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Thiopurine metabolite levels in patients with atopic dermatitis and/or chronic hand/foot eczema treated with azathioprine

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ABSTRACT

Background: Azathioprine is frequently used in severe eczema. It is converted in the liver into active metabolites, including 6-thioguanine nucleotide (6-TGN) and methylated 6-methylmercaptopurine (6-MMP). In the past, the therapeutic potential of azathioprine may have not been fully utilized. Recent investigations on inflammatory bowel disease have led to a better understanding of azathioprine metabolism and optimizing treatment.

Objective: To investigate whether measuring thiopurine metabolites in circulation can improve the effectiveness and safety of azathioprine treatment in patients with atopic dermatitis and/or chronic hand/foot eczema.

Methods: Azathioprine metabolite levels were measured in eczema patients during maintenance treatment (Part I) and dose escalation (Part II). Clinical effectiveness, hepatotoxicity, and bone marrow suppression were analyzed and TPMT genotype was assessed.

Results: A wide variation in metabolite levels in all dose groups was observed. In Part I (32 patients), there were no significant differences in 6-TGN levels between clinical responders and non-responders ($p = .806$). No hepatoxicity or myelotoxicity was observed. In Part II, all 6-TGN and 6-MMP levels increased during dose escalation. Hypermethylation was observed in 2/8 patients.

Conclusion: For individual eczema patients treated with azathioprine, routinely measuring 6-TGN and 6-MMP can be helpful in optimizing azathioprine dose, improving clinical effectiveness, and preventing side effects.

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Atopic dermatitis; azathioprine; metabolites; therapeutic drug monitoring

Introduction

Atopic dermatitis (AD) and chronic hand/foot eczema are common chronic inflammatory skin diseases. Although the majority of patients can be treated adequately with topical corticosteroids and/or phototherapy, oral immunosuppressive drugs are indicated in more severe cases.

Azathioprine (AZA) is an important off-label drug in the management of severe and difficult to treat AD. Randomized clinical trials report a decrease in clinical scores, varying from 26% to 37% decrease in SASSAD (Six Area, Six Sign Atopic Dermatitis) score and 39% decrease in SCORAD (Scoring Atopic Dermatitis) score after 12 weeks. The percentage of patients reporting adverse events in these short-term (12 weeks) randomized clinical trials ranged between 5.6% and 22.9% (1). Abnormalities in cell count, such as lymphocytopenia, were most frequently reported. In 9% of the patients, these adverse events led to withdrawal and in 9% of patients dose adjustments were made. However, in daily practice, results are less favorable. In an AD population treated with AZA in daily practice, Thomsen et al. found more than 30% treatment failure after one year of AZA treatment: 9/60 patients (15%) had to discontinue due to a lack of clinical benefit and 12/60 patients (20%) due to side effects (2).

In a retrospective daily practice study in two university hospitals in the Netherlands, also 30% (14/46) of the adult AD patients treated with AZA discontinued treatment due to side effects and/or ineffectiveness (3). In a recent drug survival study of 94 AD patients treated with AZA in daily practice, 57% discontinued treatment due to ineffectiveness (19%), side effects (36%), or both (2%) (4).

Little data are available on the efficacy of AZA in chronic hand/foot eczema. Agarwal et al. performed a randomized controlled trial in which the use of AZA ($n = 45$) was compared to topical clobetasol proprionate 0.05% ($n = 46$) in patients with chronic hand eczema. After 24 weeks of treatment, the improvement of itch and the improvement of the HECSI (hand eczema scoring index) were significantly higher in the AZA group ($p = .003$ and $p = .001$, respectively) (5).

In the past, the therapeutic potential of AZA may has not been fully utilized (6). The AZA metabolism is very complex due to the involvement of various enzymes; it is studied thoroughly in patients with inflammatory bowel disease (IBD). AZA is a thiopurine pro-drug and has no immunosuppressive activity itself. In the liver, AZA is converted into 6-mercaptopurine (6-MP). Subsequently, 6-MP undergoes metabolic transformations, *via* a

