

Relationship Between Thiopurine S-Methyltransferase Genotype/Phenotype and 6-Thioguanine Nucleotide Levels in 316 Patients With Inflammatory Bowel Disease on 6-Thioguanine

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Background: In inflammatory bowel disease (IBD), conventional thiopurine users cease treatment in 60% of cases within 5 years, mostly because of adverse events or nonresponse. In this study, the authors aimed to investigate the role of 6-thioguanine nucleotide (TGN) measurements, geno/phenotyping of thiopurine S-methyltransferase (TPMT), and their mutual relationship with TG therapy in IBD.

Methods: An international retrospective, multicenter cohort study was performed at 4 centers in the Netherlands (Máxima Medical Centre) and the United Kingdom (Guy's and St. Thomas' Hospital, Queen Elizabeth Hospital, and East Surrey Hospital).

Results: Overall, 526 6-TGN measurements were performed in 316 patients with IBD. The median daily dosage of TG was 20 mg/d (range 10–40 mg/d), and the median duration of TG use was 21.1 months (SD, 28.0). In total, 129 patients (40.8%) had a known TPMT status. In the variant-type and wild-type TPMT genotype metabolism groups, median 6-TGN values were 1126 [interquartile range (IQR) 948–1562] and 467.5 pmol/8 × 10E8 red blood cells (RBCs) (IQR 334–593). A significant difference was observed between the 2 groups ($P = 0.0001$, t

test). For TPMT phenotypes, in the slow, fast, and normal metabolism groups, the median 6-TGN values were 772.0 (IQR 459–1724), 296.0 (IQR 200–705), and 774.5 pmol/8 × 10E8 RBCs (IQR 500.5–981.5), with a significant difference observed between groups ($P < 0.001$, analysis of variance).

Conclusions: Our findings indicated that TPMT measurements at TG initiation can be useful but are not necessary for daily practice. TPMT genotypes and phenotypes are both associated with significant differences in 6-TGN levels between metabolic groups. However, the advantage of TG remains that RBC 6-TGN measurements are not crucial to monitor treatments in patients with IBD because these measurements did not correlate with laboratory result abnormalities. This presents as a major advantage in countries where patients cannot access these diagnostic tests.

Key Words: thioguanine, inflammatory bowel disease, therapeutic drug monitoring, 6-thioguanine nucleotides, thiopurine methyltransferase

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gut. It can be divided into 2 main diseases: Crohn's disease (CD) and ulcerative colitis (UC). In the past 25 years, novel biological therapies have emerged for the treatment of IBD. However, for maintaining remission, conventional thiopurines [azathioprine (AZA) and mercaptopurine (6-MP)] remain important first-line immunosuppressives.^{1,2} Three to 4 million patients with IBD are known to use thiopurines in daily practice.³ However, approximately 60% of AZA/MP users cease treatment within 5 years mostly because of adverse events or nonresponse.⁴ Various strategies have been proposed to optimize conventional thiopurine therapies. Common approaches include personalized dosing using the individual geno/phenotype of thiopurine S-methyltransferase (TPMT), reduced dosing with coprescription of allopurinol, and therapeutic drug monitoring (TDM)-directed dosing. The latter approach, that is, TDM, involves measuring 6-thioguanine nucleotides (TGNs), followed by dosing advice.^{5–7} These approaches are dependent on access to measuring TPMT and TGNs; thus, this could be difficult in countries where such facilities are not readily available.

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The authors declare no conflict of interest.

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More than 6 decades ago, the Nobel Prize Laureates Elion and Hitchings created thiopurines for the treatment of childhood leukemia,⁸ heralding the advent of designer drugs. TG was invented before MP and remains part of leukemia treatment regimens.⁹ The use of TG in IBD started 2 decades ago, and on retrospective, the TG doses, although well tolerated, were markedly high (>120 mg/d) and resulted in high rates of reversible liver injury, limiting its widespread use and potential licensing for IBD.⁹ With the evolution of analytical chemistry, there is a far better understanding of purine metabolism allowing 6-TGN measurements in erythrocytes, presenting clear benefits such as optimizing TG therapy for leukemia,¹⁰ helping determine adherence to thiopurines, and the need for allopurinol co-therapy.¹¹

