Thiopurine Methyltransferase Activity Is Correlated With Azathioprine Metabolite Levels in Patients With Inflammatory Bowel Disease in Clinical Gastroenterology Practice

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KEY WORDS: azathioprine, thiopurine methyltransferase, thioguanine, 6-mercaptopurine

ABSTRACT

Background: Genetic polymorphisms in thiopurine methyltransferase (TPMT) influence the metabolism of azathioprine (AZA) and 6-mercaptopurine (6-MP) in patients with inflammatory bowel disease (IBD).

Objective: The aim of this study is to assess the relationship between TPMT activity and 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine ribonucleotide (6-MMPN) levels in patients with IBD in clinical practice.

Methods: TPMT activity, 6-TGN levels, and 6-MMPN levels were analyzed in all patients who had a TPMT assessment and at least 2 metabolite profiles sent to Prometheus Laboratories Inc. (San Diego, CA) from June 2000 to February 2004. The use of AZA or 6-MP and the dosing of the drug were at the discretion of the patient's gastroenterologist. Linear regression was used to analyze the relationships between TPMT and 6-TGN, 6-MMPN, and 6-MMPN/6-TGN.

Results: A total of 1,021 patients were identified. Linear regression showed a significant inverse relationship between TPMT activity and 6-TGN level, $r(1017)=-0.162$, $P<0.01$, a significant direct relationship between TPMT activity and 6-MMPN level, $r(1018)=0.172$, $P<0.01$, and a significant direct relationship between TPMT activity and 6-MMPN/6-TGN ratio, $r(589)=0.141$, $P<0.01$.

Conclusions: Higher TPMT activity is associated with lower 6-TGN levels and higher 6-MMPN levels in patients with IBD who were treated with AZA or 6-MP in clinical practice.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that encompasses both Crohn's disease and ulcerative colitis. IBD is commonly treated with immunomodulators such as 6-mercaptopurine (6-MP) and its prodrug, azathioprine (AZA).

Recent investigation has led to a better understanding of the metabolism of AZA. 6-thioguanine nucleotides (6-TGN) are the active metabolites of AZA and result in inhibition of lymphocyte proliferation.[1] This may contribute to the therapeutic benefit of AZA. The enzyme thiopurine methyltransferase (TPMT) facilitates formation of 6-methylmercaptopurine ribonucleotides (6-MMPN), which are inactive metabolites and may be responsible for some toxicities of AZA. Studies have shown that 6-TGN levels are associated with clinical response when AZA or 6-MP is used in the treatment of Crohn's disease.[2,3] Elevated levels of 6-MMPN are associated with hepatotoxicity,[4] but not clinical response.

Genetic polymorphisms in TPMT influence the metabolism of AZA/6-MP in patients with IBD. Patients with absent TPMT activity are at high risk for very high levels of 6-TGN and potentially dangerous leukopenia. Patients with intermediate TPMT activity are likely to respond to a low dosage of AZA. Patients with normal TPMT activity can be treated with standard doses (1 to 1.5 mg/kg/day of 6-MP or 2 to 2.5 mg/kg/day of AZA). It should be noted that factors other than TPMT activity also contribute to leukopenia.[5]

In clinical gastroenterology practice, there are 3 general approaches to the initiation of AZA/6-MP in the treatment of IBD. With empiric dosing, the clinician begins a low dosage of the drug, such as 50 mg/day. The dosage can then be slowly increased while monitoring the white blood cell count. If leukopenia occurs, the dosage can be decreased or the drug can be discontinued. The second approach is weight-based dosing. This entails prescribing 6-MP 1 to 1.5 mg/kg/day or AZA 2 to 2.5 mg/kg/day and closely observing the patient for myelosuppression. The third approach is pharmacogenetic-based dosing. This approach includes determining the patient's TPMT genotype or phenotype prior to initiating the drug. Patients with absent TPMT activity would not receive AZA therapy, while patients with intermediate activity would receive low-dose therapy, and those with normal activity would receive standard weight-based doses. Modeling studies have suggested that checking a TPMT assay prior to initiating AZA is cost-effective.[6,7] This can be accomplished with or without checking metabolite levels after the drug is initiated. Ho et al propose an algorithm using TPMT testing prior to initiation of AZA.[8]

Small prospective studies have confirmed the inverse correlation between TPMT activity and 6-TGN levels. Some of these studies failed to confirm a direct correlation between TPMT activity and 6-MMPN levels as would be expected. Currently, pharmacogenetic-based dosing of AZA/6-MP is used by a significant number of gastroenterologists in clinical practice. The distribution of TPMT activity differs among whites, blacks, and Asians, which are all components of the diverse population in the United States and elsewhere. The aim of this study is to assess the relationship between TPMT activity and 6-TGN and 6-MMPN levels in a large number of patients with IBD in clinical practice.

