ORIGINAL RESEARCH

TPMT Genetic Variability and Its Association with Hematotoxicity in Indonesian Children with Acute Lymphoblastic Leukemia in Maintenance Therapy

Dewi Selvina Rosdiana[1]
Rianto Setiabudy[1]
Rizka Andalusia[2,3]
Djajadiman Gatot[4]
Melva Louisa [1]
Saptawati Bardosono[5]
Instiaty Instiaty[1]

[1]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [2]Dharmais National Cancer Hospital, Jakarta, Indonesia; [3]Drug Registration Directorate, National Agency for Drug and Food Control, Jakarta, Indonesia; [4]Division of Hematology-Oncology, Department of Pediatrics, Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [5]Department of Nutrition, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

Correspondence: Dewi Selvina Rosdiana
Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Jakarta, 10430, Indonesia
Tel +62 21 31930481
Fax +62 21 3920947
Email dewiselvina79@gmail.com

Purpose: Hematotoxicity monitoring in children with acute lymphoblastic leukemia (ALL) is critical to preventing life-threatening infections and drug discontinuation. The primary drug that causes hematotoxicity in ALL children is 6-mercaptopurine (6-MP). Genetic variability of the drug-metabolizing enzymes of 6-MP, thiopurine *S*-methyltransferase (*TPMT*), is one factor that might increase the susceptibility of children to hematotoxicity. The present study aimed to determine the variability in *TPMT* genotypes and phenotypes and its association with the occurrence of hematotoxicity in ALL children in maintenance therapy.

Patients and Methods: A cross-sectional study was conducted at Cipto Mangunkusumo and Dharmais National Cancer Hospital, Jakarta, Indonesia, from June 2017 to October 2018. We included ALL patients, 1–18 years, who were receiving at least one month of 6-MP during maintenance therapy according to the Indonesian protocol for ALL 2013. Direct sequencing was used to determine *TPMT*3A, *3B*, and *3C* genotypes, and LC-MS/MS analysis was performed to measure the plasma concentrations of 6-MP and its metabolites. Association analysis between the *TPMT* genotype and hematotoxicity was evaluated using the unpaired *t*-test or Mann–Whitney's test.

Results: The prevalence of neutropenia, anemia, and thrombocytopenia in ALL children during maintenance therapy was 51.9%, 44.3%, and 6.6%, respectively. We found a low frequency of *TPMT*3C, which is 0.95%. No association was found between hematotoxicity and *TPMT* genotypes or age, nutritional status, serum albumin levels, risk stratification, the daily dose of 6-MP, and cotrimoxazole co-administration. However, hematotoxicity was associated with 6-methylmercaptopurine (6-MeMP) plasma concentrations and the ratio 6-MeMP/6-thioguanine (6-TGN). We also found no association between *TPMT* genotypes and *TPMT* phenotypes.

Conclusion: The 6-MeMP/6-TGN ratio is associated with hematotoxicity in ALL children during maintenance therapy but is not strong enough to predict hematotoxicity.

Keywords: thiopurine methyltransferase, mercaptopurine, methylmercaptopurine, thioguanine, neutropenia

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising 30% of cancers occurring before 15. It accounts for 80% of all leukemia cases in children. Despite steady improvements in outcome, ALL remains the leading cause of childhood cancer death.[1,2] A complete remission rate is more than 90% after

induction therapy, but in developed countries, around 11% of children relapse while receiving maintenance therapy,[3] whereas in Cipto Mangunkusumo Hospital, Indonesia, it is 28.7%.[4]

During the maintenance phase, pediatric ALL patients received a treatment regimen containing 6-mercaptopurine (6-MP). However, 6-MP can cause life-threatening side effects such as myelosuppression, which may lead to treatment interruptions, and subsequently contribute to an increased relapse incidence.[5]

Studies have shown that the polymorphism of 6-MP drug-metabolizing enzymes, thiopurine S-methyltransferase (TPMT), has an essential role in patients' susceptibility to myelosuppression from 6-MP. Individuals with TPMT polymorphisms represent approximately 10% of the population. Numerous variant alleles of TPMT polymorphisms have been identified and associated with decreased TPMT enzyme activity levels. Reduced enzyme activity will enhance the hematotoxicity of 6-MP by increased concentrations of 6-thioguanine nucleotide (6-TGN) levels, an active metabolite of 6-MP. Consequently, 6-MP dose reduction is needed to avoid the hematotoxicity of 6-MP. Based on population studies, three alleles account for more than 95% of TPMT variants: TPMT*3A, TPMT*3C, and TPMT*2.[6] The Clinical Pharmacogenetics Implementation of the Consortium (CPIC) Guidelines recommended adjusting the starting dose of 6-MP based on the TPMT genotype to avoid hematotoxicity.[7] However, some studies in Asian population indicate the relationship between TPMT genotypes and hematotoxicity, with conflicting results. In one study, patients with TPMT heterozygotes received a less 6-MP dose compared to those with wild-type alleles. The above study concluded that the identification of the TPMT genotype might be significant.[8] In another study in an Indian population, TPMT heterozygous patients received a similar 6-MP dose, and the study showed that TPMT genotyping might not be necessary.[9]

To date, there are no data on TPMT polymorphism and its relationship with hematotoxicity in Indonesian pediatric ALL. Thus, dose adjustment for 6-MP maintenance therapy based on TPMT genotypes, which reflects ethnic allele distribution, needs to be evaluated in Indonesian pediatric ALL.

Patients and Methods
Study Design
This cross-sectional study was conducted from June 2017 to October 2018 at Cipto Mangunkusumo Hospital and Dharmais Cancer Hospital to recruit pediatric ALL patients undergoing maintenance therapy. The study was conducted following the Declaration of Helsinki. The Ethics Committee of Medical Faculty, Universitas Indonesia, approved this study. Written informed consent/assent was obtained from patients and/or parents/legal guardians.

