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ORIGINAL ARTICLE

Thiopurine S-Methyltransferase Polymorphisms in Korean Dermatologic Patients

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Background: Thiopurine S-methyltransferase (TPMT) is an important enzyme in the metabolism of thiopurines including azathioprine (AZA), 6-mercaptopurine, and 6-thioguanine. TPMT genotyping is widely used for screening of AZA-related toxicity during routine clinical practice in Korea. However, the data of TPMT genotypes and its AZA-related toxicity have not been studied in the field of dermatology. **Objective:** The aim of this study was to evaluate the genetic basis of TPMT polymorphism in Korean dermatologic patients and subsequently to investigate the relationship between mutant TPMT and adverse responses to AZA treatment. **Methods:** This study was retrospective, single-center study. One hundred forty-nine Korean dermatologic patients who underwent TPMT screening test were included. Each patient's medical records, the result of TPMT screening test, dose and treatment period of AZA, and side effects, were reviewed. Laboratory tests were assessed at each visit in order to monitor adverse drug reactions. Leukopenia grading was used in accordance with the common terminology criteria for adverse events (CTCAE) ver. 4.03. **Results:** Behçet's disease was the leading disorder among the patients. The frequency of TPMT mutation was 4.0% (6/149) among the participants in this study. Four of the six patients with genetic alterations were treated with a low-dose AZA regimen, but no AZA-related adverse events were observed. **Conclusion:** Our results suggest that 1) TPMT polymorphisms in Korean dermatologic patients are similar to those previously reported in Asian patients with the most common mutant allele being TPMT*3C and 2) AZA can be used in the patients with these polymorphisms under a careful dosing regimen. **(Ann Dermatol 29(5) 529～535, 2017)**

-Keywords-
Azathioprine, Behcet syndrome, Dermatology, Thiopurine S methyltranferase deficiency

INTRODUCTION

Thiopurine agents, such as 6-mercaptopurine, 6-thioguanine, and azathioprine (AZA), are widely used for the treatment of acute leukemias, inflammatory bowel diseases, and other immunological disorders[1,2]. AZA has been reported to be effective in treating various dermatologic diseases and is frequently used in an off-label manner for different inflammatory skin diseases, including Behçet's disease (BD), atopic dermatitis, psoriasis, and photodermatoses[3]. However, when treating patients with AZA, dermatologists should be aware of possible side effects, such as myelotoxicity, which could result in severe immunosuppression and even death, requiring discontinuation of the treatment[4]. In this context, categorization of high-risk patients before treatment may be helpful to physicians in differing clinical settings.

Clinically, thiopurine S-methyltransferase (TPMT) genotyping is used to screen for AZA-related toxicity; genetic polymorphisms in the TPMT gene can affect protein stability and may lead to decreased TPMT activity[5]. Patients with

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high TPMT activity are resistant to AZA due to the catabolizing effect of TPMT converting thiopurine drugs into 6-methylmercaptopurines (6-MMPs)[6]. On the other hand, patients with low TPMT activity have elevated 6-thioguanine nucleotide (6-TGN) levels and may be at increased risk of bone marrow (BM) suppression[5]. In Korea, TPMT genotyping is generally available and has been widely used for the screening of AZA-related toxicity during routine clinical practice. However, the data of TPMT genotypes and its AZA-related toxicity have not been studied in the field of dermatology.

The aims of this study were to evaluate the genetic basis of TPMT polymorphism in patients with dermatological diseases and subsequently to investigate the relationship between mutant TPMT and adverse responses observed in Korean patients treated with AZA for various dermatologic conditions.

MATERIALS AND METHODS

Patients

Retrospective Clinical Data Retrieving System data (Severance Hospital, Seoul, Korea) were used to obtain clinical and laboratory information from patients who visited our dermatology clinic and underwent a TPMT screening test between June 2013 and July 2014. A total of 149 Korean patients with various dermatoses were identified, and 123 of them received treatment with AZA. In these 123 patients, a review of each patient's medical records, including age, sex, comorbidities, medications, the result of the TPMT screening test, dose and treatment period of AZA, and side effects, was performed. This retrospective study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea (IRB no. 4-2015-025).