The enzyme TPMT is responsible for the metabolism of TG and its active TGN metabolites. This results in the formation of inactive methyl-TG and active methyl-TGNs.¹² Since its initial use for IBD in 2001, TG has been rediscovered for IBD therapy¹³ and currently holds a provisional license in the Netherlands.^{14,15} This has been achieved by administering a lower daily clinical dose of TG in IBD (20 mg/d) than those in initial reports for IBD and leukemia. Similar to other immunosuppressives, adverse events remain an important reason for patients to cease TG treatment in IBD, observed in 11%–20%^{16,17} of cases; however, these adverse events are reversible with no reported deaths. To further improve safety, TDM and TPMT geno/phenotyping might add value to reduce adverse events if the facility is available at the treatment center. However, to date, no relationship has been identified between 6-TGN levels and abnormal laboratory values.^{18,19} In this study, we aimed to investigate the role of 6-TGN measurements, TPMT geno/phenotyping, and their mutual relationship with TG therapy in IBD.

METHODS

Study Design and Patient Population

An international retrospective, multicenter cohort study was performed at 4 centers in the Netherlands (Máxima Medical Centre) and the United Kingdom (Guy's and St. Thomas' Hospital, Queen Elizabeth Hospital, and East Surrey Hospital). Patients were identified using local hospital pharmacy dispensing records dating from 2003 to 2020, as TG is only distributed by the hospital pharmacy. Patients were included if they were diagnosed with CD, UC, or IBD-unclassified (IBD-U) according to clinical, endoscopic, and/or histological criteria, and if they were treated with TG, either as monotherapy or concomitant therapy with biologicals. All patients had at least one 6-TGN measurement. All patients with 6-TGN levels below 100 pmol/8 × 10E8 red blood cells (RBCs) were excluded to reduce the influence of noncompliance.

Data Collection

Patient characteristics and laboratory measurements were retrieved from the electronic medical patient dossier, including age, sex, weight, IBD diagnosis, year of diagnosis, and previous use of thiopurines. The start date, dose, and duration of TG use were also collected. The laboratory

measurements included TPMT genotype or phenotype, 6-TGN level, hemoglobin level, mean corpuscular volume (MCV), leukocytes, thrombocytes, alkaline phosphatase (AP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), C-reactive protein (CRP), and calprotectin.

TPMT and 6-TGN

The genotype of TPMT was determined at the Máxima Medical Centre. TPMT genotyping was based on the method by Schütz et al.²⁰ The phenotype of TPMT was determined in the United Kingdom. TPMT levels below 25 U/mL were considered slow metabolizers and levels above 65 U/mL were considered fast metabolizers.²¹ The phenotypical TPMT level was determined by the method described by Breen et al.²² Until 2016, in the Netherlands, 6-TGN concentrations were determined in erythrocytes by a modified high-performance liquid chromatography method of Lennard and Singleton.¹⁰ The lower limit of quantification for 6-TGN metabolite levels was 40 pmol/8 × 10E8 RBCs. Blood samples for thiopurine metabolite measurement were immediately stored in a refrigerator (2–8°C) to ensure metabolite stability and were subsequently sent to the Department of Clinical Pharmacy and Toxicology of the Zuyderland Medical Center (Sittard-Geleen, the Netherlands), where the erythrocytes were washed, counted, and stored at –20°C until further required. As of 2016, 6-TGN concentrations in erythrocytes were determined at the Department of Clinical Pharmacy of Catherina Hospital (Eindhoven, the Netherlands) using an ultra-high-performance liquid chromatography-tandem mass spectrometer method, which was cross-validated with the Sittard method.²³ In patients from the United Kingdom, 6-TGN measurements were performed at the Purine Research Laboratory at St Thomas' Hospital using the Dervieux method.²⁴ The reference level in the United Kingdom is 235–450 pmol/8 × 10E8 RBCs.