We included children diagnosed with ALL, aged 1–18 years, who underwent 6-MP treatment for at least one month according to the Indonesian protocol for ALL 2013.[10] Patients were excluded if they had a severe infection, received colony-stimulating factors, allopurinol, mesalazine, olsalazine, or sulfasalazine on their treatment regimen. The sample size estimation for the polymorphism study was determined based on reference, and Hong et al stated that the minimal sample size in the polymorphism study was 100.[11]

The blood samples of each patient who underwent 6-MP 50 mg/m^2/day for at least one month were drawn once, in the morning (a day before receiving vincristine intravenously), to evaluate TPMT genotyping, to quantify 6-TGN and 6-methylmercaptopurine (6-MeMP) concentrations, as well as for hematology data. We recorded the demography of all eligible patients. Hematology data were categorized according to the Common Terminology Criteria for Adverse Events v3.0.[12] Later, we correlated the metabolite levels with the hematological data obtained simultaneously.

Drug Regimen in Maintenance Therapy
Maintenance therapy was initiated at treatment week 13 for standard risk (SR) ALL or 18 for high risk (HR) ALL and continued until 2.5 years after diagnosis. The 6-MP starting dose was 50 mg/m^2/day. Some patients received breaking tablets (the same daily dose of 6-MP), and others were given different doses on alternating days to achieve specific doses (modified daily dose of 6-MP). During maintenance therapy, patients also received low-dose oral (20 mg/m^2/week) pulse methotrexate (MTX) and vincristine intravenously every fifth and sixth week with dexamethasone orally 6 mg/m^2/day (HR ALL) or 4 mg/m^2/day (SR ALL), for two weeks. During the first year of the maintenance phase, patients received pulses of intrathecal MTX at seven-week intervals until six courses.[10]

TPMT Genotyping
Genomic DNA was isolated from 200 μL of buffy coat from peripheral blood using the Genomic DNA Mini Kit (Geneaid Biotech Ltd, Taipei, Taiwan) according to the

manufacturer's instructions. Genotyping was performed using polymerase chain reaction on the LightCycler® 480 Real-Time PCR System (Roche, Basel, Switzerland) for mutations in exon 7 (G460A) and exon 10 (A719G) (*TPMT*3A, *3B*, and *3C*) followed by direct DNA sequencing using the primers as previously described by Dervieux et al[13].

Phenotyping

The blood sample of each patient who underwent 6-MP treatment for at least one month was tested for 6-TGN and 6-MeMP concentrations. LC-MS/MS was used to quantify the levels of 6-TGN and 6-MeMP, according to the method previously published.[14] The blood sample was drawn in patients who underwent 6-MP treatment for at least one month because the steady-state level of mercaptopurine metabolites was reached after 2–4 weeks of administration.[15]

Statistical Analysis

We used SPSS 20 software (New York, USA) for statistical analysis. The Kolmogorov–Smirnov test was used to evaluate the normality of data distribution. Association analysis between the *TPMT* genotype and hematotoxicity and subgroup analysis were assessed using the unpaired *t*-test, Mann–Whitney's test, the chi-square test, or Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) curve analysis was used to determine the predictive 6-MP metabolite levels and hematotoxicity risk.

Results
Patient Characteristics and Prevalence of Hematotoxicity

A total of 106 ALL patients were included in our study. The baseline characteristics of these subjects are shown in Table 1. The median age at study participation was 6.21 years. In all, 53% of the subjects were male, and 54.7% presented with high-risk disease. More than 95% of the subjects had normal to obese nutritional status and normal serum albumin levels. Subjects with concomitant cotrimoxazole were 46.2%, and 52.8% received the same daily dose of 6-MP.

The prevalence of hematotoxicity was 71.7%, the majority in grade 1 (Table 2). The prevalence of neutropenia, anemia, and thrombocytopenia was 51.9%, 44.3%, and 6.6%, respectively. Ten subjects (9.4%) presented with grade 3–4 neutropenia (absolute neutrophil count [ANC] < 1000 cells/mm^3).

TPMT Genotyping and Phenotyping

TPMT genotyping is shown in Table 3. The result of the *TPMT* genotype was that 104 subjects (98.1%) were *1/*1* (wild type), and two subjects (1.9%) were *1/*3C*. The frequency of *TPMT*3C* was 0.95%. *TPMT*3A* and *3B* were not found.

The median 6-TGN levels, 6-MeMP levels, and ratio 6-MeMP/6-TGN levels were 19.12 (6–234.04), 44.54 (3.5–3167.01), and 3.1 (0.06–100.64) pmol/8×10^8 RBC, respectively. The association of hematotoxicity with genotypes and several factors are shown in Table 4. There was no association between *TPMT* genotypes and hematotoxicity (p = 1.000). There were no statistical differences in 6-TGN levels between the group with hematotoxicity and that without hematotoxicity (p = 0.938; Figure 1). In contrast, there were statistically significant differences in 6-MeMP levels (p = 0.004; Figure 2) and ratio 6-MeMP/6-TGN levels (p = 0.022; Figure 3) with hematotoxicity. We tried to predict the risk of hematotoxicity based on the ratio of 6-MeMP/6-TGN levels using ROC curve analysis. The area under the curve was 0.66. The 6-MeMP/6-TGN ratio threshold at 0.6 was shown to have the best sensitivity (71.1%) and specificity (53.3%).