Analysis of the TPMT genotype

Whole blood specimens were collected into EDTA tubes, and genomic DNA was isolated from peripheral blood leukocytes using the QIAsymphony DSP DNA Mini kit (QIAGEN GmbH, Hilden, Germany) and QIAsymphony SP (QIAGEN GmbH, Hombrechtikon, Switzerland) according to the manufacturer's instructions.

Three exons, TPMT*3B exon 6 (460G>A Ala154Thr), TPMT*3C exon 9 (719A>G Tyr240Cys) and TPMT*6 exon 7 (539A>T Tyr180Phe), of the TPMT gene were amplified on a model C1000/S1000^{TM} thermal cycler (BIO-RAD, Singapore) using biotin attached modified oligonucleotide primers. Pyrosequencing was performed with PyroMark Q24 MDx (QIAGEN GmbH, Sollentuna, Sweden) using PyroMark Gold Q24 kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. To identify sequence variations, patients' sequences were compared with respective reference sequences using the PyroMark software ver. 2.0 (QIAGEN GmbH, Hilden, Germany).

Laboratory monitoring and adverse events

Laboratory tests were reviewed at each visit in order to monitor adverse drug reactions. Routine blood examinations, including a complete blood cell count, erythrocyte sedimentation rate, albumin, transaminase, amylase, lipase, and C-reactive protein, were carried out. Any records of adverse events and symptoms that patients complained about were reviewed within the follow-up period. The time interval in which the adverse events developed after the administration of AZA was also investigated. In addition, medications which are known to cause drug interaction when used concomitantly with AZA were analyzed.

BM suppression

Leukopenia was defined as a white blood cell (WBC) count $<4{,}000/mm^3$, categorized as follows: grade 1, WBC count $\geq 3{,}000/mm^3$ and $<4{,}000/mm^3$; grade 2, WBC count $\geq 2{,}000/mm^3$ and $<3{,}000/mm^3$; grade 3, WBC count $\geq 1{,}000/mm^3$ and $<2{,}000/mm^3$; grade 4, WBC count $<1{,}000/mm^3$. This classification was used in accordance with the common terminology criteria for adverse events (CTCAE) ver. 4.03.

Statistical analysis

Discrete variables were described using frequency (percentages), and continuous variables were reported using median (range) or mean (standard deviation). The chi-square test was performed to evaluate the occurrence of AZA-induced BM suppression in patients subgroups categorized according to the use of aminosalicylates, and one-way ANOVA test was performed to analyze relationship between onset and severity of leukopenia. IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used, with $p<0.05$ considered statistically significant.

RESULTS

Clinical characteristics of the patients

A total of 149 patients were studied. The average patient age was 48.6 ± 13.2 years, and the male to female ratio was 1:1.98. There were 116 (77.3%) cases of BD, which was the most common disease among patients evaluated for TPMT mutations, followed by atopic dermatitis (6.7%), lichen planus (2.7%), and chronic eczema (2.7%). In the

Table 1. Baseline characteristics of analyzed patients

Factors	TPMT wild type (n=143)	TPMT heterozygotes (n=6)	Total (n=149)
Demographics			
Age (yr)	48.6±13.1	48.5±19.5	48.6±13.2
Sex			
Male	47 (32.9)	3 (50.0)	50 (33.6)
Female	96 (67.1)	3 (50.0)	99 (66.4)
Disease			
Behçet disease	111	5	116 (77.3)
Atopic dermatitis	10	0	10 (6.7)
Lichen planus	4	0	4 (2.7)
Chronic eczema	3	1	4 (2.7)
Allergic contact dermatitis	3	0	3 (2.0)
Dermatomyositis	2	0	2 (1.3)
Psoriasis vulgaris	2	0	2 (1.3)
Chronic urticaria	2	0	2 (1.3)
Others*	6	0	6 (4.0)
Administration of AZA			
Number of patients	119 (83.2)	4 (66.7)	123 (82.6)
Dosage (mg/d)	62.02±22.68	43.75±12.50	61.42±22.72
Duration (d)	1,211.05±929.66	1,074.25±654.23	1,206.61±924.20

Values are presented as mean±standard deviation, number (%), or number only. TPMT: thiopurine S-methyltransferase, AZA: azathioprine. *These include alopecia areata, erythema multiforme, seborrheic dermatitis, vitiligo, onychomycosis, and skin graft versus skin disease.