Statistical Analysis

Data were presented as numbers with percentages, medians with interquartile range (IQR), or means with standard deviations. Depending on the type of parameter, distribution, parametric or nonparametric tests, including the Mann–Whitney *U* test, Wilcoxon signed-rank test, Kruskal–Wallis, and Student *t* test or analysis of variance (ANOVA), were used to test for differences within and between groups. This study was conducted according to the Strengthening of Reporting of Observational Studies in Epidemiology statement.²⁵ IBM SPSS Statistics (version 25.0, IBM, Armonk, NY) was used for the statistical analysis. A *P* value of less than 0.05 was considered statistically significant.

Ethical Considerations

According to the guidelines of the UK Health Research Authority, as data were collected as part of routine clinical care and were evaluated retrospectively, the study was considered a review of daily clinical practice, and ethical approval was not required.²⁶ This study was conducted in accordance with the Declaration of Helsinki.²⁷ All data in this study were anonymized.

RESULTS

Cohort Characteristics

In total, 526 6-TGN measurements were performed in 316 patients with IBD. Of these 316 patients with IBD, 195 (62%) were women, with a median age of 45 years (IQR 34–58.5). Furthermore, CD, UC, and IBD-U were diagnosed in 154 (48.8%), 147 (46.5%), and 15 (4.7%) patients, respectively. The median daily dosage of TG was 20 mg/d (range 10–40 mg/d), and the duration of TG use was 21.1 months (SD 28.0). The distribution of TG dosages 10, 20, 30, and 40 mg/d for all measurements were 82 (15.6%), 404 (76.8%), 12 (3.7%), and 28 (5.3%) patients, respectively. In this cohort, 86 of the 316 (27%) patients were thiopurine-naïve before initiating TG. The mean 6-TGN level in thiopurine-naïve patients was 593.8 (SD 387.2), whereas in patients who were previously treated with thiopurines, the 6-TGN level was 625.2 (SD 421.4). This difference was not statistically significant. Both TPMT genotypical and phenotypical analyses were performed for individual patients. In total, 129 patients (40.8%) had a known TPMT status. The distribution of TPMT genotypes was *1/*1 in 67 (94.4%), *1/*3A in 3 (4.2%) patients, and *1/*2 in 1 patient (1.4%). According to the phenotypical TPMT level, 43 (74.2%) were deemed normal metabolizers (≥ 25 , ≤ 65 mU/L), 12 (20.7%) were fast metabolizers (> 65 mU/L), and 3 (5.1%) patients were slow metabolizers (< 25 mU/L). The description of the entire patient population is summarized in Table 1.

TPMT Genotype/Phenotype and TGN Levels

For TPMT phenotypes, in the slow, fast, and normal metabolism groups, the median 6-TGN values were 772.0 (IQR 459–1724), 296.0 (IQR 200–705), and 774.5 pmol/8 \times 10E8 RBCs (IQR 500.5–981.5). A significant difference was

observed between groups ($P < 0.001$, ANOVA). Furthermore, a significant difference ($P < 0.05$, t test) was observed between individual groups. The distribution of 6-TGN levels by the TPMT phenotype is shown in Figure 1. In the variant-type and wild-type TPMT genotype metabolism groups, the median 6-TGN values were 1126 (IQR 948–1562) and 467.5 pmol/8 \times 10E8 RBCs (IQR 334–593). A significant difference was observed between the 2 groups ($P = 0.0001$, t test). The distribution of 6-TGN levels by the TPMT genotype is shown in Figure 2.

Laboratory Parameters and TGN Levels

The median values for hemoglobin, MCV, leukocytes, thrombocytes, AP, ALAT, ASAT, CRP, and calprotectin were 8.4 mmol/L (IQR 7.7–9.0), 91 (IQR 88–94), 6.8×10^9 (IQR 5.6–8.5), 280×10^9 (IQR 229–345), 78 U/L (IQR 63–91.5), 22 U/L (IQR 16–31), 22 U/L (IQR 22–30.8), 2.5 mg/L (IQR 1–6), and 375 mcg/g (IQR 103–1295), respectively. Linear regression models of laboratory parameters against the 6-TGN level did not show any statistical significance. None of the laboratory parameters investigated showed any relationship with 6-TGN levels per TG dose category. The comparison between 6-TGN levels and laboratory parameters is summarized in Table 2 and Figure 3.