Subgroup analysis was carried out based on the occurrence of anemia, grade 3–4 neutropenia, and thrombocytopenia (Table 5). Based on subgroup analysis, there was a statistical difference of 6-MeMP levels between subjects with anemia and anemia. A ROC curve analysis to determine the predictive 6-MeMP levels and the risk of anemia resulted in an area under the ROC curve of 0.61. The best sensitivity and specificity were defined for the 6-MeMP level threshold at 44.54 pmol/8 × 10^8 erythrocytes, 61%, and 60%. There was also a significant difference in 6-MeMP levels between subjects with and without grade 3–4 neutropenia. Based on the ROC curve analysis, the area under the curve was 0.77. The 6-MeMP level threshold at 60 pmol/8 × 10^8 erythrocytes was shown to have the best sensitivity (90%) and specificity (62%). There was also a significant difference in the 6-MeMP/6-TGN ratio between subjects with and without grade 3–4 neutropenia (Figure 4). Based on the ROC curve analysis, the area under the curve was 0.75. The 6-MeMP/6-TGN ratio threshold at 3.1 was shown to have the best sensitivity (90%) and specificity (54%).

There was no association in hematotoxicity prevalence analyzed based on patients' characteristics (age, nutritional

Table 1 Patients Characteristics

Characteristics		n	(%)
Age (years)			
Median (Min-Max) 1 > 10 ≥ 10	6.21 (2–18)	 81 25	 76.4 23.6
Gender			
Male Female		56 50	52.8 47.2
Risk stratification			
High risk (HR) Standard risk (SR)		58 48	54.7 45.3
Nutritional status			
Underweight Normal–Obese		2 104	1.9 98.1
BMI (kg/m^2)			
Median (Min–Max)	17.26 (12.94–28.04)		
Albumin (g/dl)			
Mean (± SD) Normal Hypoalbuminemia (<3,5g/dL)	4.54 (± 0.35)	 105 1	 99.1 0.9
SGOT (U/L)			
Median (Min-Max)	29.5 (12–189)		
SGPT (U/L)			
Median (Min-Max)	29.5 (7–204)		
Duration of maintenance therapy (weeks)			
Median (Min-Max)	40 (19–106)		
The daily dose of 6-MP			
Same as guideline Modified		56 50	52.8 47.2
Cotrimoxazole			
Yes No		49 57	46.2 53.8
Hematology			
Hb Levels (g/dL) Median (Min–Max)	11.6 (7.9–14.6)		
ANC (cells/µL)			
Median (Min-Max)	2386 (108–8268)		

(*Continued*)

Table 1 (Continued).

Characteristics		n	(%)
Platelets (/µL)			
Mean (± SD)	328,320.75 (±110,472.02)		
Hematology parameters before the maintenance phase			
Hb level (g/dL)			
< 11.5 ≥ 11.5		50 55	47.2 51.9
ANC (cells/µL) < 2500 ≥ 2500		 59 46	 55.7 43.4
Platelets (/µL) < 150,000 ≥ 150,000		 5 100	 4.7 94.3

Abbreviations: SD, standard deviation; ANC, absolute neutrophil count; HR, high Risk; SR, standard risk; BMI, body mass index.

status, serum albumin levels, risk stratification, a daily dose of 6-MP, and co-administration cotrimoxazole). Multivariate logistic regression analysis was not carried out because none of the bivariate analysis was significant (Table 4). No relationship between *TPMT* genotypes and phenotypes was found. Analysis using Mann–Whitney's test did not show statistically significant differences between both metabolites (6-TGN levels and 6-MeMP levels) and the *TPMT* genotype, p = 0.075 and p = 0.935, respectively (Figure 5).

Discussion

Several studies have shown that genetic variability in *TPMT* is essential to determine the susceptibility of ALL patients to hematotoxicity due to 6-MP.[7] Yet, studies in Asian populations have been inconclusive. In the present study, we found the lack of a relationship between *TPMT *3A, *3B*, and *3C* and the occurrence of hematotoxicity. However, we found strong associations between the *TPMT* phenotypes and grade 3–4 neutropenia prevalence.

The present study results showed that the allele frequencies among patients were similar to those found in other Asian populations, with *TPMT*3C* as the primary variant allele (0.95%). In Asian populations, the overall prevalence of *TPMT* mutant alleles is lower (~1.7%) and is

Table 2 Hematotoxicity Grading According to CTCAE

Hematotoxicity	Total n (%)	Grades			
		1	2	3	4
Anemia	47/106 (44.3)	36 (34)	10 (9.4)	1 (0.9)	0
Neutropenia	55/106 (51.9)	33 (31.1)	2 (11.3)	7 (6.6)	3 (2.8)
Thrombocytopenia	7/106 (6.6)	5 (4.7)	0	0	2 (1.9)

Abbreviations: ANC, absolute neutrophil count; CTCAE, Common Terminology Criteria for Adverse Events.

Table 3 Genotyping of Subjects

Genotyping	n	(%)
Wild type	104	98.1
Mutant		
TPMT*3A	0	0
TPMT*3B	0	0
TPMT*3C (heterozygotes)	2	1.9

predominantly represented by the *TPMT*3C* variant (1.6%).[7]

No association was found between the three *TPMT* variants studied (*3A, *3B*, and *3C*) and hematotoxicity. The 97.4% of subjects with hematotoxicity were of wild-type *TPMT* genotypes. Similarly, in subgroup analysis, the *TPMT* genotype showed no significant association with the incidence of anemia, grade 3–4 neutropenia, or thrombocytopenia. All the patients who experienced grade 3–4

Table 4 Association Between Hematotoxicity with *TPMT* Genotypes, Phenotypes, and Several Variables