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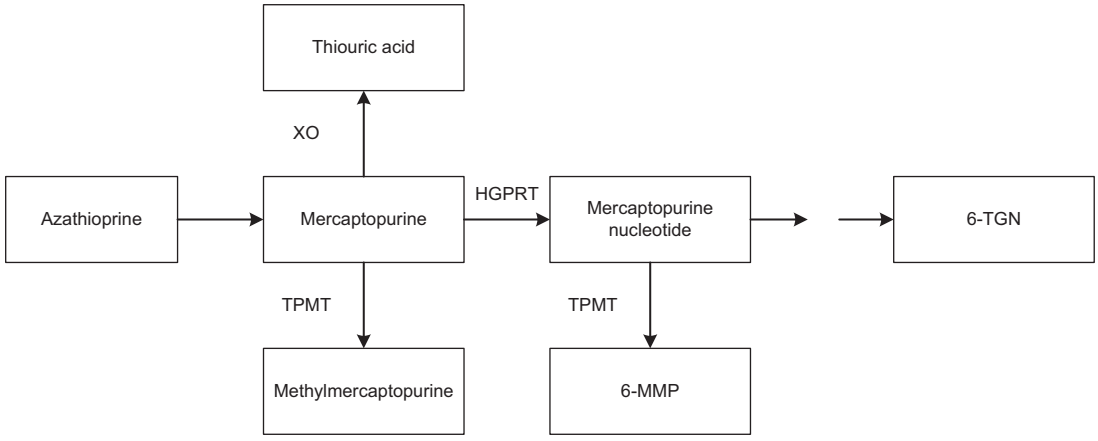

Figure 1. Azathioprine metabolism (simplified). 6-MMP: methylated 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; HGPRT: hypoxanthine-guanine phosphoribosyl transferase; TPMT: thiopurinemethyltransferase; XO: xanthine oxidase.

complex enzymatic pathway, resulting in a variety of pharmacologically active metabolites (Figure 1) (7). The most important metabolites are 6-thioguanine nucleotide (6-TGN) and methylated 6-methylmercaptopurine (6-MMP). The immunosuppressive effect of AZA is caused by the 6-TGN metabolites. Intracellular 6-TGN is incorporated into DNA instead of guanine nucleotides, which, after a strand breakage, triggers cell-cycle arrest and apoptosis. This results in the inhibition of nucleotide and protein synthesis, and ultimately in the inhibition of lymphocyte proliferation (8–10). However, the main mechanism of immunomodulation is by inducing T-cell apoptosis by modulation cell (Rac1) signaling (11). The therapeutic onset after thiopurine initiation is delayed as it takes 12–17 weeks for TGNs to be incorporated into DNA (12,13). This explains the delayed clinical efficacy in AD, which is usually reached after 3–4 months.

Recent investigations, especially in IBD, have led to a better understanding of the AZA metabolism and optimization of treatment (6,14,15). Therapeutic drug monitoring can reveal noncompliance or under-dosing and can be used as a practical tool to optimize AZA therapy (6). Data on the AZA metabolism and therapeutic drug monitoring of AZA metabolites in patients with chronic inflammatory skin diseases are scarce. Recently, Reynolds et al. suggested, based on their unpublished experience in individual patients with AD, that measuring 6-TGN levels particularly in patients with heterozygote thiopurine S-methyltransferase (TPMT) activity may be useful (16). The aim of the present study is to investigate whether measurement of thiopurine metabolites is helpful in improving effectiveness and safety of AZA treatment in patients with AD and/or chronic hand/foot eczema.

Methods

Study population

This study was exempted from review by our institutional review board. In this prospective mono-center study, AZA metabolite levels were measured in adult patients with AD and/or hand/foot eczema during maintenance treatment (≥100 mg/day, for more than three months) (Part I). The criteria of Hanifin and Rajka and of Williams et al. were used for the diagnosis of AD (17,18). The following patient characteristics were collected: age, sex, type of eczema (AD or hand/foot eczema), AZA dose, and the concomitant use of oral corticosteroids. Additionally, AZA metabolites were measured structurally in another small group of patients from start of AZA treatment and during dose escalation (Part II).

Table 1. Measurement of azathioprine metabolites: Dervieux versus Lennard method.

		Analysis method	
	Lennard	Dervieux	Unit
Therapeutic			
6-TGN	230–450	600–1200	pmol/8 × 10^8 RBC
6-MMP/6-TGN ratio	5–25	2–10	pmol/8 × 10^8 RBC
Toxic			
6-TGN	>450	>1200	pmol/8 × 10^8 RBC
6-MMP	>5700	>5700	pmol/8 × 10^8 RBC

Values used by the Dutch Society of Clinical Pharmacy, based on studies using Lennard method in inflammatory bowel disease patients and the conversion factor 2.6 for deriving the Dervieux values (33).