Dosage and TGN Levels

In this study, 6-TGN levels correlated with the TG dosage in 487 measurements. The median 6-TGN levels for 10, 20, 30, and 40 mg/d were 404 (IQR 268.5–641.5), 552.5 (IQR 364–803), 510 (IQR 333–670.5), and 677 pmol/8 \times 10E8 RBCs (IQR 471.5–1435.5), respectively. A statistically significant difference was observed between the groups (ANOVA $P < 0.0001$). The TG levels after administration of 40 mg/d differed significantly when compared with levels obtained with other dosages. Furthermore, TG levels with 20 mg/d were significantly different when compared with those with 10 mg/d. The distribution of 6-TGN levels by TG dosing is shown in Figure 4.

DISCUSSION

This retrospective international, multicenter analysis of prospective databases investigated 6-TGN measurements and TPMT geno/phenotyping for TG therapy in IBD in daily practice. More than 500 individual 6-TGN measurements in 316 TG-treated patients with IBD were correlated with dose, TPMT genotype/phenotype, and laboratory parameters. This study identified an association between 6-TGN levels and TPMT genotype and phenotype. Although a wide range of 6-TGN levels was detected per 6-TG dose category, a correlation was noted. However, there was no relationship between 6-TGN levels and laboratory parameters.

TPMT Genotype/Phenotype

In this study, we compared both genotypes and phenotypes. In both groups, a significant difference was observed between metabolism groups. The genotypical variant types (ie, slower metabolism) of TPMT revealed significantly higher 6-TGN levels. No previous studies have

TABLE 1. Description of the Cohort Characteristics (n = 316)

Parameter	Outcome
Age, median (IQR)	45 y (34–58.5)
Male:Female	121:195
IBD type (CD, UC, and IBD-U)	CD: 154 (48.8%) UC: 147 (46.5%) IBD-U: 15 (4.7%)
Thiopurine-naïve patients	86 (27.2%)
Daily dosage of TG, median (range)	20 mg/d (10–40 mg/d)
Duration of TG use, mean (SD)	21.1 months (28.0)
Patients with known TPMT status	129 (40.8%)
TPMT genotypes	
*1/*1	67 (94.4%)
*1/*3A	3 (4.2%)
*1/*2	1 (1.4%)
TPMT phenotypes	
Slow metabolizer (< 25 U/L)	3 (5.8%)
Fast metabolizer (> 65 U/L)	12 (11.5%)
Normal metabolizer (≥ 25 , ≤ 65 U/L)	43 (82.7%)
Country (Netherlands:United Kingdom)	245: 71