Variables		Hematotoxicity		p-value	OR	95% CI
		+ (Grade 1–4)	– (Normal)			
		(n = 76)	(n = 30)			
Genotypes[a]	Wild type	74	30	1.000		
	Mutant	2	0			
Age[b] (years)	Median (min–max)	6 (2–18)	7.29 (2.42–16.83)	0.707		
Age[c] (years)	1–< 10	60	21	0.328	0.622	0.239–1.619
	10–18	16	9			
Risk Stratification[c]	HR	43	15	0.540	0.767	0.329–1.791
	SR	33	15			
BMI[b] (kg/m²)	Median (min–max)	16.7 (3.3–28,04)	18.08 (12.94–25.62)	0.116		
Nutritional status[a]	Underweight	1	1	0.488	2.586	0.157–42.732
	Normal-obesity	75	29			
Albumin levels[d]	Mean (± SD) (g/dL)	4.52 (±0.33)	4.59 (±0.37)	0.305		−0.07–0.220
Albumin levels[a]	Normal	75	30	1.00		
	Hypoalbumin	1	0			
The daily dose of 6MP[c]	Same	38	18	0.353	1.500	0.636–3.537
	Modified	38	12			
Cotrimoxazole[c]	No	41	16	0.954	0.976	0.418–2.276
	Yes	35	14			

Notes: [a]Fisher's Exact test; [b]Mann Whitney test; [c]Chi-Square test; [d]Unpaired *t*-test.
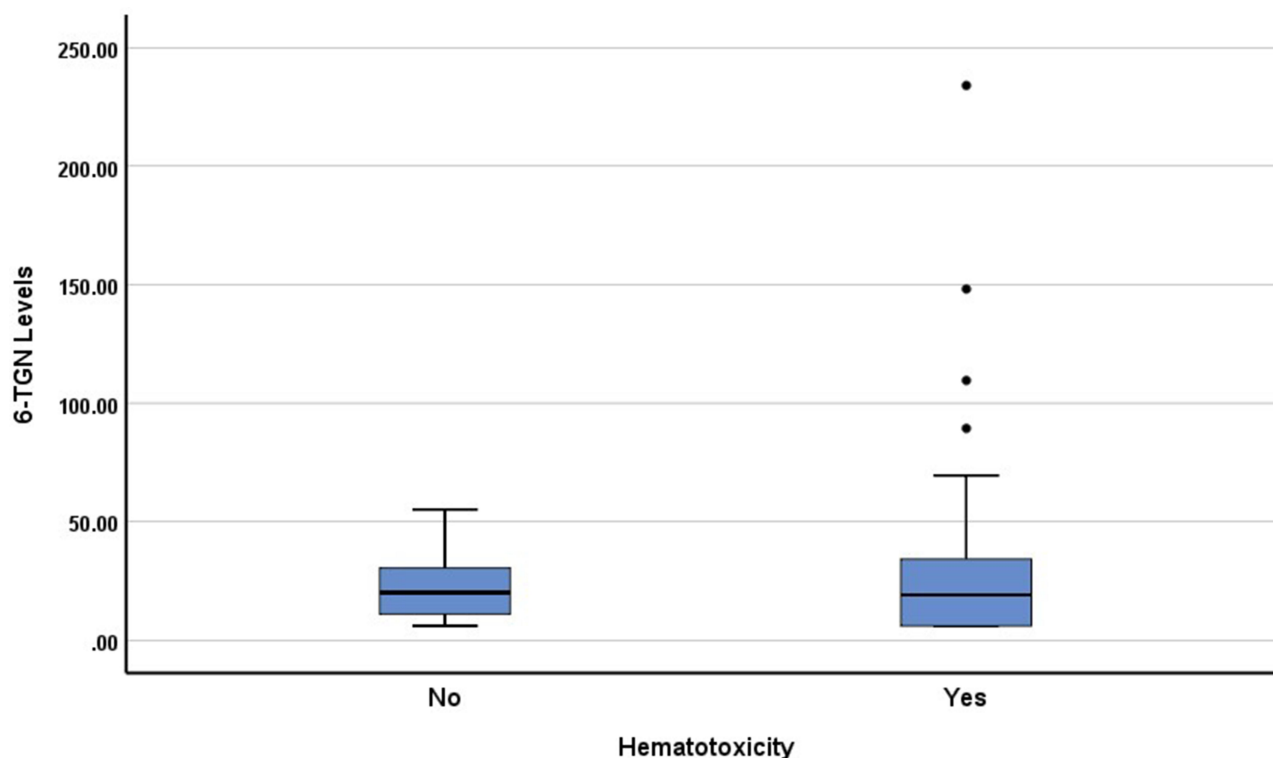**Abbreviations:** RBC, red blood cells; SD, standard deviation.

Figure 1 Box plots of 6-TGN levels in patients with and without hematotoxicity. The 6-TGN levels were not significantly different in both groups (p=0.938).

neutropenia (ANC<1000 cells/m^3) in this study had wild-type genotypes. However, both subjects with heterozygous *TPMT*3C* (*TPMT *1/*3C*) alleles belong to the group of subjects who are experiencing hematotoxicity. The results of this study are in agreement with those of Levinsen et al[16] who also found no difference in myelotoxicity in subjects with high *TPMT* activity (wild type) compared with those with intermediate *TPMT* activity (heterozygous mutants). These results are in accordance with other studies in Asian populations.[17–22] No *TPMT* mutant alleles were even found in patients experiencing hematotoxicity.[19] The result of our study indicates that the *TPMT* genotype alone could not explain the high incidence of hematotoxicity in ALL children in Indonesia.