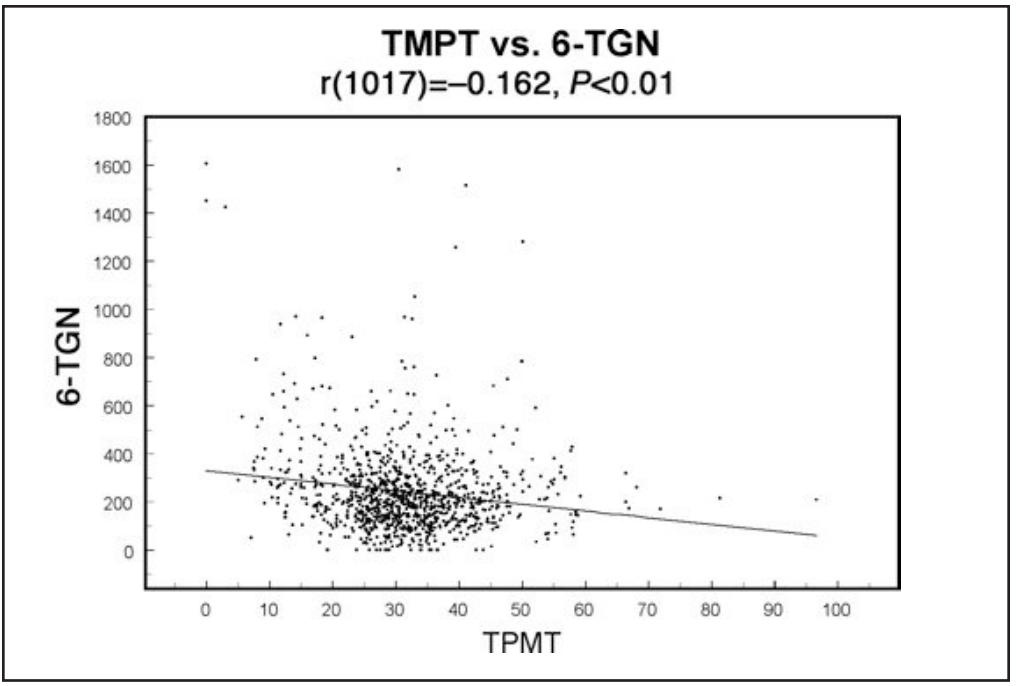

Figure 1. Plot of 6-TGN level (pmol/8 x10^8erythrocytes) by pretreatment TPMT activity (Enzyme Units). 6-TGN = 6-thioguanine nucleotide; TPMT = thiopurine methyltransferase.

MATERIALS AND METHODS

Prometheus Laboratories Inc. (San Diego, CA) has been measuring TPMT activity and AZA metabolite profiles (which include 6-TGN and 6-MMPN levels) commercially since 1999. These tests have been widely used by gastroenterologists in their management of patients with IBD. In this observational study, all patients who underwent a measurement of TPMT activity and at least 2 measurements of AZA metabolites by Prometheus Laboratories Inc. between June 2000 and February 2004 were selected. Because many clinicians use metabolite levels to optimize the dose of AZA, this study limited the analysis to patients who had more than 1 metabolite measurement to only consider those who have been dose-optimized by their clinicians. Only the most recent metabolite profile was analyzed. A total of 1,021 patients met these criteria.

The data were analyzed without patient identifiers. The selection of AZA or 6-MP and the dosage of the drug were at the discretion of the clinician. All clinical management of the patient remained with the primary physician.

Linear regression was used to analyze the relationships between TPMT activity and 6-TGN level, TPMT activity and 6-MMPN level, and TPMT activity and 6-MMPN/6-TGN ratio.

RESULTS

A total of 1,021 patients were identified. TPMT activity was <23.6 Enzyme Units (EU) in 168 patients (16.5%), which corresponds to low or intermediate activity, and normal in the remaining 853 patients (83.5%). 6-TGN levels ranged from 0 to 1451 pmol/8 x10^8 erythrocytes, while 6-MMPN levels ranged from 0 to 26,820 pmol/8 x10^8erythrocytes.

