The utility of thiopurine methyltransferase enzyme testing in inflammatory bowel disease

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OBJECTIVE: To assess the levels of red blood cell thiopurine methyltransferase (TPMT) in subjects with inflammatory bowel disease (IBD) and to determine how these levels impacted thiopurine dosing.

METHODS: A retrospective chart review was performed on all adult IBD patients (n=423, 88.2% Caucasian) who had TPMT levels measured by 11 participating gastroenterologists in Manitoba between 2008 and 2010. In addition to the retrospective data, white blood cell count, dose and reason for discontinuation were analyzed for the first six months of therapy. Patients receiving ≥2.0 mg/kg of azathioprine (AZA) or ≥1.0 mg/kg of 6-mercaptopurine (6-MP) were considered to be 'substantially' dosed.

RESULTS: Of the 423 patients, 8.3% had intermediate levels and 93.4% had normal levels of TPMT. Only one subject had a low level. A total of 216 patients had sufficient data to be included for full analysis. Patients with intermediate TPMT levels were generally started at lower doses of thiopurine than patients with normal TPMT levels (mean [± SD] 1.0±0.6 mg/kg versus 1.8±0.5 mg/kg). Of the subjects with normal TPMT levels, only 37.8% were dosed with ≥2.0 mg/kg of AZA. Each month, approximately 5% of subjects were leukopenic. These subjects received a mean overall AZA dose of 1.9±0.3 mg/kg and had a mean white blood cell count of 3.8±0.4×10^9/L.

CONCLUSIONS: Normal TPMT levels did not prevent the development of leukopenia, although life-threatening leukopenia was rare. Physicians are not using TPMT levels to substantially dose thiopurines at the outset, which may limit the speed at which adequate doses are reached to facilitate remission.

Key Words: Crohn disease; Leukopenia; Ulcerative colitis; Thiopurines; Thiopurine methyltransferase

Inflammatory bowel disease (IBD) is a chronic and relapsing immune-mediated disease of the gastrointestinal tract, characterized by two distinct subtypes, ulcerative colitis (UC) and Crohn disease (CD). Our understanding of these IBD subtypes has evolved significantly in the past decade and, although the etiology and pathogenesis of IBD has not been fully elucidated, treatments have improved dramatically.

While many different therapies exist, the thiopurine drugs, namely azathioprine (AZA) and 6-mercaptopurine (6-MP), have shown benefits in the IBD population (1,2). Initially introduced for the treatment of childhood leukemia, these drugs play a key role in the maintenance of remission (1,3). They have also shown some benefit in patients with fistulizing disease and steroid-dependent illness (4,5). However, treatment with these drugs comes with a price.

While the long-term threat of lymphoma in thiopurine users is of great concern, the most concerning – and potentially life-threatening – immediate adverse event from thiopurine treatment has been myelosuppression. After oral ingestion, AZA is converted to 6-MP, which is then metabolized via three competing enzymatic pathways. Key enzymes within these pathways are xanthine oxidase, hypoxanthine guanine phosphoribosyltransferase (HPRT) and thiopurine methyltransferase (TPMT). Patients with little to no TPMT activity experience a loss of the inactivation pathways and there is preferential conversion of the thiopurines by HPRT to their active form, specifically, 6-thioguanine nucleotides. Using a standard dose in TPMT-deficient patients can lead to life-threatening myelosuppression.

TPMT genetic polymorphism was first described by Weinshilboum and Sladek in 1980 (6). Enzyme expression is inherited in an autosomal codominant fashion, with significant correlation between TPMT genotype and phenotype (7,8). Within Manitoba, TPMT phenotype testing became widely available to physicians in 2008. TPMT phenotyping, which is the measurement of red blood cell TPMT activity (as opposed to the genotyping of leukocyte DNA) avoids the complexity of defining the genetic polymorphisms within a patient complement. The genetic polymorphism in Caucasians has

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been described and is the basis of clinically available TPMT genotype testing; however, there are less published data on enzyme levels in large populations.