analysis of TPMT genotyping, 143 cases (96.0%) were wild type (*1/*1), and 6 patients (4.0%) had at least one mutated TPMT allele. A total of 123 patients were treated with AZA, including four patients with a TPMT mutation. The mean dose of AZA was 61.42±22.72 mg/d, 62.02±22.68 mg/d, 43.75±12.50 mg/d in the total population, wild-type patients, and TPMT-mutated patients, respectively. The mean total duration of AZA treatment was 1,206.61 days, 1,074.25 days, and 1,211.05 days in the total population, wild-type patients, and TPMT-mutated patients (Table 1). Comorbidities in AZA-treated patients were evaluated, in which gastrointestinal BD was the most common, followed by hypertension (Supplementary Table 1).

Adverse drug reaction during AZA treatment

Among the 123 patients treated with AZA, an adverse drug reaction occurred in 47 patients (38.2%). Among these patients, hepatitis occurred in 2 (4.3%) patients, fatigue in 3 (6.4%), the common cold in 1 (2.1%), and eczema herpeticum in 1 (2.1%) patient. Leukopenia comprised 81.5% (n=40) of all adverse reactions, with most patients experiencing a mild degree of leukopenia. Patients displayed grade 1 leukopenia (30), grade 2 leukopenia (8), or grade 4 leukopenia (2); none displayed grade 3 leukopenia. There were no adverse events reported among those treated with AZA, even in the four patients with a TPMT mutation (Table 2). Among the medications that are known to cause drug interaction with AZA, only aminosalicylate was taken concurrently with AZA in some of the patients. The analysis evaluating the effect of concomitant usage of aminosalicylates on the occurrence of AZA-induced BM suppression did not show statistical significance (Supplementary Table 2).

Table 2. ADRs during azathioprine treatment

ADR	Wild type (n=119)	TPMT heterozygotes (n=4)
Leukopenia	40 (81.5)	0
Grade 1	30	0
Grade 2	8	0
Grade 3	0	0
Grade 4	2	0
Hepatitis	2 (4.3)	0
Fatigue	3 (6.4)	0
Cold	1 (2.1)	0
Eczema herpeticum	1 (2.1)	0
Total	47	0

Values are presented as number (%) or number only. ADR: adverse drug reaction, TPMT: thiopurine S-methyltransferase.

The time interval of BM suppression after AZA treatment

In 40 patients who experienced leukopenia, the mean time interval of occurrence of leukopenia was 1,044.75±941.55 days, and its range varied from 20 to 3,635 days. However, two patients who developed severe leukopenia (grade 4) showed short time interval of occurrence (20 and 40 days), and the onset of BM suppression had a tendency to be shorter with increased grade of leukopenia despite of statistically insignificance (p=0.11). In each group, the mean time interval was 1,208 days, 686.25 days, and 30 days in the grade 1 leukopenia, grade 2 leukopenia, and grade 4 leukopenia, respectively (Fig. 1).

Patient characteristics of those with a TPMT mutation

The TPMT genotype of most patients was *1/*1 (wild type). Six patients had a mutant TPMT genotype, and the distribution was as follows. Four patients with BD and one patient with chronic eczema had *1/*3C (heterozygous), and one patient with BD had *1/*6 (heterozygous). Of note, the four patients heterozygous for a TPMT mutation were treated with AZA with an initial and maintenance dosage in the 25 to 50 mg per day range, and the total treatment duration ranged from 361 to 1,836 days. Regardless of the presence of a TPMT mutation, none of patients treated with AZA experienced additional inconvenience, and their laboratory assessments all remained stable, without any definite adverse events, including leukopenia (Table 3).