Measurements of AZA metabolites

6-TGN and 6-MMP levels in red blood cells (RBC) were measured in erythrocytes, as a surrogate marker of the levels in leukocytes, using the Dervieux-method (19). The majority of previously published studies used the Lennard method (comparison of the values shown in Table 1).

Metabolites were analyzed by the Clinical Pharmaceutical and Toxicological laboratory of the Department of Pharmacy, University Medical Center Utrecht, The Netherlands.

Outcomes

Part I

6-TGN and 6-MMP steady-state levels were measured and ratios between these metabolites were calculated in all patients. The correlation between the AZA dose and the metabolite levels was investigated.

6-TGN and 6-MMP levels and 6-MMP/6-TGN ratios between responders and non-responders were compared. Responsiveness was based on the Investigator Global Assessment (IGA, 6-point scale) (20). A responder was defined as IGA 0–2. Non-responders were defined as an IGA 3–5 or concomitant treatment with oral corticosteroids. The optimal 6-TGN level to achieve disease control was defined as the median 6-TGN level in the responder group.

TPMT genotype was determined. Safety results, including hepatotoxicity and bone marrow suppression, were analyzed. Clinically significant hepatotoxicity was defined as an alanine aminotransferase (ALT) or aspartate aminotransferase (AST) two times the upper limit of normal (ALT: >45 U/L in men and >35 U/L in women; AST: >35 U/L in men, >30 U/L in women) (21).

Bone marrow suppression was defined as white blood cell count $<4 \times 10^9$/L and/or thrombocytopenia (platelet count $<150 \times 10^9$/L).

Part II

AZA metabolites were measured in a small group of patients from the start of treatment and during dose escalation. Patients started with AZA 50 mg/day, followed by metabolite measurement after 2 weeks (T1). TPMT genotype was determined using TaqMan analysis. When hepatotoxicity or bone marrow suppression was not observed, AZA dose was increased to 100 mg/day, followed by metabolite measurement 2 weeks later (T2). Finally, the dose was increased to 150 mg/day, again followed by metabolite measurement 2 weeks later (T3).

The following patient characteristics were collected in these patients: age, sex, type of eczema (AD or hand/foot eczema), 6-TGN, and 6-MMP levels at T1, T2, and T3.

Because the therapeutic effect after AZA initiation is delayed up to four months, clinical efficacy was not evaluated at this early time point.

Statistical analysis

The data of the total group were evaluated. Subgroup analyzes for responders and non-responders were made. All statistical analyzes were performed using SPSS version 21.0 (IBM Corp., Armonk, NY; 2012). Frequencies, percentages, and medians with inter quartile ranges (IQR) were calculated.

The Mann–Whitney *U* test and the Chi Square test were used to calculate whether there were statistically significant differences between responders and non-responders, regarding sex, age, dose, 6-TGN levels, and 6-MMP levels. The ANOVA test, corrected for multiple testing by Bonferroni, was used to calculate whether there were statistically significant differences in 6-TGN and 6-MMP levels between the different AZA doses.

Results

Part I

Patient characteristics

Thirty-five patients were treated with AZA. Three patients (8.6%) had 6-TGN levels <50 pmol/8×10^8 RBC and 6-MMP levels <250 pmol/8×10^8 RBC, suggesting noncompliance to AZA. These patients were excluded from further analysis: 32 patients remained for further analysis (Table 2).

The median age at metabolite measurement was 48.0 years [IQR 43.0–56.7]. Twenty-three patients (71.9%) had the diagnosis AD. The other nine patients had isolated hand- and/or foot-eczema (eight dyshidrotic eczema, one hyperkeratotic eczema). Twenty-four patients (75.0%) used monotherapy AZA (dose range 100–200 mg/day). Eight patients (25.0%) used concomitant oral corticosteroids (dose range 5–30 mg/day)

Table 2. Patient characteristics (Part I).

	All patients ($n = 32$)
Male, *n* (%)	15 (46.9)
Age at AZA metabolite measurement (years), median [IQR]	48.0 [43.0–56.7]
Patients with AD, *n* (%)	23 (71.9)
Patients with isolated hand/foot eczema, *n* (%)	9 (28.1)
AZA dose in mg/day, median [IQR]	150.0 [100.0–187.5]
Concomitant use of oral corticosteroids, *n* (%)	8 (25.0)

AD: atopic dermatitis; AZA: azathioprine; IQR: inter quartile range.

Metabolite levels

AZA dose varied from 100 to 200 mg/day with a median dose of 150 mg/day [IQR 100.0–187.5] (Figure 2).