Linear regression showed a significant inverse relationship between TPMT

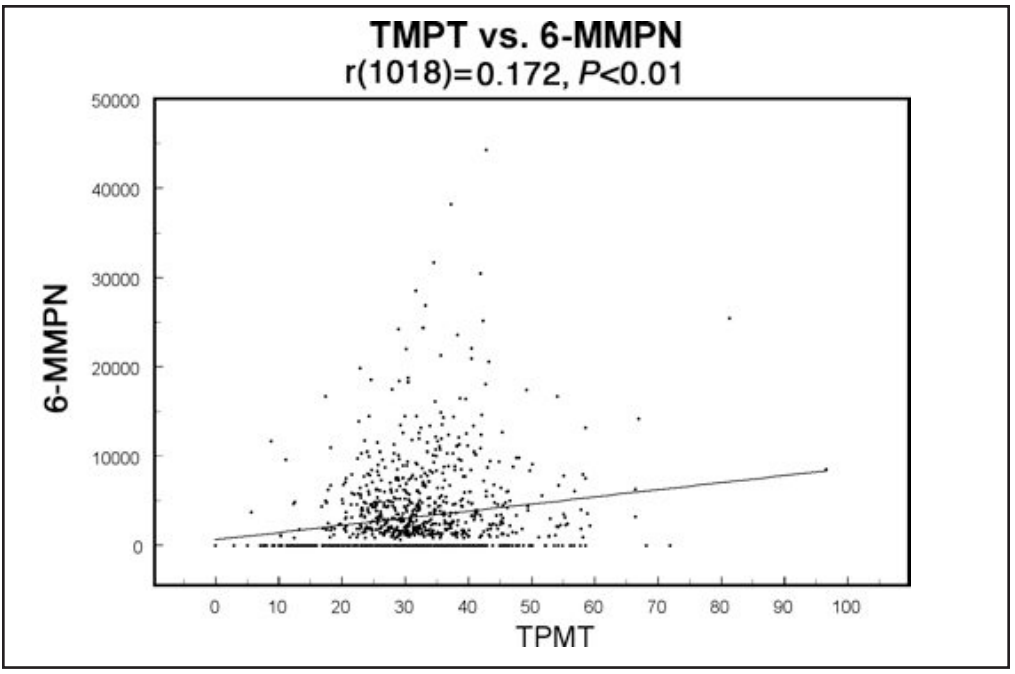

Figure 2. Plot of 6-MMPN level (pmol/8 x10^8 erythrocytes) by pretreatment TPMT activity (EU). 6-MMPN = 6-methylmercaptopurine ribonucleotide; TPMT = thiopurine methyltransferase.

activity and 6-TGN level, $r(1017)=–0.162$, $P<0.01$ (Figure 1), a significant direct relationship between TPMT activity and 6-MMPN level, $r(1018)=0.172$, $P<0.01$ (Figure 2), and a significant direct relationship between TPMT activity and 6-MMPN/6-TGN ratio, $r(589)=0.141$, $P<0.01$) (Figure 3).

DISCUSSION

There is disagreement about how to best approach the dosing of AZA in IBD. There is debate about the value of checking TPMT activity prior to initiating the drug. The 2 clinical areas in which knowledge of TPMT activity may be useful are: (1) predicting adverse events and (2) predicting clinical efficacy. It is clear that patients with absent TPMT activity or who are homozygous for TPMT mutations are at increased risk for myelosuppression. It is not clear whether TPMT activity can predict the clinical efficacy of AZA.

6-TGN levels have been associated with clinical response. The present study assesses the relationship between TPMT activity and 6-TGN levels, 6-MMPN levels, and 6-MMPN/6-TGN ratio in patients in clinical practice. Although small prospective studies have shown a correlation between TPMT and 6-TGN levels, correlation with 6-MMPN levels has not been shown previously. The present study used a large, unselected database of 1021 patients who have had TPMT activity measurements and at least 2 measurements of AZA metabolites evaluated by Prometheus Laboratories Inc. to assess this correlation with patients with IBD in clinical practice. A statistically significant inverse correlation was found between TPMT activity and 6-TGN levels. Additionally, a statistically significant direct correlation was found between TPMT activity and 6-MMPN levels. Although the strength of these relationships is not strong ($r=–0.162$ and $r=0.172$, respectively), this reflects a

