-TGN levels were sub-therapeutic and their azathioprine dose was increased. We did not observe a significant correlation between azathioprine (monotherapy) dose and 6-TGN levels ($r=0.3$, $P=0.20$) or a significant correlation between WBC and TGN levels ($r=-0.18$, $P=0.3$; Fig. 2,3). This may be due to the small sample size, as those correlations have been well documented in other studies.

Reduced enzyme activity results in an increased risk of myelosuppression. It is therefore recommended to check TPMT levels in every child prior to the initiation of therapy (normal range 25-50 pmol/h/mgHb). Cytopenia can occur even in the presence of normal TPMT activity; therefore, blood monitoring is required in all patients [4]. Dose reduction or alternative treatments can be considered where an abnormal TPMT status is identified [8].

Side effects and ADRs, including gastroenterological disturbance, skin rash and pancreatitis, are common

Table 2 Excluding children on biologics

Characteristic	Mean \pm SD
Age (years)	12.4 \pm 3.4
Azathioprine dose (mg/kg)	1.4 \pm 0.5
Time metabolites measured (days)	61 \pm 45
White blood cells $\times 10^9$ (/L)	8.1 \pm 3.4
Lymphocytes $\times 10^9$ (/L)	1.8 \pm 0.9
Neutrophils $\times 10^9$ (/L)	5.1 \pm 3.2

SD, standard deviation

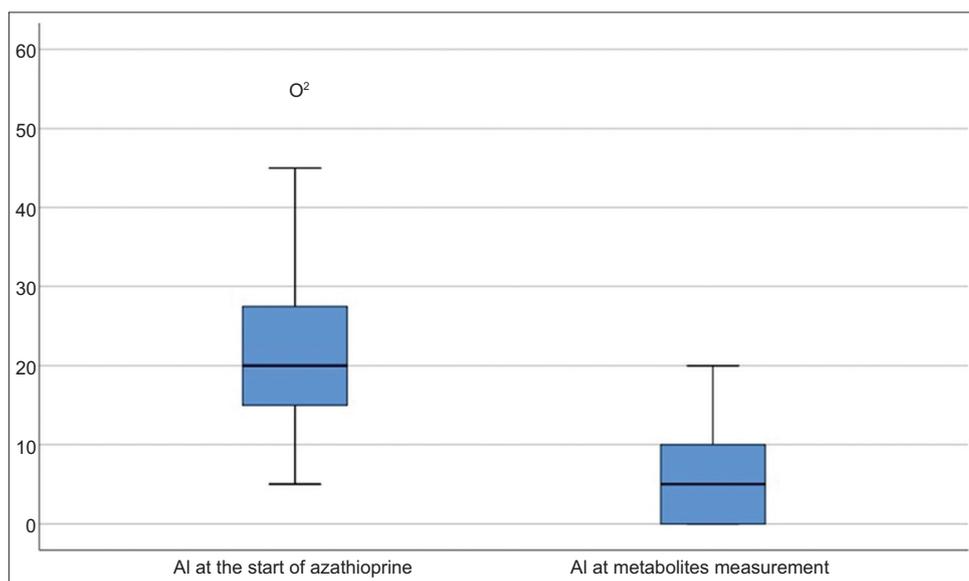


Figure 1 Activity index (AI) at the start of azathioprine treatment and at the time of metabolite measurement

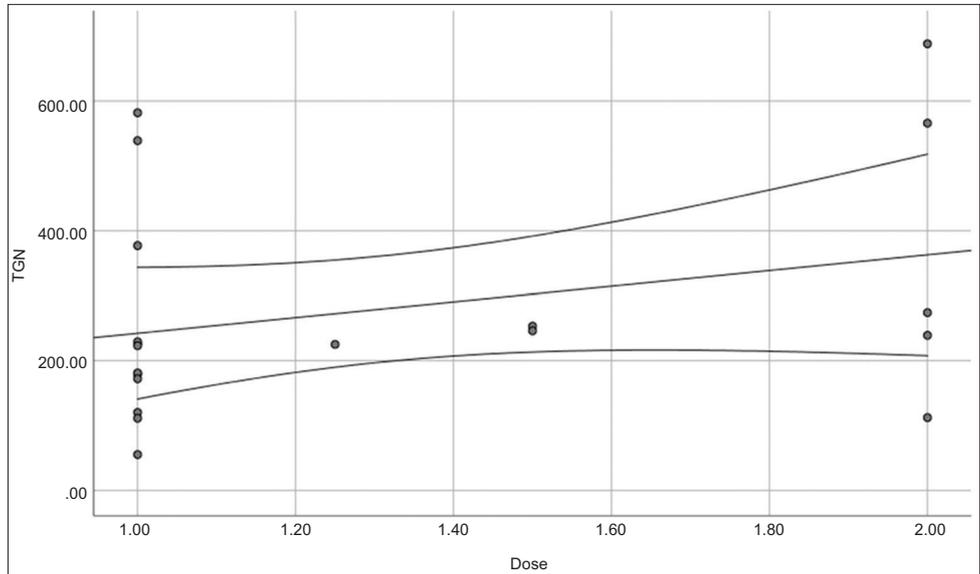


Figure 2 Scatterplot for azathioprine dose and thioguanine (TGN) level ($r=0.3$ $P=0.20$)