Twenty-eight patients had an extensive metabolizer TPMT genotype, three patients had an intermediate metabolizer TPMT genotype (*1/*3A). In one patient the TPMT genotype determination failed.

There was a wide variation in metabolite levels in all dose groups. 6-TGN levels varied between 42 and 696 pmol/8×10^8 RBC in patients using 100 mg/day, between 99 and 656 pmol/8×10^8 RBC in patients using 150 mg/day and between 142 and 861 pmol/8×10^8 RBC in patients using 200 mg/day. 6-MMP levels varied between 205 and 3411 pmol/$8 \times$ x10^8 RBC in patients using 100 mg/day, between 275 and 26,027 pmol/8×10^8 RBC in patients using 150 mg/day, and between 126 and 4173 pmol/8×10^8 RBC in patients using 200 mg/day. In six (18.8%) patients (all using 150 mg/day) the 6-MMP level was >5700 pmol/8×10^8 RBC (toxic level). The steady state 6-MMP/6-TGN ratio varied from 0.9 to 81.2 in patients using 100 mg/day, from 1.2 to 262.9 in patients using 150 mg/day, and from 0.5 to 18.9 in patients using 200 mg/day. The metabolite levels of the three TPMT*1/*3A patients are highlighted in Figures 2 and 3.

6-MMP levels were statistically significantly different among patients with various AZA doses ($p = .0431$), but not after correction for body weight (data not shown). 6-TGN levels were significantly higher in the three patients with an intermediate metabolizer TPMT genotype ($p = .01$) compared to patients with an extensive metabolizer TPMT genotype, but no 6-TGN levels above the upper limit of 1200 were observed.

Responders versus non-responders

In total, 16 patients were characterized as responder and 16 patients as non-responder (Table 3, Figure 3).

Responders were significantly older (median 50.9 years versus 44.3 years in the non-responder group) at the time of the metabolite measurement ($p = .029$). There were no significant differences in AZA dose (mg/day) ($p = .628$), 6-TGN levels ($p = .806$), 6-MMP levels ($p = .763$), or 6-MMP/6-TGN ratios ($p = .940$) between responders and non-responders.

The median 6-TGN level in the responder group was 197.0 pmol/8×10^8 RBC.

Safety

No patient met the criteria of hepatotoxicity. Six patients had 6-MMP levels >5700 pmol/8×10^8 RBC (toxic level, Table 3) with a median duration of treatment until metabolite measurement of 0.82 years [IQR 0.27–2.16]. Two of these patients (al extensive metabolizer TPMT genotype) had slightly increased ALT levels at time of metabolite measurement (83 U/L in one male patient and 42 U/L in one female patient).

None of the 32 patients met the criteria of bone marrow depression. None of the patients had 6-TGN levels >1200 pmol/8×10^8 RBC (toxic level, Table 3).

Part II. AZA metabolites were measured consecutively in eight patients (three men, five women) from start of AZA treatment and during dose escalation (Table 4, Figure 4). Median age at start of AZA treatment was 46.2 years [39.9–50.9]. Five patients were diagnosed with AD, three patients with isolated hand/foot eczema. Seven patients had an extensive metabolizer TPMT genotype. The TPMT genotype was missing in one patient. 6-TGN and 6-MMP values at the different measurements varied widely. All levels of 6-TGN and 6-MMP increased during dose escalation up to T3