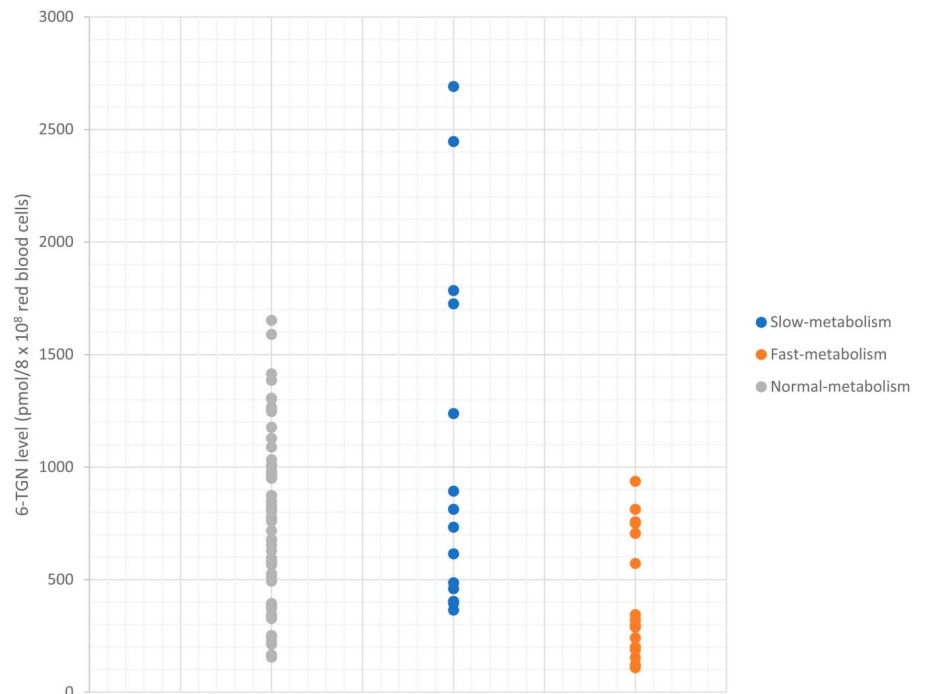


FIGURE 1. Relationship between 6-TGN levels and slow (n = 14), fast (n = 17), and normal (n = 81) TPMT phenotypes in multiple TGN measurements. A significant difference can be observed between the groups (P = 0.0001, ANOVA).

assessed the relationship between TPMT genotype and response or side effects in patients with TG-treated IBD. In this study, no relationship was observed between TPMT

genotype/phenotype or any investigated laboratory result abnormalities. The relationship between TPMT genotype/phenotype and response remains to be established.

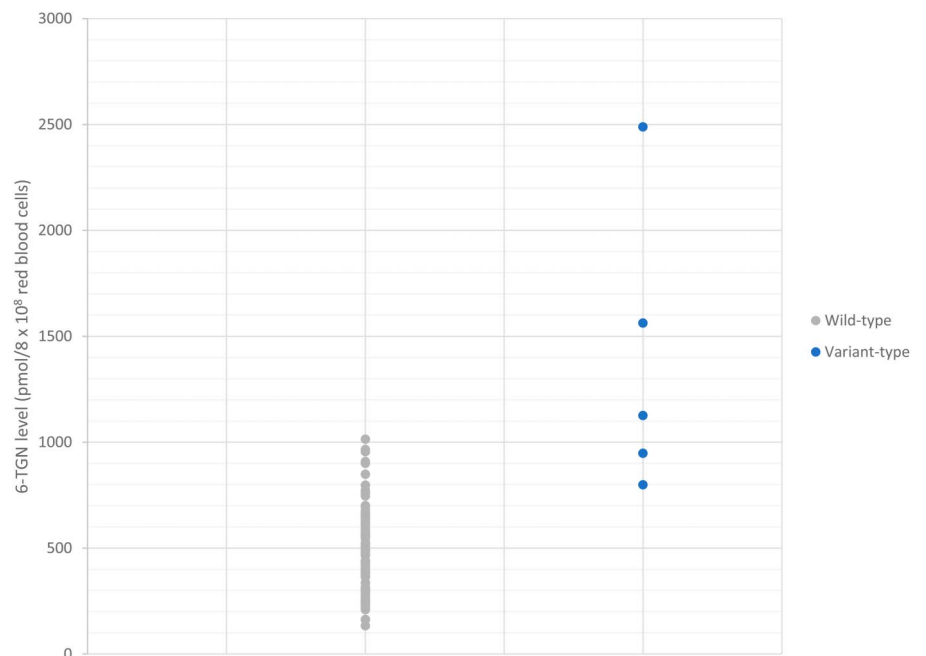


FIGURE 2. Relationship between 6-TGN levels and variant-type (n = 5) and wild-type (n = 122) TPMT genotypes in multiple 6-TGN measurements. A significant difference can be observed between groups (P = 0.0001, ANOVA).

TABLE 2. Relationship Between Laboratory Parameters and 6-TGN Values

Parameter	N of Abnormal Measurements	6-TGN in Abnormal Lab Values (Mean ± SD)	N of Normal Measurements	6-TGN in Normal Lab Values (Mean ± SD)	P
Hemoglobin <7.5 mmol/L	76	564.2 ± 327.7	353	576.0 ± 360.3	0.79
MCV <80 fL	7	564.0 ± 253.0	442	595.3 ± 381.4	0.83
Leukocytes <4.2 × 10 ⁹ /L	28	496.4 ± 267.7	424	604.5 ± 387.0	0.15
Thrombocytes <150 × 10 ⁹ /L	14	577.8 ± 165.6	343	581.3 ± 363.5	0.97
ALAT >45 U/L	42	611.4 ± 357.9	381	595.2 ± 393.5	0.80
ASAT >40 U/L	56	599.0 ± 401.3	366	598.6 ± 375.8	0.99
AP > 140 U/L	16	543.63 ± 247.3	411	600.0 ± 382.9	0.45
CRP >5 mg/L	123	596.6 ± 371.5	282	621.0 ± 408.5	0.57
Calprotectin >250 µg/g	48	528.7 ± 257.2	40	575.5 ± 312.2	0.45