However, in Caucasian pediatric ALL, Relling et al found that hematotoxicity was higher in heterozygous mutant patients than wild-type patients. In that study, the most frequent allele found was *TPMT*3A* and not *TPMT*3C*.[23]

TPMT enzyme activity differs between alleles. The *TPMT*3A* allele is the most common mutant allele responsible for low *TPMT* activity in the Caucasian, Mediterranean, American, Middle Eastern, and Mexican populations. *TPMT*3A* encodes a protein with two single-nucleotide polymorphisms (SNPs), ie, G460A in exon 7 and A719G in exon 10, leading to amino acid substitutions at codon 154 (Ala>Thr) and codon 240 (Tyr>Cys), resulting in low activity. *TPMT*3A* had more than 200-fold reduced enzyme activity than the wild type. While the *TPMT*3C* mutant allele, the most common mutant allele in Asia and Africa, contains an SNP (A719G in exon 10), it produces intermediate enzyme activity compared with the wild type.[24–26] The variation of *TPMT* enzyme activity in mutant alleles caused a significant difference in the level of 6-TGN in *TPMT* genotypes *1/*3A*, which was significantly higher than *TPMT* genotype patients *1/ *3C*.[27] In our study, the proportion of subjects with heterozygous mutant alleles was low, 1.9% (2/106). We only found *TPMT*3C* mutant alleles, which caused a slight decrease in *TPMT* activity. The allele is one reason for the difference in hematotoxicity based on the *TPMT* genotype in our study.

In the Asian population, the frequency of the *TPMT* mutant allele is low and dominated by *TPMT*3C*.[7] However, similar to our results, hematotoxicity associated with mercaptopurine administration in Asian patients is common.[18–21] *TPMT* deficiency cannot explain the high
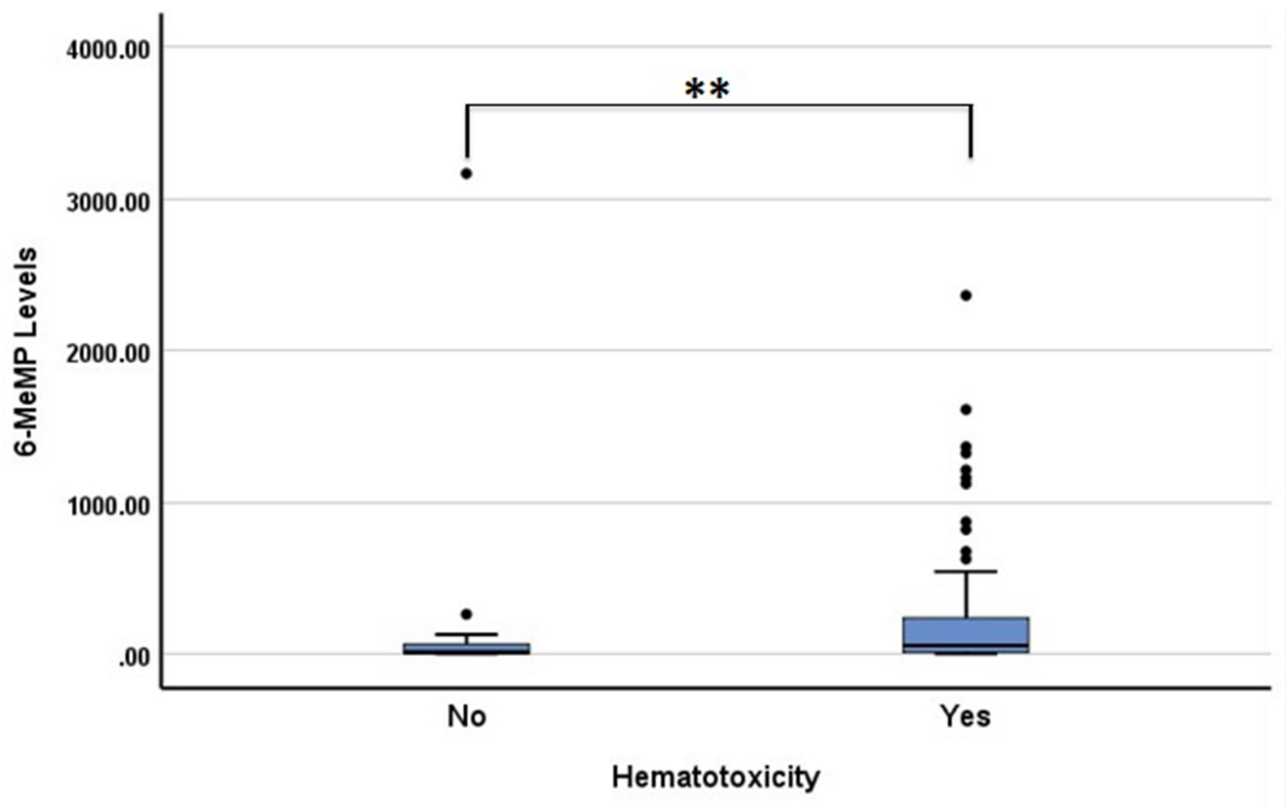
Figure 2 Box plots of 6-MeMP levels in patients with and without hematotoxicity. The 6-MeMP levels were higher in patients with hematotoxicity (**p<0.01).
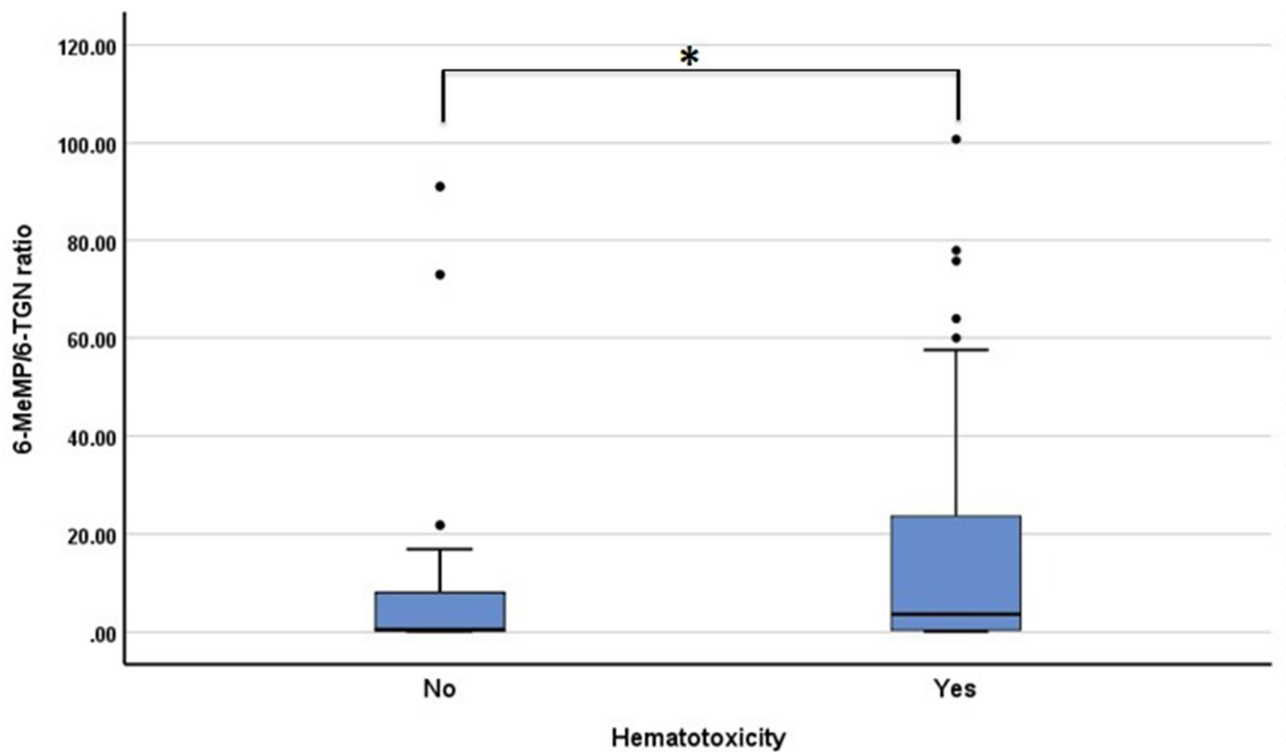