WBC changes in TPMT heterozygotes during AZA treatment

Laboratory findings were reviewed during AZA treatment in the four patients heterozygous for a TPMT mutation. Treatment periods varied from 1 to 6 years. During follow-up, WBC counts were consistently above 4,000/mm^3

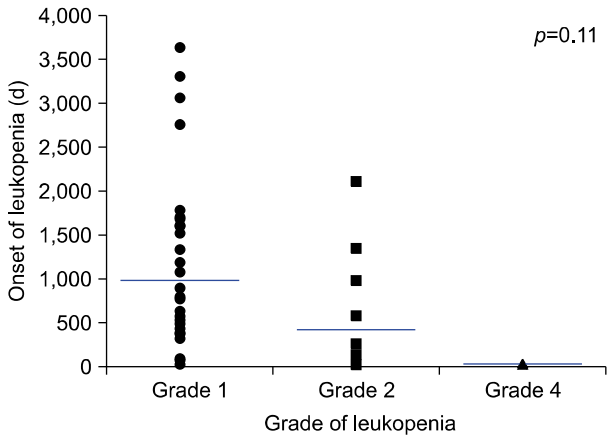

Fig. 1. The time interval of bone marrow suppression after azathioprine (AZA) treatment. Total 40 patients with leukopenia were analyzed; grade 1 leukopenia (30), grade 2 leukopenia (8), and grade 4 leukopenia (2). Onset of leukopenia relates to time from the start of AZA treatment to the detection of leukopenia (medians are indicated).

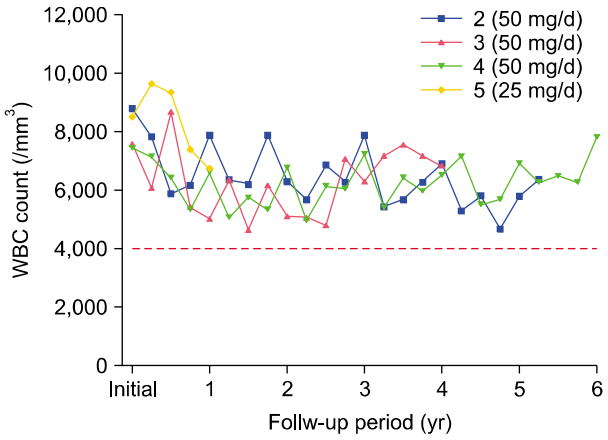

Fig. 2. Changes in leukocyte counts over the follow-up interval in azathioprine (AZA)-treated patients heterozygous for a thiopurine S-methyltransferase (TPMT) mutation (n=4). The red dashed line indicates the threshold minimal value for grade 1 leukopenia according to the common terminology criteria for adverse events (CTCAE) ver. 4.03. The follow-up period ranged from 1 to 6 years. None of the AZA-treated patients developed leukopenia during the follow-up period. WBC: white blood cell.

Table 3. Characteristics of patients with TPMT mutations (n=6)

Patient no.	Age (yr)/ sex	Disease	TPMT mutation	Nucleotide variation	Use of AZA	Daily dosage (mg)	Total duration (d)	Total dosage (mg)	ADR
1	49/M	Behçet's disease	*1/*3C	c.719A>G	−				-
2	53/M	Behçet's disease	*1/*3C	c.719A>G	+	50	1,310	65,500	None
3	76/F	Behçet's disease	*1/*3C	c.719A>G	+	50	850	42,500	None
4	49/F	Behçet's disease	*1/*3C	c.719A>G	+	50	1,836	91,800	None
5	15/F	Chronic eczema	*1/*3C	c.719A>G	+	25	361	9,025	None
6	49/M	Behçet's disease	*1/*6	c.539A>T	−				-

TPMT: thiopurine S-methyltransferase, AZA: azathioprine, ADR: adverse drug reaction, M: male, F: female.

in all four patients, which represents the criteria for a grade 1 leukopenia (Fig. 2). In addition, no cases of BM toxicity defined as neutropenia, thrombocytopenia, or anemia were reported during the treatment period (data are not shown).

DISCUSSION

AZA has been commonly used for more than 30 years to manage various dermatoses, and the drug is still substantially affordable. Dermatologists commonly prescribe AZA for atopic dermatitis, psoriasis, photodermatosis, immunobullous disease, and various other dermatologic conditions[3]. One of the severe side effects of AZA treatment that must be managed is myelosuppression, which should be closely monitored. AZA is a type of pro-drug that is activated after serial metabolism by multiple enzymes. Among those enzymes, alterations in TPMT are known to be correlated with AZA toxicity[6].

Recently, other pharmacogenetic markers, such as inosine triphosphate pyrophosphatase and nucleoside diphosphate linked moiety X-type motif 15, have been suggested for screening of AZA-related toxicity[7,8]. However, clinical application of screening tests for these markers is still a long way from actually being marketed. Accordingly, TPMT genotyping is commonly used for screening of AZA-related toxicity during routine clinical practice in Korea.