With TPMT enzyme testing, the objective measure of red blood cell TPMT activity can be used to identify patients with little or no TPMT activity and to avoid thiopurines in their treatment. Alternatively, for a patient with normal enzyme activity, physicians can prescribe thiopurines relatively safely. Furthermore, dosing to recommended levels can be considered a dose of 2.0 mg/kg for AZA and 1.0 mg/kg to 1.5 mg/kg for 6-MP. For the purposes of understanding whether initial TPMT levels were associated with 'substantial' doses, a dose ≥2.0 mg/kg for AZA and ≥1.0 mg/kg of 6-MP were considered to be 'substantial'.

The aims of our retrospective cohort study were fourfold. First, we sought to identify the prevalence of low (≤10) nmol/g hemoglobin (Hb)/h, intermediate (>11 nmol/g Hb/h but ≤35 nmol/g Hb/h) and normal (>35 nmol/g Hb/h). TPMT levels within a convenience sample of IBD patients. Second, we assessed to what extent 'substantial' doses (≥2.0 mg/kg AZA; ≥1.0 mg/kg 6-MP) were prescribed at drug initiation in patients with intermediate and normal TPMT levels. Third, we evaluated the prevalence and mean time to leukopenia (white blood cell [WBC] count <4.5×10^9/L) over the first six months of therapy. Finally, we identified adverse events and determined the number of subjects who subsequently discontinued thiopurine therapy.

METHODS

Subjects

In 2008, a database was initiated with the aim of collecting all TPMT levels checked in Manitoba, including all TPMT levels ordered by various physicians (including non-gastroenterologists) across the province. TPMT levels from patients treated by 11 of 16 participating gastroenterologists practicing in both private practice and tertiary care settings were used for the purpose of the present study. A retrospective chart review was performed on each patient to determine the variables of interest, including IBD diagnosis (CD versus UC), birthdate and age, sex, ethnicity, initial thiopurine (AZA or 6-MP) dose, weight, and initial and follow-up WBC counts. IBD diagnosis was established by each gastroenterologist according to clinical, endoscopic and histological findings, and documented in the chart. In cases for which ethnicity was not clearly described in the chart, physicians were asked to recall the information.

Data were analysed for the first six months of therapy (WBC count and dose) and any reasons for cessation of therapy during that time were also recorded. Reasons why patients may have had enzyme levels checked but were not initiated on therapy were also included. Leukopenia was defined as a WBC count <4.5×10^9/L because this value is the conventional definition used by laboratories in Manitoba. Patients with WBC counts less than this would have their blood work automatically flagged by laboratories for physicians.

There were 452 subjects at least 18 years of age who had TPMT levels checked by the 11 gastroenterologists. Of these subjects, 29 were excluded because their diagnosis was not IBD. The remaining sample included 423 subjects (UC, n=228; CD, n=195) and was used to analyze prevalence data of low, intermediate and normal TPMT levels as well as baseline cohort characteristics such as age, sex, IBD subtype and ethnicity. While the optimal dose of thiopurines in IBD is truly unknown, generally, the target doses are considered to be 2.5 mg/kg for AZA and 1.0 mg/kg to 1.5 mg/kg for 6-MP. For the purposes of understanding whether initial TPMT levels were associated with 'substantial' doses, a dose ≥2.0 mg/kg for AZA and ≥1.0 mg/kg of 6-MP were considered to be 'substantial'.

The study was approved by the Health Research Ethics Board of the University of Manitoba, Winnipeg, Manitoba.

Analysis of TPMT activity

Red blood cell TPMT activity was determined as described previously (9-11). Briefly, whole blood lysates from EDTA-treated blood were incubated with 6-thioguanine and the methyl donor S-adenosyl-L-methionine to produce the methylated product 6-methylthioguanine. The reaction was stopped with perchloric acid and the 6-methylthioguanine was quantified by high-performance liquid chromatography fluorescence analysis (excitation 310 nm, emission 390 nm) using 4-aminooacetophenone as an internal standard. One of two technicians performed all assays.

Statistical analysis

For all quantitative variables, means and SDs were calculated. Medians and ranges are provided in all accompanying tables. For categorical variables, percentages were calculated.