Figure 3 Box plots of 6-MeMP/6-TGN ratio in patients with and without hematotoxicity. The 6-MeMP/6-TGN ratio was higher in patients with hematotoxicity (*p<0.05).

Table 5 Association Between Anemia, Neutropenia Grade 3–4, Thrombocytopenia, and Other Variables

Variables	Anemia (Yes/No)			Grade 3–4 Neutropenia (Yes/No)			Thrombocytopenia (Yes/No)		
	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI
Genotypes[a] (Wild type or mutant)	0.194			1.000			0.128	16.33	0.906–294.4
Phenotypes[b]									
6-MeMP/6-TGN Ratio	0.295			0.011*			0.980		
6-TGN levels pmol/8x10^8 RBC	0.560			0.794			0.995		
6-MeMP levels pmol/8x10^8 RBC	0.042*			0.005*			0.792		
Age[b] (years)	0.085			0.545			0.303		
Risk Stratifications[c] (HR/SR)	0.911	0.957	0.443–2.068	0.343	0.486	0.119–1.991	0.055	0.469	0.215–1.022
BMI[b] (kg/m^2)	0.001*			0.252			0.472		
Albumin levels[d] (g/dL)	0.443			0.815		−0.256–0.202	0.007*		0.098–0.618
Daily dose of 6MP[c] (Same/Modified)	0.947	0.974	0.452–2.099	1.000	1.133	0.308–4.170	1.00	0.830	0.176–3.902
Cotrimoxazole[c] (Yes/No)	0.911	0.957	0.443–2.068	0.749	0.756	0.200–2.849	1.00	0.864	0.184–4.064

Notes: [a]Fisher's Exact test; [b]Mann Whitney test; [c]Chi Square test; [d]Unpaired t-test; *p<0.05.
Abbreviations: RBC, red blood cells; SD, standard deviation.

hematotoxicity in patients in Asia.[28] *TPMT* genotyping cannot predict hematotoxicity in 6-MP administration in Asian patients.[29]