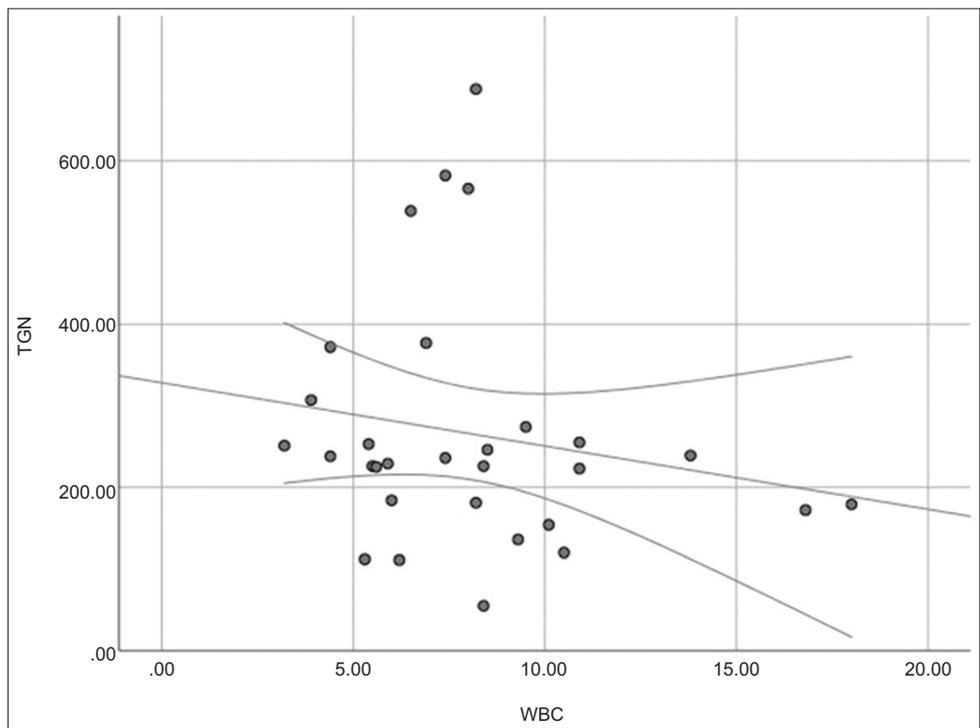


Figure 3 Scatterplot for white blood cell (WBC) counts and thioguanine (TGN) level ($r=-0.18$ $P=0.3$)

with thiopurine treatment, requiring dose adjustment or discontinuation in up to 25% of adult patients [16], with 56% of ADRs occurring within 1 month and 92% within 3 months of treatment. A multifaceted approach is required to maximize the response to and safety of these drugs. Pre-treatment TPMT status and regular monitoring of blood counts on treatment is considered the minimum standard for patients treated with thiopurine. Using 6-TGN levels above $235 \text{ pmol}/8 \times 10^8$ RBC as a surrogate marker of efficacy is well documented

and is associated with maintaining clinical remission [17]. It is also important to minimize the idiosyncratic side-effects by using an appropriate dosing regimen. Although the current dosing regimen for thiopurine therapy is body-weight based, there is increasing evidence to support the use of metabolite measurement to adjust thiopurine doses. Similarly to our findings, Hibi *et al* reported that low-dose thiopurine was effective and safe in maintaining remission in adults with ulcerative colitis [18]. They described a significantly lower

incidence of myelosuppression in the low-dose cohort compared with the standard-dose group.

6-TGN levels have been shown to increase with thiopurine dose escalation and decrease with dose reduction, and a study by Nguyen *et al* explored this relationship, the results of which support the use of metabolite levels to reliably inform and guide thiopurine dosing [9]. 6-TGN levels are also useful in identifying cases of non-adherence to thiopurine therapy, and it has been suggested that routine observation of the metabolites can help in improving adherence rates [14]. A further study demonstrated that the majority of patients had a favorable outcome when their treatment was directed by metabolite measurement and that this can lead to better optimization of thiopurine treatment [19].

A subjectively reported barrier to routine metabolite measurement was the cost of the test and reimbursement issues; however, as demonstrated by Dubinsky *et al*, the overall cost may be reduced [13]. In children who find the side-effects of thiopurine therapy intolerable, the next treatment step, such as biologics, can be costly.

The data collected from this cohort show that, in a subset of children with IBD, early monitoring of thiopurine metabolites can aid clinical decisions regarding azathioprine dosing, and suggests that therapeutic thiopurine metabolites can be achieved with lower than the recommended target dose.

We acknowledge the limitations of this study, including the small sample size and retrospective design. We also acknowledge the short follow-up period, and that some children could still be experiencing the effect of induction therapy, making it difficult to comment on the effect of azathioprine alone and therefore to extrapolate any assumptions on safety. Therefore, we do not suggest replacing weight-based azathioprine dosing with metabolite measurement, but rather that this should be used as an adjunct to help optimize therapy, minimize unwanted adverse effects and assist in the timely guidance of clinical decision making.

Summary Box

What is already known:

- Thiopurines are standard treatment for children with inflammatory bowel disease (IBD)
- Weight-based dosing is the current recommended regime for thiopurine treatment in these patients
- Thiopurine metabolites are increasingly used to monitor treatment

What the new findings are:

- This study describes a proactive approach to dosing and monitoring children on thiopurine therapy
- This paper suggests standard weight-based dosing may not be applicable to children with IBD

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