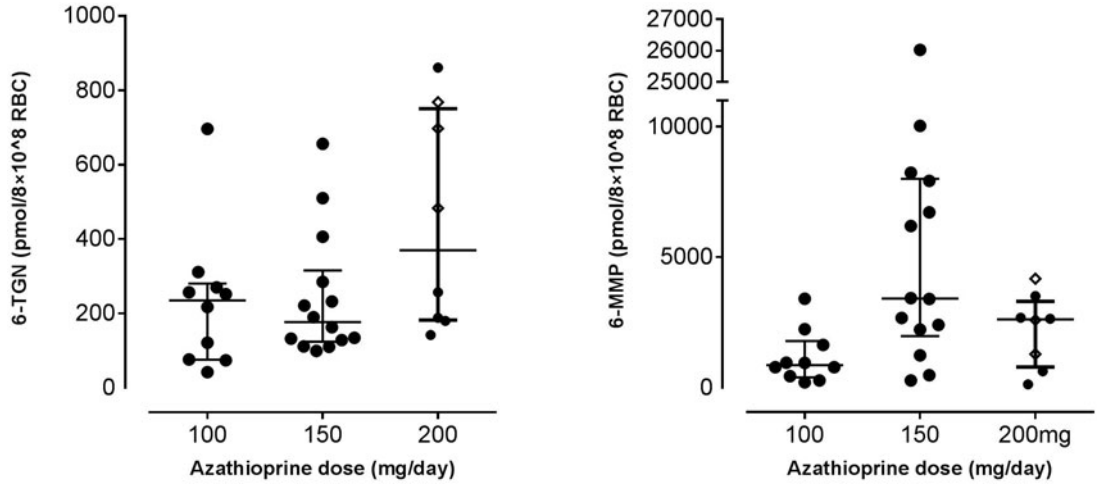

Figure 2. Azathioprine dose (100, 150, and 200 mg/day) and steady-state metabolite (6-TGN and 6-MMP) levels (Part I) (median with IQR). ◇ = Intermediate metabolizer TPMT genotype. A, azathioprine dose (mg/day) and 6-TGN level; B, azathioprine dose (mg/day) and 6-MMP level. 6-MMP levels were statistically significantly different among patients with various AZA dose ($p = .0431$); 6-TGN levels were not significantly different among patients with different AZA dose ($p = .0682$). 6-MMP: methylated 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; IQR: inter quartile range; RBC: red blood cell.

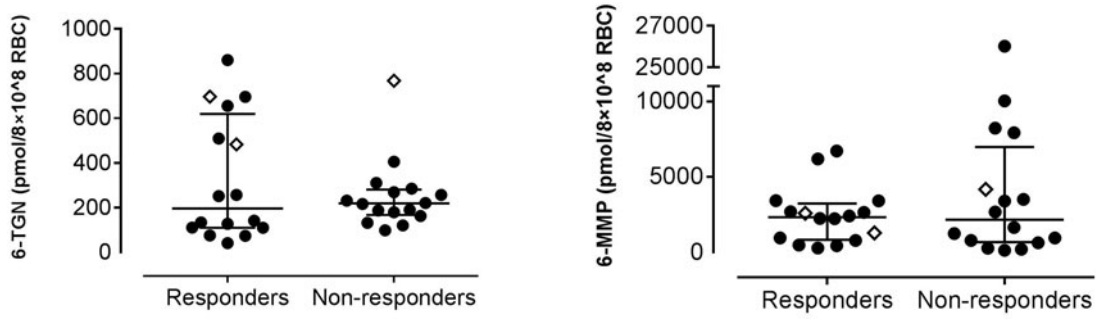

Figure 3. Responders versus non-responders and steady-state metabolite levels (Part I) (median with IQR). ◇ = Intermediate metabolizer TPMT genotype. A, 6-TGN level; B, 6-MMP level. There was no significant difference in 6-TGN levels ($p = .806$) and 6-MMP levels ($p = .763$) between responders and non-responders. 6-MMP: methylated 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; IQR: inter quartile range; RBC: red blood cell.

(150 mg/day). In one patient (patient 5), AZA dose was not increased to 150 mg/day due to subjective side effects.

Thiopurine hypermethylation (low 6-TGN with high 6-MMP) was observed in patients 2 and 3.

Discussion

This study aimed to investigate whether measurement of thiopurine metabolites is helpful in improving effectiveness and safety of AZA treatment in patients with AD and/or chronic hand/foot eczema.

A wide inter- and intra-individual variation in both 6-TGN and 6-MMP levels in patients with AD or chronic hand/foot eczema during AZA maintenance treatment was observed. Metabolite levels between individuals were not significantly related to the AZA dose, but within individuals higher AZA dose led to higher metabolite levels. Metabolite levels were not significantly different between responders and non-responders.

Several past studies investigated thiopurine metabolites and their relation to clinical efficacy and side effects in IBD. Although it is hypothesized that 6-TGN is responsible for the immunosuppressive effect of AZA, several studies in IBD patients failed to find a statistically significant correlation between metabolite levels, and clinical response (6,22–25).

A prospective study of steroid-dependent patients with IBD showed that dose optimization of AZA to achieve 6-TGN levels $\geq$250 pmol/8 $\times$ 10^8 RBC was significantly associated with a higher rate of disease remission (Lennard method) (26).

Other studies in IBD suggest a therapeutic range in 6-TGN concentration between 235 and 490 pmol/8 $\times$ 10^8 RBC (Lennard method) as significantly more exacerbations were found in patients below the threshold of 235 pmol/8 $\times$ 10^8 (6,27–29). The risk for leucocytopenia increased with 6-TGN levels above 490 pmol/8 $\times$ 10^8 RBC (6,29,30).