In this study, 106 patients who received 6-MP 50 mg/m^2/day for at least one month showed highly varied metabolite levels of 6-TGN and 6-MeMP. Our result is in line
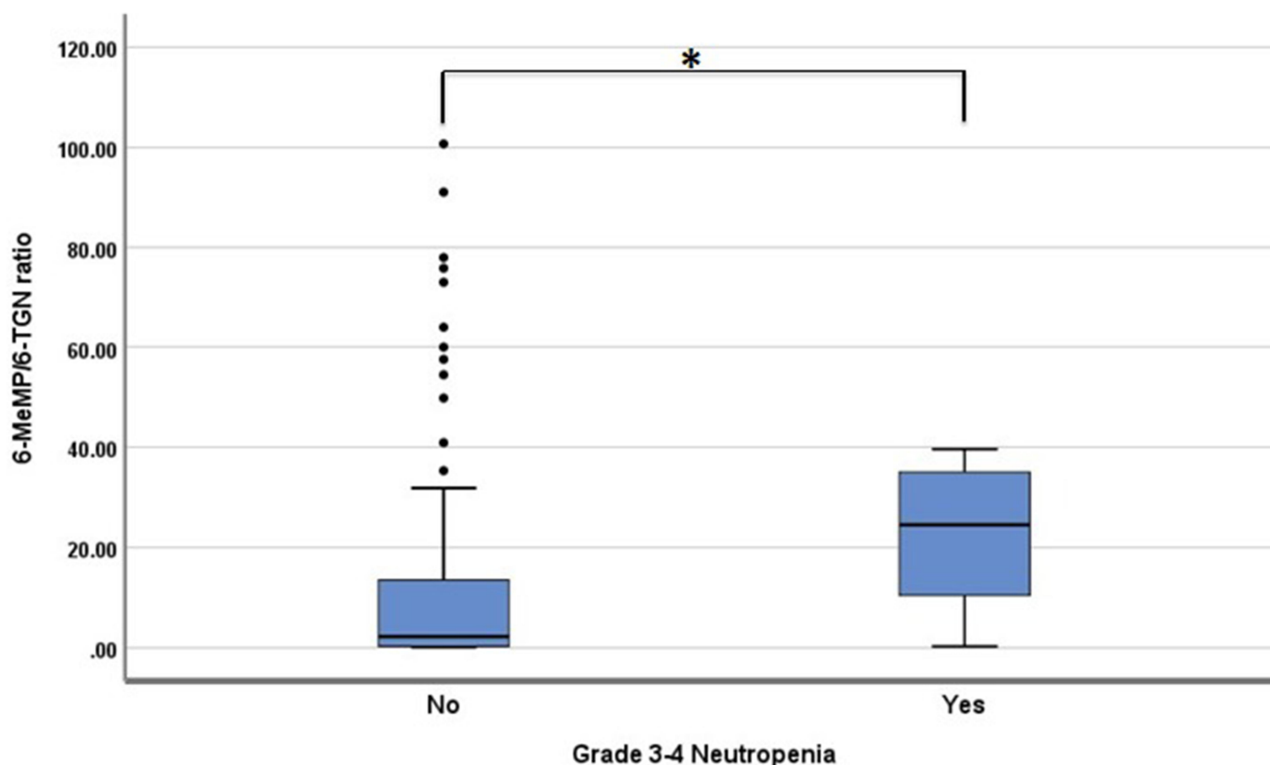

Figure 4 Box plots of 6-MeMP/6-TGN ratio in patients with and without grade 3–4 neutropenia. The 6-MeMP/6-TGN ratio was higher in patients with grade 3–4 neutropenia (*p<0.05).
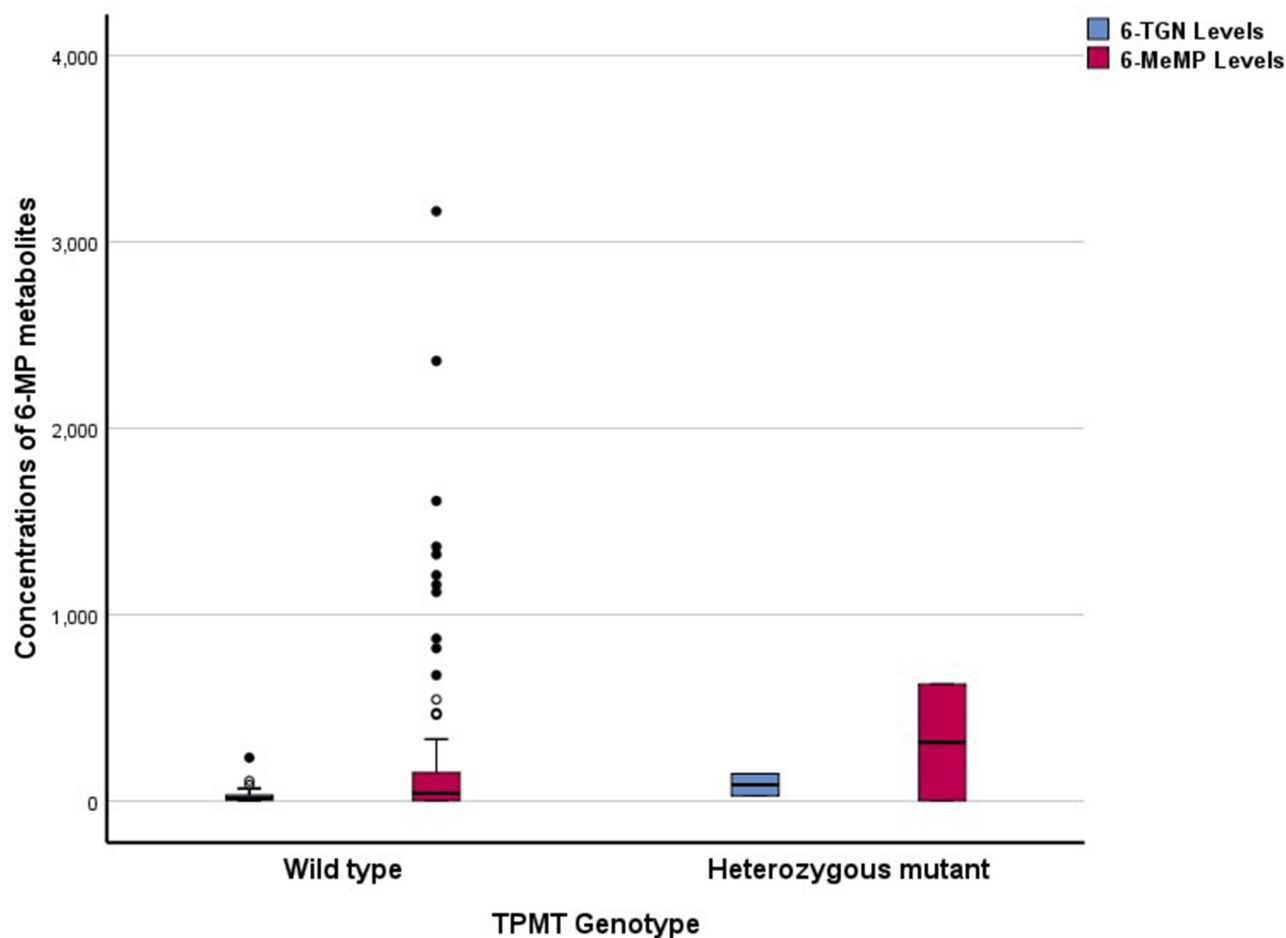
Figure 5 Erythrocyte 6-TGN and 6-MeMP concentrations (pmol/8x10^{8} RBC) by TPMT genotype (wild type, n=104; mutant, n=2).