TPMT is an important enzyme that metabolizes thiopurines. When AZA is absorbed by cells, the pro-drug is rapidly converted to its active metabolites, such as 6-mercaptopurine, 6-thioinosine monophosphate, and 6-TGN. TPMT catabolizes these active metabolites into inactive forms like 6-MMP[9]. Reduced enzymatic activity of TMPT is associated with increased 6-TGN levels, which may result in direct incorporation of 6-TGN into DNA and cause cytotoxicity and immunosuppression by inhibition of RNA, DNA, and protein synthesis[9,10].

Although allelic variations in TPMT affect protein stability or enzyme activity, there are >30 TPMT alleles. The wild-type allele is TPMT*1, and the majority of alleles related to low enzyme activity of TPMT are represented by *2, *3A, *3B and *3C; TPMT*2 results in 26%, TPMT*3A results in 1.6%, TPMT*3B results in 1.7% and TPMT*3C results in 17% of enzymatic activity compared with wild type *in vitro*[11,12]. In Caucasians, TPMT*3A is the most common mutant allele; however, in East Asia the TPMT*3C is most frequently reported gene polymorphism[13,14].

In our study, similar to previous data reported involving Asian patients, the most common mutant allele was TPMT*3C. Additionally, the frequency of the TPMT*3C allele in our study was 3.4% (5/149), consistent with the reported Korean data in other inflammatory diseases (frequency of 2.4%～4.4%)[15-18]. We also detected one TPMT*6 allele in a patient with BD. This rare TPMT*6 mutant allele has been previously reported in one Malaysian blood sample in 2002[19]. Several additional TPMT*6 mutations have been reported only in Korean patients; whereas, no TPMT*6 alleles have been found even in Japanese or Chinese patients, who were thought to have similar genotypic features with Koreans[15,20,21]. The clinical relevance of the TPMT*6 allele has not yet been clarified, but this variant might be a candidate for a TPMT pharmacogenetics study, especially in Korea.

We evaluated for a possible relationship between TPMT gene mutations and adverse response to AZA. Because there were no reported AZA-related adverse events in our patients with TPMT mutations, we could not investigate the relationship between the TPMT genotype and adverse events, including leukopenia. In a similar study of the patients with systemic lupus erythematosus analyzed the relationship between TPMT mutations and their adverse effects, mean dosage of AZA in TPMT mutated patient was low (60.71 ± 22.72 mg/d) and there was no statistically significant correlation between TPMT genotype and AZA toxicity[15].

In this study, adverse events were found only in patients with wild-type TPMT. The majority of reported events were grade 1 leukopenias, and most adverse events were not severe with only two cases of mild hepatitis, which did not require discontinuation of AZA. However, in two of forty patients with leukopenia, severe leukopenia (grade 4) occurred abruptly. Though, there was no significant difference found between the grade and onset of leukopenia, the onset had a tendency to be shorter with increased grade of leukopenia. Since the onset of myelosuppression is unpredictable and severe leukopenia occurred in a short onset time of 6 weeks in this study, blood count should be monitored frequently in the early period of AZA use.

In addition, concomitant medications including allopurinol, aminosalicylate (sulfasalazine and mesalazine), cotrimoxazole, angiotensin converting enzyme inhibitors, and ribavirin, which are known to cause drug interaction with AZA, may increase the risk of AZA-related BM toxicity. In a previous study, concurrent administration of AZA with aminosalicylate for treatment of inflammatory bowel disease led to a significant increase in the frequency of leukopenia[22]. In this study, there were patients taking aminosalicylate, thus we also evaluated the association of the medication and AZA-related toxicity. However, the effect of concomitant usage of aminosalicylates on the occurrence of AZA-induced BM suppression did not show

any statistical significance (Supplementary Table 2).