6-TGN and 6-MMP levels are not routinely measured during AZA treatment in dermatological diseases. However, attempts have been made in a small number of studies to optimize clinical efficacy of AZA by measuring metabolite levels. Recently, Reynolds et al. suggested, based on their unpublished experience in individual cases, that measuring 6-TGN levels particularly in patients with heterozygote TPMT activity may be useful (16).

In 2009, el Azhary et al. investigated the optimal levels of 6-TGN for disease remission in patients with immunobullous disease treated with AZA (Dervieux method). They found a mean optimal level of 6-TGN of 190.7 pmol/8 $\times$ 10^8 RBCs (31). Limited disease (involving only the oral or genital mucosa, the face, or limited areas on the chest) was found to require less 6-TGN to achieve clinical improvement in their study, with a mean of 145.3 pmol/8 $\times$ 10^8 RBCs.

In 2013, Caufield et al. reported on a case series of 12 children with recalcitrant AD treated with AZA (32). They found in 11 responders, 6-TGN levels ranging from 45 to 358 pmol/8 $\times$ 10^8 RBC

Table 3 Responders versus non-responders (Part I).

	Responders ($n = 16$)	Non-responders ($n = 16$)	p value
Male, *n* (%)	7 (43.8)	8 (50)	.723
Age at AZA metabolite measurement (years), median [IQR]	50.9 [46.7–61.3]	44.3 [26.0–52.1]	.029
AZA dose (mg/day), median [IQR]	150.0 [100.0–187.5]	150.0 [112.5–187.5]	.628
Steady-state 6-TGN level (pmol/8 $\times$ 10^8 RBC), median [IQR]	197.0 [110.3–619.5]	219.0 [167.3–281.3]	.806
Number of patients with 6-TGN levels >1200, *n* (%)	0 (0.0)	0 (0.0)	–
Steady-state 6-MMP level (pmol/8x10^8 RBC), median [IQR]	2324.0 [829.8–3228.5]	2156.5 [668.3–6982.5]	.763
Number of patients with 6-MMP levels >5700, *n* (%)	2 (12.5)	4 (25.0)	.365
Steady-state 6-MMP/6-TGN ratio, median [IQR]	5.6 [3.2–27.7]	7.0 [3.2–25.5]	.940

AZA: azathioprine; 6-MMP: methylated 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; IQR: inter quartile range.

Table 4 6-TGN and 6-MMP levels in eight patients during dose escalation (Part II).

	All patients ($n = 8$)
Male, *n* (%)	3 (37.5)
Patients with AD, *n* (%)	5 (62.5)
Patients with isolated hand/foot eczema, *n* (%)	3 (37.5)
6-TGN level (pmol/8 $\times$ 10^8 RBC), median [IQR]	
T1 (2 weeks, 50 mg/day)	70.0 [50.3–91.8]
T2 (4 weeks, 100 mg/day)	170.0 [130.5–193.3]
T3[a] (6 weeks, 150 mg/day)	262.5 [164.5–359.3]
6-MMP level (pmol/8 $\times$ 10^8 RBC), median [IQR]	
T1 (2 weeks, 50 mg/day)	284.0 [250.0–301.3]
T2 (4 weeks, 100 mg/day)	788.5 [393.5–1154.5]
T3[a] (6 weeks, 150 mg/day)	1384.0 [932.5–3253.3]

[a]In one patient, dose was 125 mg/day due to subjective side effects.
AZA: azathioprine; 6-MMP: methylated 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; IQR: inter quartile range.

during stable disease (not clear whether the Dervieux or Lennard method was used).

The above mentioned studies on AZA treatment in dermatological disease and our results (showing a median 6-TGN level in responders 197.0), suggest that 6-TGN levels needed to induce clinical efficacy might be lower compared to IBD. Though, differences in measurement methods (Dervieux versus Lennard) may have contributed to these differences as well, because some studies using Dervieux methods in IBD patients also reported lower 6-TGN levels for therapeutic response (33).

The majority of the non-responders in our study had low 6-TGN levels compared to responders (Figure 2), but this difference was not significantly different. This may be a consequence of the small sample size and the large variation in 6-TGN levels in the responders. Dose escalation was not performed in the non-responder group for a number of reasons, such as subjective side effects and the potential risk of hepatotoxicity.