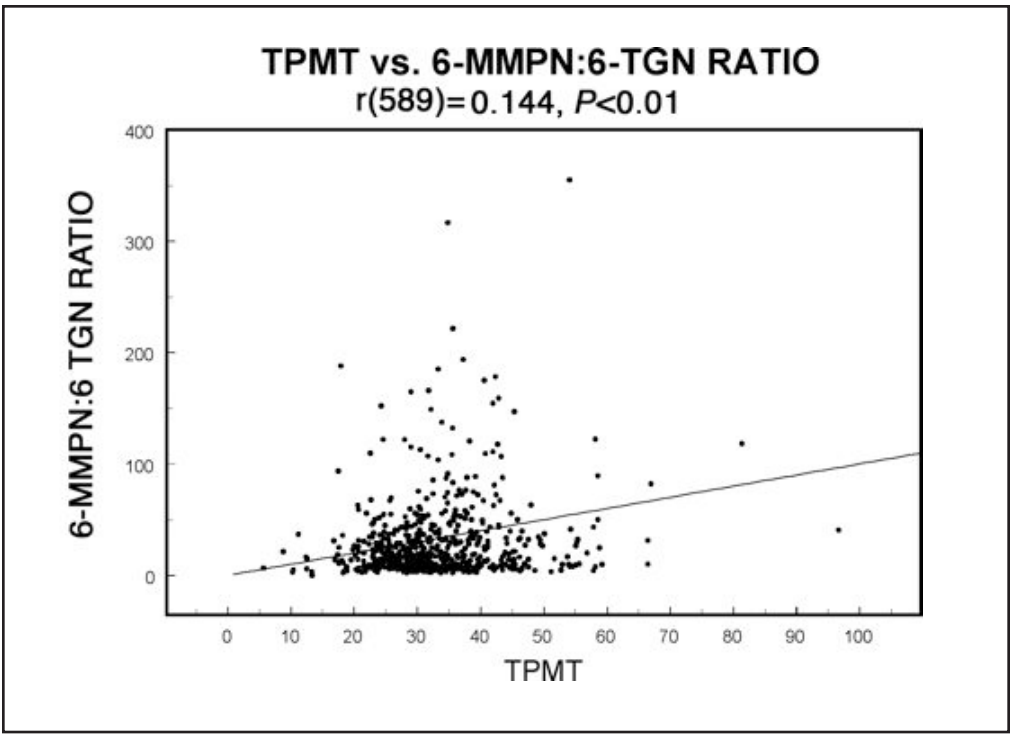

Figure 3. Plot of 6-MMPN/6-TGN ratio by pretreatment TPMT activity (EU). 6-MMPN = 6-methylmercaptopurine ribonucleotide; 6-TGN = 6-thioguanine nucleotide; TPMT = thiopurine methyltransferase.

large population with nonstandardized drug dosing. To correct for the variable dosing patterns of numerous clinical gastroenterologists, the relationship between TPMT activity and 6-MMPN/6-TGN ratio was also evaluated, and this also was statistically significantly correlated (r=0.141).

If 6-TGN levels can be considered a surrogate for clinical response, then these data suggest that TPMT activity may be useful for predicting clinical response. Derijks et al[9] found a correlation between TPMT activity and 6-TGN, but not 6-MMPN, in a prospective study of 30 patients with IBD. In a study of 142 patients with IBD, Cuffari et al[10] found that those with below average TPMT activity were more likely to clinically respond to AZA. Therefore, it may be that patients with higher TPMT activity will be less likely to respond to AZA and may benefit from more aggressive drug dosing. It will take a prospective, randomized, controlled trial to determine whether knowledge of TPMT activity prior to initiation of the drug improves clinical response.

Measuring the TPMT genotype will allow identification of patients with homozygous mutations or compound heterozygous mutations in those who lack enzyme activity. This will allow identification of those at increased risk for adverse events. However, measuring the TPMT phenotype will allow identification of those with absent TPMT activity as well as documenting high TPMT activity, which is correlated with future metabolite levels. Since higher TPMT activities are associated with lower 6-TGN levels and, perhaps, lower clinical response, one may use the presence of high TPMT activity to justify more

aggressive drug dosing. This would allow higher 6-TGN levels and may increase clinical response.

Although TPMT activity is statistically significantly associated with the 6-MMPN/6-TGN ratio, the correlation is relatively weak (r=0.141) with a lot of patient variability. A weak correlation would be expected for the correlation with 6-TGN or 6-MMPN when nonstandardized drug dosing is used in patients with normal TPMT activity. However, the weak correlation with the 6-MMPN/6-TGN ratio suggests that there are patient factors other than TPMT activity that influence the metabolism of AZA and the 6-TGN and 6-MMPN levels. This patient variability needs to be recognized, and all patients need appropriate monitoring regardless of the TPMT activity or metabolite levels.

Limitations to the present study include the lack of standardized dosing of AZA or 6-MP and the lack of clinical correlation. However, these data reflect the usage of these tests in real-life clinical practice. Prospective, randomized, controlled trials are currently underway to formulate evidence-based algorithms for the initiation of antimetabolite therapy in IBD.

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