with various studies conducted previously.[5,30,31] The study of Relling et al[23]. in 180 ALL pediatric patients who received 6-MP therapy during the maintenance phase, gave highly variable 6-TGN levels, with an inter-patient variation coefficient of 78% and an intra-patient variation coefficient of 37.9%.

The therapeutic level range of 6-TGN in inflammatory bowel disease (IBD) patients is around 235–450 pmol/8 × 10^8 erythrocytes.[32] In pediatric ALL patients, a range of 6-TGN therapeutic levels has not been determined. In our study, the 6-TGN levels varied significantly in a wide range, as in other studies. Nevertheless, the levels obtained in this study were lower, 6–234.04 pmol/8 × 10^8 erythrocytes, compared with the Chrzanowska et al study,[30] <60–833 pmol/8 × 10^8 erythrocytes, despite the patient receiving the same 6-MP dose of 50 mg/m^2/day. In the Bhatia et al study,[5] patients received a higher dose of 6-MP, 75 mg/m^2/day. Still, the 6-TGN levels in the study were 0.3–714.1 pmol/8 × 10^8 erythrocytes, similar

to the study of Chrzanowska et al[30]. Several factors can contribute to inter-individual variations in 6-MP metabolite levels: differences in 6-MP absorption; a variety of *TPMT* enzyme activities, which are influenced by genetic polymorphisms, interactions with other drugs, and patient compliance.[6,33–36] Although the 6-TGN levels in this study were relatively low, the myelosuppression effect, as indicated by the ANC values, was in the range of 108–8268 cells/mm^3. Our study's ANC value was not much different from that reported by Bhatia et al[5]; with higher 6-TGN levels, ANC ranged from 600 to 8100 cells/mm^3.

We found higher 6-MeMP levels and a higher ratio of 6-MeMP/6-TGN levels in patients with hematotoxicity and grade 3–4 neutropenia. However, the sensitivity and specificity values were not strong enough to predict the risk of hematotoxicity and grade 3–4 neutropenia.

To date, no studies have been done to assess the relationship between the ratio of 6-MeMP/6-TGN levels and

hematotoxicity in pediatric ALL patients. Nevertheless, there were studies in patients with IBD. They found no difference in the ratio 6-MeMP/6-TGN levels, 6-TGN levels, and 6-MeMP levels in IBD patients with or without leukopenia.[37] Another study showed that the 6-MeMP/6-TGN level ratio >11 were included in the thiopurine hyper-methylator and for those patients necessary to reduce 25–33% 6-MP dose.[36]

TPMT phenotyping to predict hematotoxicity is associated with some limitations, ie, the therapeutic range of 6-TGN levels and 6-MeMP levels for ALL is not yet determined due to inter-and intra-individual variations, higher cost, and because the measurement results are affected by patient compliance.

We found no association between *TPMT* genotypes and *TPMT* phenotypes. In this study, 6-TGN levels tended to be higher, and 6-MeMP levels tended to be lower in heterozygous mutant patients, which was in line with another study.[13] However, the difference did not reach statistical significance due to the small number of patients with heterozygous mutants found in this study.

This study found no association of patients' characteristics with 6-MP hematotoxicity. The genetic factor, such as polymorphism, contributed to 6-MP hematotoxicity. *TPMT* polymorphism plays an essential role in the metabolism of 6-MP and affects the efficacy and safety of the treatment. However, the frequencies of *TPMT* variants in most Asian populations, including Indonesia, are low. More recently, the CPIC Guidelines recommended testing the genotype of the nudix enzyme (nucleoside diphosphate-linked moiety X)-type motif 15 (*NUDT15*) for 6-MP initial dose adjustment to avoid hematotoxicity in Asian patients.[38] The frequency of *NUDT15* mutant alleles in Asia was more than 10%.[29]

Based on this study's results, routine *TPMT* genotyping for adjusting the initial 6-MP dose in Indonesian pediatric ALL patients provides limited clinical benefits. Further studies are needed to confirm the role of *NUDT15* in pediatric ALL patients in Indonesia.

Conclusion

In the present study, we found no association between *TPMT*3A, *3B*, and *3C* genotype variations and hematotoxicity in Indonesian pediatric ALL patients in maintenance therapy containing 6-mercaptopurine. Yet, there was an association between the ratio of 6-MeMP/6-TGN and the occurrence of grade 3–4 neutropenia.

Abbreviations

ALL, acute lymphoblastic leukemia; CTCAE, Common Terminology Criteria for Adverse Events; 6MP, 6-mercaptopurine; 6MeMP, 6-methylmercaptopurine; 6TGN, 6-thioguanine nucleotide; TPMT, thiopurine S-methyltransferase; SD, standard deviation; ANC, absolute neutrophil count; HR, high risk; SR, standard risk; RBC, red blood cells; BMI, body mass index.

Disclosure

The authors report no conflicts of interest in this work.

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