Literature on the use of AZA in IBD patients reports an increased risk for hepatotoxicity in case of 6-MMP levels higher than 5700 pmol/8 $\times$ 10^8 RBC. In the present study, none of the patients met the criteria of hepatotoxicity, though high 6-MMP levels (>5700 pmol/8 $\times$ 10^8 RBC) were found in 6/32 patients (two clinical responders and four non-responders). These patients may be at risk of developing hepatotoxicity when AZA is continued for longer periods.

The metabolism of AZA is partly influenced by genetic polymorphisms in thiopurine S-methyltransferase (TPMT). This results in inter-individual variation in the metabolism of thiopurines; both with respect to side effects and efficacy (13). High TPMT activity is associated with lower 6-TGN levels and high 6-MMP levels, which may result in decreased immunosuppressive activity and an increased risk of hepatotoxicity. Patients with high TPMT activity may be underdosed with standard AZA dose, which could explain, at least partially, non-responsiveness to AZA. Increasing daily AZA dose in these patients will not lead to higher effectiveness because of a preferential 6-MMP production.

TPMT activity is not the only predictor for the risk of hepatotoxicity. Other metabolites, other enzymatic conversions within the metabolic pathway or interference medication (like aminosalicylates, non-steroidal anti-inflammatory drugs, anticoagulant, diuretics, and infliximab) may also influence the production of 6-MMP metabolites (34). Furthermore, low TPMT activity is associated with high 6-TGN levels, resulting in an increased risk of myelotoxicity. However, TPMT activity does not predict myelotoxicity in all patients. Therefore, monitoring haematological parameters remains crucial (35). Reports indicate that between 50% and 75% of thiopurine-related leucopenia occurs in patients with normal TPMT activity (13,36,37). Finally, although TPMT activity is generally measured only once before starting therapy, some studies have shown that TPMT levels are not static and induction of activity can occur during treatment (31,32). Therefore measurement of 6-TGN and 6-MMP instead of TPMT activity seems to be more relevant to predict/monitor the risk of hepato- and myelotoxicity. In this study, information on TPMT genotype was missing in two patients (one in Part I and one in Part II). Since these patients displayed normal complete blood counts and AZA metabolite levels, the TPMT genotype in these patients was assumed to be normal.

Recent studies on AZA in IBD and AD have reported high percentages of treatment failure due to sides effects and/or inefficacy (2,3,4,38). We suggest that this may, at least partly, be related to a skewed thiopurine metabolism profile. The so-called 'ultramethylators' (hypermethylators) preferentially produce excessive amounts of 6-MMP, whereas 6-TGN levels remain very low (6-MMP/6-TGN range above 20) (38,39,40). We observed this phenomenon in both parts of this study. In part I of this study, 6/32 patients (18.8%), all using AZA 150 mg/day, had high 6-MMP levels (>5700 pmol/8 $\times$ 10^8 RBC). Three of them had 6-TGN levels <235 pmol/8 $\times$ 10^8 RBC, suggesting thiopurine hypermethylation or preferential 6-MMP production. In Part II of this study, thiopurine hypermethylation was observed in patients 2 and 3 (25%). These hypermethylators are at risk of side effects and non-responsiveness, resulting in AZA discontinuation after several months of treatment. TPMT activity does not predict the risk of hypermethylation. Thus, metabolite levels should be routinely monitored from start of treatment and during dose escalation in order to detect hypermethylators early in AZA treatment.

Studies in IBD patients showed that concomitant use of allopurinol can result in a shift of the AZA metabolism towards the production of 6-TGN, reducing the relative formation of 6-MMP (41–44).

Three patients were excluded because they had 6-TGN levels <50 pmol/8 $\times$ 10^8 RBC and 6-MMP levels <250 pmol/8 $\times$ 10^8 RBC, indicating that they had not been taking their medication. The (suspected) noncompliance rate was 8.5%. One patient confirmed noncompliance; in the other two patients compliance remained unclear. One of these two patients still had an active eczema; the other was in clinical remission. It should be taken into consideration that low 6-TGN and 6-MMP levels may be a