RESULTS

Descriptive data are presented in Table 1 for the 423 subjects whose data were used to assess the distribution of TPMT levels. The mean (± SD) age was 42±15.6 years and 52% were female. The mean TPMT level was 59.9±16.5 nmol/g Hb/h (UC 58.3±16.1 nmol/g Hb/h; CD 61.9±16.9 nmol/g Hb/h). The majority (n=373 [88.2%]) of subjects were Caucasian. Of the Caucasian subjects, one (0.3%) had a low TPMT level, 31 (8.3%) subjects had intermediate levels and 341 (93.4%) subjects had normal levels. All subjects with low or intermediate TPMT levels were Caucasian with the exception of one Métis subject (mixed Caucasian and Aboriginal ancestry) and one First Nations subject (full Aboriginal ancestry).

Of the initial sample, 207 subjects were excluded from the remainder of the analysis. The most common reason for exclusion was that TPMT levels were checked in anticipation of the need for thiopurine therapy but therapy was not initiated (186 of 207 [89.9%]). Twenty-one subjects (10.1%) had incomplete charts and were missing key data (such as baseline dose). Ultimately, 216 subjects had baseline thiopurine doses and usable data for at least a portion of the first six months of therapy. The mean age among the present cohort was 42±15.9 years (52% female) in the UC group and 42±17.1 years (45% female) in the CD group. Again, the majority (>90%) of subjects were Caucasian in both CD and UC groups. These descriptive data were similar to the larger cohort described above.

Of these 216 subjects, 20 (9.3%) subjects had intermediate TPMT levels and 196 (90.7%) had normal TPMT levels. Initial AZA and 6-MP starting doses are shown in Table 2. Subjects with intermediate
TPMT levels were generally started at lower doses than those with normal TPMT levels (1.0±0.6 mg/kg versus 1.8±0.5 mg/kg). Only two subjects (10%) with intermediate TPMT levels were dosed substantially (≥2 mg/kg AZA; ≥1 mg/kg 6-MP) at baseline and they both completed six months of treatment without problems. The mean baseline WBC count was 8.6±1.8×10⁹/L in the intermediate level group and 9.5±5.4×10⁹/L in the normal level group. Seventy-four (37.8%) subjects with normal TPMT levels were dosed substantially at baseline (36.2% of UC subjects and 48.1% of CD subjects).

Although 216 subjects began treatment with thiopurines, 42 (19.4%) did not complete even one month of treatment. The most common reason for cessation of treatment was development of ‘flu-like’ illness (n=21 [50.0% (10% of the overall cohort)]). This included fatigue, abdominal pain, nausea, vomiting, diarrhea and headache. Five patients (11.9% [2.3% of the overall cohort]) discontinued treatment because of pancreatitis, of which two cases required hospitalization. One patient (2.3% [0.5% of overall cohort]) developed elevated liver enzyme levels and one patient developed a rash (2.3% [0.5% of overall cohort]). Hence, 28 subjects (13% of the overall cohort) who initiated thiopurines discontinued therapy within one month due to intolerance. Six subjects (14.2% [3.7% of overall cohort]) did not have a baseline WBC; 104 patients with normal TPMT levels did not have baseline WBC count data.

### Table 2

<table>
<thead>
<tr>
<th>Subjects with intermediate TPMT levels (n=20)</th>
<th>Ulcerative colitis</th>
<th>Crohn disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azathioprine (n=19)</td>
<td>1.1 (0.3–2.2)</td>
<td>0.9 (0.6–2.0)</td>
<td>1.1 (0.3–2.2)</td>
</tr>
<tr>
<td>6-Mercaptopurine (n=1)</td>
<td>None</td>
<td>0.7 (0.2–1.3)</td>
<td>0.7 (0.2–1.3)</td>
</tr>
<tr>
<td>Baseline WBC, ×10⁹/L</td>
<td>9.9 (6.7–17.6)</td>
<td>6.4 (3.8–7.5)</td>
<td>7.5 (3.8–17.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects with normal TPMT levels (n=196)</th>
<th>Baseline WBC, ×10⁹/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azathioprine (n=182)</td>
<td>1.7 (0.4–2.6)</td>
</tr>
<tr>
<td>6-Mercaptopurine (n=14)</td>
<td>1.0 (0.8–1.3)</td>
</tr>
<tr>
<td>Baseline WBC, ×10⁹/L</td>
<td>8.6 (3.8–60.4)</td>
</tr>
</tbody>
</table>