The laboratory parameters are divided into abnormal and normal values, and their respective mean 6-TGN values with SDs are provided. The independent *t* test was used to compare both groups. A *P* value of 0.05 was considered statistically significant. No laboratory parameter showed statistical significance.

In all patients with fast metabolism in our TPMT phenotype analysis, the daily dosage of TG was 40 mg/d. In the normal and slow metabolism groups, all patients were administered 20 mg/d or lower, indicating that dose adjustments might be justifiable in daily practice. In a recent study¹⁷ evaluating 193 patients with IBD treated with TG as rescue therapy, it was reported that in all 12 patients with primary nonresponse, fast TPMT metabolism was observed (mean TPMT level of 91.6 ± 26.9 mU/L). Combined with the findings observed in this study

that fast metabolism (TPMT level >65 mU/L) was associated with lower 6-TGN levels, this indicates that dose adjustments might be beneficial in these patients. Therefore, phenotypical TPMT measurements at TG initiation can be useful in our opinion. Patients with fast TPMT metabolism could start with higher dosages (30 or 40 mg/d), which might reduce the number of primary nonresponders in this group of patients. This can be achieved by splitting the daily TG dosage over 2 periods during the day, which could help maintain an adequate therapeutic level

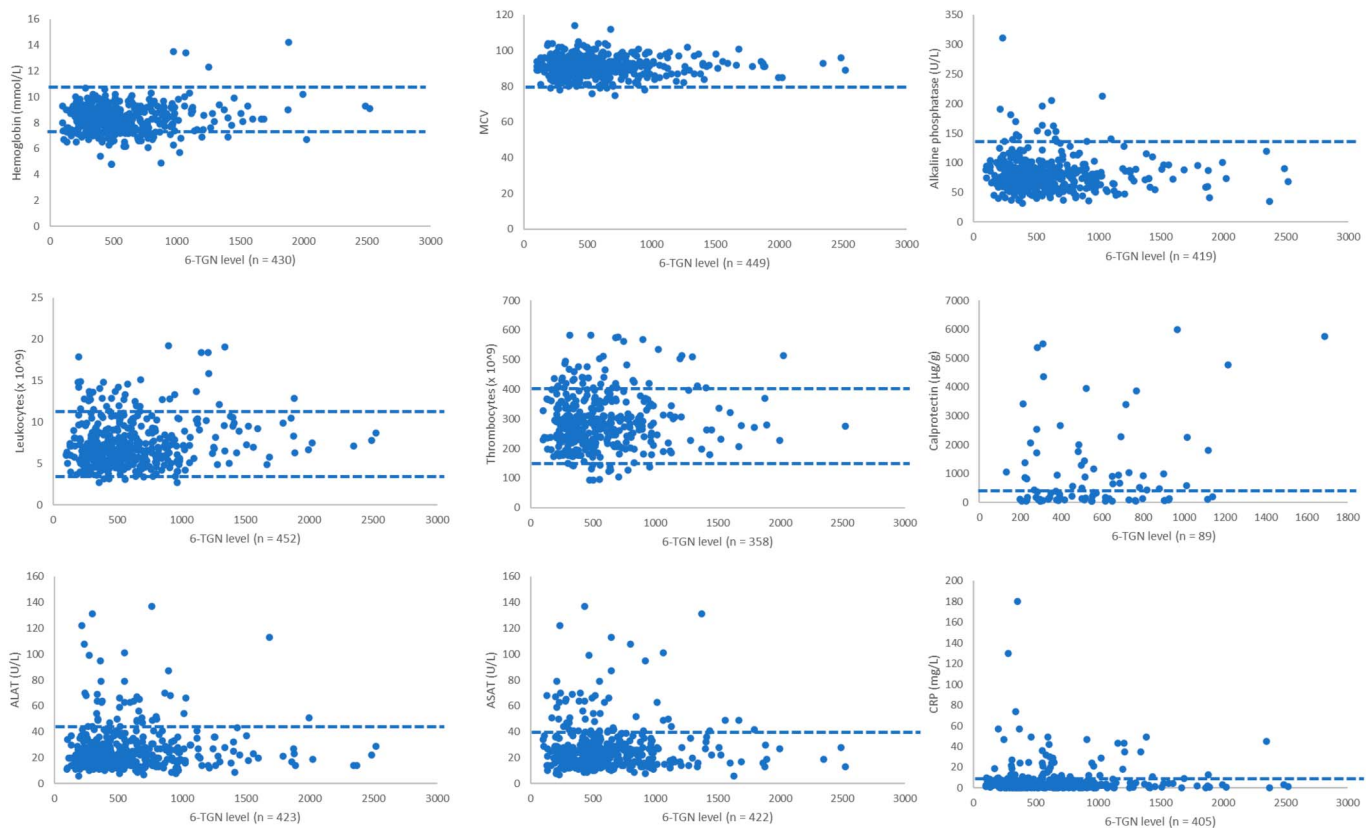


FIGURE 3. Relationship between laboratory parameters and 6-TGN levels (pmol/8 × 10⁸ RBCs). No statistically significant relationship can be observed with linear regression models between any of the laboratory parameters and 6-TGN levels.

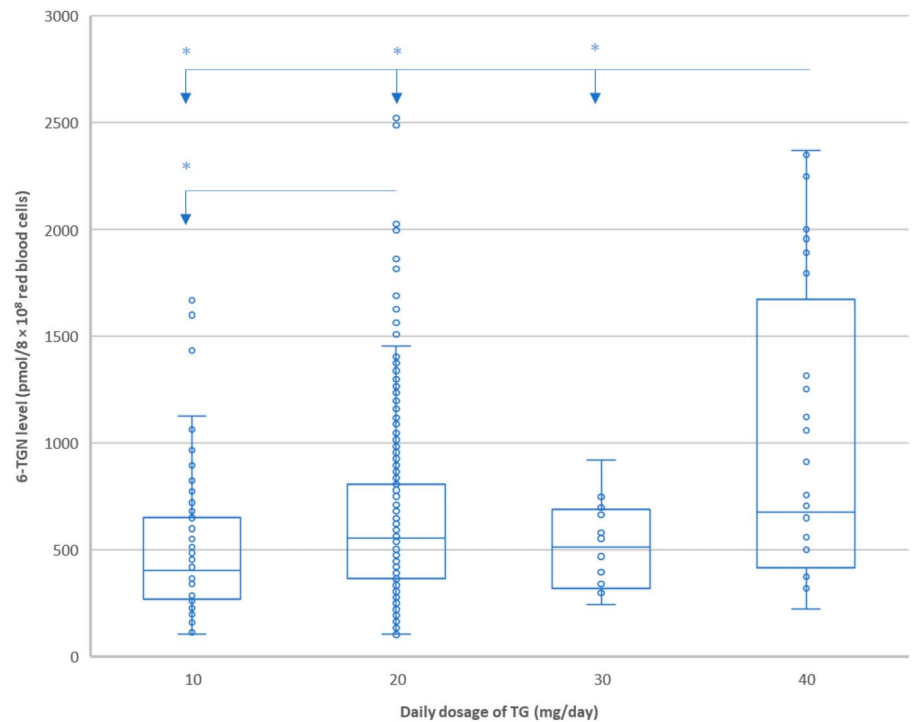


FIGURE 4. Relationship between 6-TGN levels (pmol/8 × 10⁸ RBCs) and TG dose (n = 526). 6-TGN levels for all dosages differ significantly from that of 40 mg/d ($P < 0.001$). The 6-TGN level with 20 mg/d differs significantly when compared with 10 mg/d ($P = 0.004$). *Statistically significant ($P < 0.05$).