The AZA dosage for patients with TPMT mutations was ≤50 mg/d. This range of AZA dosage is generally used to treat dermatologic disease but might be too low to cause a myelosuppressive effect. Several studies have reported that some individuals with a heterozygous genotype exhibit high enzyme activity; whereas, some homozygous wild type subjects exhibit an intermediate phenotype[6]. A possible hypothesis that can explain these phenomena is that the single nucleotide polymorphisms discussed so far are not the only factors regulating the TPMT's catalytic activity, and the genotype at the major locus, which regulates TPMT activity, accounts for only approximately two-thirds of the total variance in population genetic studies[10]. Various other factors, like promoter polymorphisms, drug interactions, diagnosis, and environmental factors, could be related. Although TPMT genotyping could not completely explain the high frequency of BM suppression during AZA treatment, obtaining TPMT genotypes in all patients before starting AZA treatment could still be reasonable, due to the mechanism-based theory and previously reported results of increased myelosuppression risk in patients with low or absent TPMT enzyme activity. It is reported that all patients with inactive homozygous TPMT genotype who were treated with conventional doses of AZA (1.5 mg/kg/d) experience severe myelosuppression and 30%～60% of patients with heterozygous TPMT genotype developed severe myelosuppression with standard doses of AZA[23]. In similar studies with conventional dose of AZA in rheumatologic diseases and inflammatory bowel diseases, there was increased risk of myelosuppression in heterozygous TPMT group[24-26]. Notably, such patients typically receive a higher AZA dosage than dermatological patients (1.5～2.5 mg/kg/d, generally over >100 mg/d). Since there were no adverse events in any of the four TPMT heterozygous patients treated with AZA in our study, we carefully recommend that the low-dose AZA generally used to treat dermatologic conditions can be cautiously used under close monitoring even in patients with heterozygous minor alleles.

However, this study has some limitations. This was a retrospective study conducted in a single center, and only a small number of patients possessing a mutant TPMT genotype were assessed. Furthermore, we could not evaluate for additional metabolites that are related to examining TPMT enzymatic activity. Also, because this is a retrospective study, there may be selection bias, in which a lower dosage of AZA was used in the TPMT mutation group than the wild type group, therefore, this report does not imply that conventional dosage of AZA in patients with TPMT mutations is as safe as those in naïve patients. In addition, the majority of the patient population consists of BD, which may have possible influences on the results. Thus the differences between patients with and without BD should be also studied in the further evaluation. Accordingly, prospective, randomized, controlled trials are needed to determine a more definite correlation between the TPMT genotype and risk of adverse events associated with usage of AZA in patients with dermatologic disorders.

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SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-29-529-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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Supplementary Table 1. Comorbidities in azathioprine treated patients (n=83)

	With BM suppression	Without BM suppression	Total
Gastrointestinal Behçet's disease	6	11	17 (20.5)
Neuro-Behçet's disease	1	5	6 (7.2)
Hypertension	6	5	11 (13.3)
Diabetes mellitus	2	2	4 (4.8)
Chronic renal failure	0	1	1 (1.2)
Old pulmonary tuberculosis	1	0	1 (1.2)
Crohn's disease	1	2	3 (3.6)
Ulcerative colitis	1	1	2 (2.4)
Chronic hepatitis	2	0	2 (2.4)
Old pulmonary tuberculosis	1	0	1 (1.2)
Asthma	1	1	2 (2.4)
Sjogren's syndrome	1	0	1 (1.2)
Myasthenia gravis	0	1	1 (1.2)
Osteopenia and Osteoporosis	2	8	10 (12.0)
Spinal stenosis	1	2	3 (3.6)
Atrial fibrillation	1	0	1 (1.2)
Deep vein thrombosis	1	4	5 (6.0)
Arteriovenous malformation	0	1	1 (1.2)
Artherosclerosis	0	1	1 (1.2)
Benign prostatic hyperplasia	0	2	2 (2.4)
Thyroid cancer	2	1	3 (3.6)
Ovarian cancer	0	1	1 (1.2)
Depression	0	2	2 (2.4)
Migrane	0	2	2 (2.4)

Values are presented as number only or number (%). BM: bone marrow.

Supplementary Table 2. Relationship between aminosalicylate usage and AZA-induced BM suppression

Usage of aminosalicylate	BM suppression		Total	*p*-value
	Present	Absent		
Aminosalicylate(+)	6 (37.5)	10 (62.5)	16	0.648*
Aminosalicylate(−)	34 (31.8)	73 (68.2)	107	

Values are presented as number (%) or number only. AZA: azathioprine, BM: bone marrow. * $\chi^2 = 0.208$.