Data presented as median (range). *Six patients with intermediate TPMTs did not have a baseline WBC; 140 patients with normal TPMT levels did not have baseline WBC count data.

Lack of follow-up (seven subjects [25% (7% of entire cohort)]) and ‘flu-like’ illness (six subjects [21% (6% of entire cohort)]). On average, 5% of subjects were leukopenic each month, with a mean overall AZA dose of 1.9±0.3 mg/kg and a mean WBC of 3.8±0.4×10⁹/L (Table 3, Figure 1). One subject taking 6-MP became leukopenic at one month; this same subject again became leukopenic at four and five months. Two other subjects taking 6-MP became leukopenic at three months, one of whom became leukopenic again at five months. These three subjects were taking doses of 6-MP >1 mg/kg. By six months, seven patients underwent dose reductions because of leukopenia.

Sixteen subjects with intermediate TPMT levels completed at least one month of therapy and 11 completed a full six months of therapy. All of these subjects were taking AZA. Of the five subjects who did not complete six months of therapy, four had stopped treatment by three months (lost to follow-up, hospitalization for pancytopenia (WBC 2.27×10⁹/L, Hb 93 g/L, platelets 78×10⁹/L), gastrointestinal symptoms and increased liver enzyme levels). By four months, two subjects had become leukopenic (mean WBC count 4.2±0.4×10⁹/L) and, by five months, two other subjects were leukopenic (mean WBC count 4.3±0.1×10⁹/L). By six months, five of 16 subjects with intermediate TPMT levels became leukopenic (Figure 1). Only one of five subjects who became leukopenic had to stop treatment because of leukopenia.

### Table 3

| Azathioprine (AZA) doses for subjects with leukopenia over the first six months of therapy, stratified according to thiopurine methyltransferase (TPMT) level |
|-----------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Subjects with normal TPMT level | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
| AZA dose, mg/kg | 1.5 (1.0–2.6) (n=9) | 2.0 (1.0–2.4) (n=7, dose held in 3 subjects) | 2.0 (1.0–2.4) (n=11, dose held in 1 subject) | 2.0 (0.9–3.0) (n=8, dose not assessed in 1 subject) | 2.0 (1.0–2.6) (n=7, dose not assessed in 1 subject) | 2.3 (1.0–2.7) (n=9, dose not assessed in 1 subject) |

| Subjects with leukopenia taking AZA ≥2 mg/kg, n | 3 | 5 | 8 | 5 | 7 |

| Subjects with intermediate TPMT level | AZA dose, mg/kg | 0.5 (n=1) | – | – | 2.4 (2.2–2.5) (n=2) | 1.9 (0.5–2.5) (n=4) | 2.5 (n=1) |

Data presented as median (range) unless otherwise indicated.

Figure 1: Percentage of leukopenic patients each month. Patients with normal thiopurine methyltransferase (TPMT) levels are illustrated in grey. Patients with intermediate TPMT levels are illustrated in black. The number of patients in each group is shown on the x-axis (number of patients in normal TPMT group, number of patients in intermediate TPMT group). WBC: White blood cell count.
DISCUSSION
Thiopurines have been shown to be effective at maintaining remission in CD and, while there are less robust data for effectiveness, they are widely used in UC as well (1). Furthermore, recent data suggest that the combination of AZA and infliximab was more effective at maintaining remission out to one year than infliximab alone (12). Hence, these drugs will likely continue to have an important role in the management of IBD. Because of the potential for myelosuppression that is typically dose related, it has become recommended practice to estimate a patient’s TPMT activity (either by phenotyping or genotyping) before initiating therapy (13,14). It is our practice to use phenotyping for the measurement of TPMT enzyme levels. If levels are low, thiopurine therapy can be avoided because the risk for cytopenia would be too great. If the levels are intermediate, then dosing can be cautiously initiated and slowly increased while careful blood count monitoring is undertaken (monthly). Finally, if TPMT levels are normal, substantial dosing can be initiated. Because it takes approximately three weeks to reach homeostasis and, therefore, maximize the pharmacodynamic effect, more robust dosing at the outset can facilitate more rapid achievement of remission. With regard to this practice, our study highlights a number of key findings.