without inducing a potential toxic concentration, a possibility with higher dosages administered once daily.^{28,29} However, the TPMT phenotype is affected by various factors such as blood transfusions or inhibitors of TPMT (eg, mesalazine, furosemide, and acetylsalicylic acid).^{30–32} Therefore, TPMT genotypical testing seems more reliable than phenotypical testing.^{33,34}

6-TGN Measurements: Relationship With Laboratory Parameters and Dosing

In the current study, no relationship was observed between 6-TGN levels and laboratory parameters investigated. The mean 6-TGN levels in patients who had abnormal laboratory values were similar to those in patients who presented normal laboratory values. This is in line with earlier reports that assessed 6-TGN levels and laboratory parameters. Derijks et al³⁵ and Meijer et al³⁶ did not detect the occurrence of leukopenia in patients with high levels (>1000 pmol/8 × 10⁸ RBCs) of 6-TGNs; the same result was observed in this study. This is a major safety advantage with TG therapy when compared with conventional thiopurines (AZA/MP).¹¹ 6-TGN levels correlated with dosing; the 6-TGN level was significantly higher in patients administering a dose of 40 mg/d when compared with all other dosages (10, 20, and 30 mg/d). Furthermore, a significant difference in the 6-TGN level was observed between 20 mg/d and 10 mg/d. No significant difference was observed between 30 mg/d and 10 or 20 mg/d. This may be attributed to the relatively small number of patients in the 30 mg/d group. The relationship between 6-TGN levels and dosing was previously described in 28 patients with IBD treated with TG.³⁵

van Gennep et al³⁷ performed a systematic review and meta-analysis on the risk factors for thiopurine-induced leukopenia in patients with IBD. A higher AZA/MP-induced risk of

leukopenia was observed in TPMT variants (OR 3.9, 95% CI 2.5–6.1). The mean 6-TGN levels were higher in patients with IBD with leukopenia (204–308 pmol/8 × 10⁸ RBCs (Lennard method)¹⁰ and 397 pmol/8 × 10⁸ RBCs (Dervieux method)²⁴ than in patients with IBD without leukopenia (170–212 pmol/8 × 10⁸ RBCs (Lennard method)¹⁰ and 269 pmol/8 × 10⁸ RBCs (Dervieux method).²⁴ However, this relationship between TPMT variants and high 6-TGN levels with leukopenia was not observed in this study in patients with TG-treated IBD. It seems that the need for 6-TGN testing is not as high in patients with TG-treated IBD when compared with patients with AZA/MP-treated IBD.

Strengths and Limitation

To date, this has been the largest study performed to assess the need for TDM in patients with IBD treated with so-called low-dose TG. This study contains both genotypical and phenotypical TPMT data, which allows for comparison of both TPMT methods in correlation with 6-TGN levels. All laboratory parameters were simultaneously measured with 6-TGN levels, allowing direct comparison. One of the limitations was that it was impossible to retrospectively determine which patients were noncompliant with TG. This may have caused lower 6-TGN levels and, therefore, may have affected the outcomes in this study. This may explain why higher TG dosages or slow metabolizers may present 6-TGN levels within the normal range. We strived to eliminate the effects of noncompliance as much as possible. In our analysis, we removed all patients with 6-TGN levels below 100 pmol/8 × 10⁸ RBCs to reduce the influence of noncompliance. Owing to the current strict privacy regulations in Europe, we were unable to consult the patient or the pharmacist regarding TG compliance. Furthermore, the lack of

clinical data in our data set made it difficult to compare 6-TGN levels with clinical outcomes.

CONCLUSIONS

Our findings suggest that TPMT measurements at the initiation of TG can be useful but is not necessary in clinical settings. TPMT genotype and phenotype are both related to significant differences in 6-TGN levels between metabolic groups. The TPMT genotype is the preferred method as it is less prone to presenting biased results. Laboratory parameters, especially leukocyte counts, were not affected by 6-TGN measurements. Moreover, leukopenia occurs infrequently with TG therapy. 6-TGN measurements relate to dosing and can be used to assess compliance and possibly also responses in patients with IBD. However, the advantage of TG remains that erythrocyte 6-TGN measurements are not necessary to monitor treatment in patients with IBD because these measurements do not correlate with laboratory result abnormalities. This is a major advantage for patients who do not have access to such diagnostic tests.

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