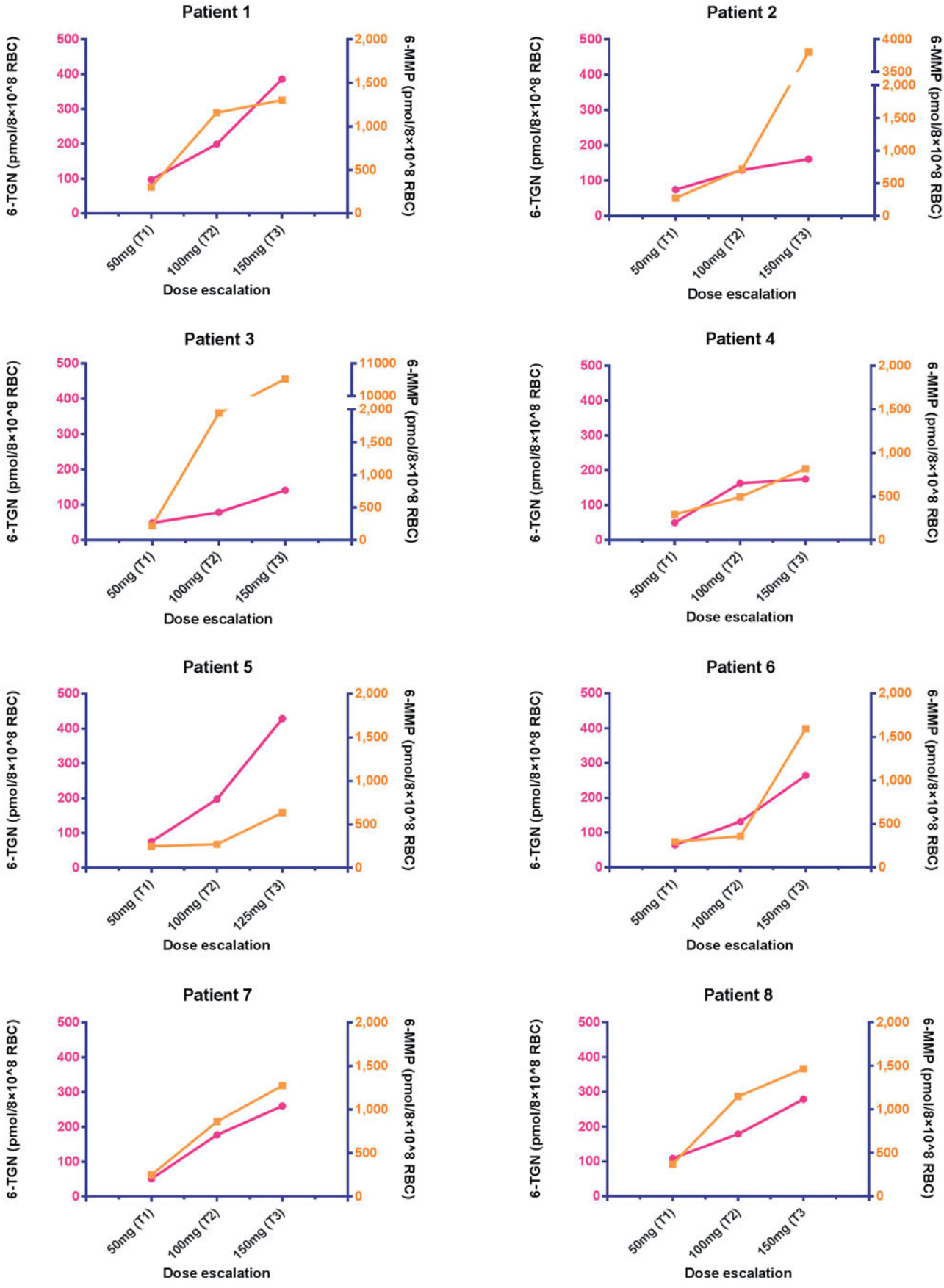

Figure 4. 6-TGN and 6-MMP levels in patients treated with AZA treatment during dose escalation.

result of noncompliance; however, a patient-dependent variation in the AZA metabolism (e.g. malabsorption, yet unknown enzyme defects or extremely high enzyme activity in the thiopurine pathway) cannot be excluded (6).

Limitations

Concomitant medication (including aminosalicylates, non-steroidal anti-inflammatory drugs, anticoagulants, diuretics, and infliximab) may interfere with the thiopurine metabolism, but in this study no information on concomitant medication (except for oral corticosteroids) was collected (34).

In order to use a clinical parameter that was useful in both AD and chronic hand/foot eczema, the IGA was selected to define response to treatment. This clinical score is less detailed than for instance the EASI (Eczema Area and Severity Index) score that is commonly used as a score for AD severity.

Conclusion

For individual AD patients treated with AZA, routinely measuring 6-TGN and 6-MMP levels can be helpful in optimizing AZA dose, improving clinical effectiveness, and preventing side effects.

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