First, we report that in our majority Caucasian population, 92% had normal TPMT levels, 8% had intermediate levels and <1% had low levels. These data are relatively consistent with the original data on TPMT genotype reported by Weinshilboum and Sladek (6), in which 11% of the population harboured heterozygous and 0.3% homozygous TPMT mutations, which correlate with intermediate and low TPMT phenotypic activity, respectively, and reports by others (7,15) who additionally noted that for Caucasians, the genotype and enzyme levels correlated reasonably well (15,16). Of our patient population, we found that among those with a normal enzyme level, only 37.8% were dosed with AZA >2.0 mg/kg and 65.0% were dosed with AZA >1.5 mg/kg. Hence, clinicians were missing the opportunity to initiate substantial dosing to achieve the desired effect more quickly in these thiopurine users. We also found that in spite of having normal TPMT activity, each month, 5% of patients developed leukopenia during the course of therapy, and that the leukopenia could occur as long as six months postinitiation of thiopurine therapy. This highlights the importance of regular blood count monitoring regardless of the patient’s TPMT activity. Additionally, because one subject had sufficiently abnormal liver enzyme levels that warranted therapy discontinuation, we believe this supports the need to check liver enzyme levels with initial blood testing, which we perform at one month.

Similar to other studies, we found that drug intolerance with thiopurines is an issue. In our study, we found that 28 (13%) patients who received thiopurine therapy had discontinued the use of these drugs during the first month due to drug intolerance. This was somewhat independent of TPMT enzyme level because only four of these patients had intermediate TPMT levels. Similarly, in a meta-analysis of double-blinded randomized controlled trials, the incidence of adverse events sufficiently severe to cause withdrawal from thiopurine therapy was approximately 8.9% (17). In our study, there were only 111 of 216 (51%) subjects who remained on therapy at six months. While there were several reasons for therapy discontinuation, our data are from a number of different gastroenterology practices and likely reflect a ‘real world’ experience.

There were several limitations to our study. First, there were missing data, including dosing and WBC, at baseline and for several months of follow-up for a number of study subjects. This made it difficult to accurately quantify the number of leukopenic patients as well as to reliably describe dosing practices in all subjects and, hence, we restricted our analysis to those for whom all data were available. This also serves as a reminder for clinicians to be vigilant with their patients about regular blood count monitoring and to know the starting point. Second, because the data reflect the experience of 11 of 16 Manitoba gastroenterologists, starting doses were far from uniform and, hence, the timing and/or incidence of leukopenia may have been underestimated if subjects were more uniformly dosed with more substantial doses. Even for subjects with normal TPMT enzyme levels, their AZA dosing was initiated at <1.5 mg/kg approximately 35% of the time. Finally, the different prescribing practices of individual clinicians, regardless of patient weight or degree of illness, were evident throughout the present study. Physicians were not individually queried about their dosing patterns and, in one practice, patients were dosed at 50 mg for the first week of therapy, followed by 100 mg the next week and then 150 mg, up to the desired target dose. The rational for this approach may be explained by physician comfort level, the desire to avoid gastrointestinal side effects or the desire to avoid leukopenia in nonadherent patients who may not follow-through with blood counts. Finally, there were different clinical approaches to varying degrees of leukopenia. Some subjects had doses held when they became leukopenic whereas other subjects continued their drug but were simply monitored more closely. Because the present study was retrospective in nature, there was no protocol at the time directing when to stop therapy and when to continue.

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