

# THIOPURINE METHYLTRANSFERASE SCREENING BEFORE AZATHIOPRINE PRESCRIPTION: A PHYSICIAN SURVEY

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## ABSTRACT

### Background

In patients treated with azathioprine, deficient thiopurine methyltransferase (TPMT) activity has been associated with haematologic toxicity.

### Objectives

To determine how many Saskatchewan rheumatologists routinely pre-screen TPMT activity before prescribing azathioprine. Further, to retrospectively review TPMT activity levels from pre-screening in one patient cohort.

### Methods

All rheumatologists practicing in the province of Saskatchewan were surveyed by questionnaire. The questionnaire scrutinized prevalence of routine TPMT screening pre-azathioprine initiation, response to abnormal test results, monitoring frequencies, starting dosages, and attitudes towards potential consensus guidelines or practice standards. For the second objective of this study, health region laboratory database retrieval identified 42 patients who had undergone TPMT phenotype testing within a single rheumatology practice. Chart review of all 42 patients was performed to verify test results.

### Results

In a survey of all eleven Saskatchewan rheumatologists, 55% reported routinely pre-screening, compared to 45% who never screen pre-azathioprine. Half of those who pre-screen, report avoidance of azathioprine in both patients with deficiency and those with intermediate activity levels. The majority of respondents indicated they would adjust their practice to conform to future national rheumatology clinical guidelines. A retrospective review in one practice revealed TPMT activity values consistent with deficiency, carrier status, and normal ranges in 2.4%, 21.4%, and 76.2% of patients pre-screened, respectively.

### Conclusions

Provincial rheumatologists were divided on the practice of pre-screening TPMT status prior to initiation of azathioprine. Azathioprine may be underutilized by half of those who pre-screen. A need for practice guidelines was recognized by the participants. In this patient group, diminished TPMT activity was observed in 23.8% of those who were tested.

**Key Words:** *Thiopurine methyltransferase, rheumatology, azathioprine, toxicity*

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There is increasing awareness of reactions. For azathioprine, prescreening of socioeconomic costs related to adverse drug thiopurine methyltransferase (TPMT) enzyme

activity has been proposed to facilitate an individualized approach to prescribing/dosing strategies and minimize haematological toxicity.<sup>1</sup> TPMT is an enzyme participating in metabolism of thiopurine agents, including azathioprine.

Azathioprine being a pro-drug, requires conversion to 6-mercaptopurine (6-MP) for activation. At least three enzymes react with 6-MP. Xanthine oxidase metabolises 6-MP to 6-thiouric acid, hypoxanthine guanine phosphoribosyl transferase (HPRT) metabolises 6-MP to active 6-thioguanine nucleotide (6-TGN), and TPMT methylates 6-MP to the inactive 6-methyl mercaptopurine. Deficiency of TPMT activity lessens competition for the remaining metabolic pathways, and may result in increased active 6-TGN levels and potential increased toxicity.<sup>2</sup>

The gene for TPMT has been mapped to chromosome 6p22.3. Twenty-eight variant alleles have been identified, most associated with decreased activity. In North American Caucasians, TPMT \*3A is the most frequent allele mutation observed. Differences in the dominant variant alleles and variant allele frequency have been identified between ethnic groups.<sup>2-4</sup> Screening for the presence of variant alleles may be done by either genotyping or phenotyping. The latter reflects the level of enzymatic activity and does not characterize the TPMT alleles. Homozygosity for a deleterious mutation results in deficiency of TPMT activity; heterozygosity results in varying levels of reduced enzyme activity. A trimodal distribution of TPMT activity levels has been described in a general population (Rochester, Minnesota, USA). The majority (89%) had normal enzyme activity levels, 11% demonstrated low enzyme activity, and 0.3% had deficient enzyme activity.<sup>5</sup> Various laboratory techniques for establishment of TPMT genotype and phenotypes have been evaluated and demonstrated high sensitivity and specificity, although concerns have been raised in selections of reference standards for some studies.<sup>6</sup>

Deficiency or low activity levels of TPMT have been associated with haematologic toxicity related to azathioprine. TPMT pre-testing has been advocated as a means to identify people with deficiency in whom azathioprine should be avoided altogether, those with decreased enzyme levels in whom caution, very close observation

and lower doses may be appropriate, and those in whom full doses may be employed with the use of regularly scheduled laboratory monitoring.<sup>1</sup> However, there is controversy in the literature in relation to effectiveness of TPMT pre-screening.<sup>7</sup> It has been less clear whether intermediate activity has the same risk as deficiency. The recent TARGET study has concluded there is no increased risk of myelosuppression in this intermediate activity group.<sup>8</sup> However, systematic reviews of the literature concluded this group is at increased risk of myelosuppression, albeit a lesser risk than seen in the homozygous/absent-activity individuals.<sup>7,9</sup>

Azathioprine is an agent widely used in treatment of rheumatic disease.<sup>10</sup> In the absence of clear guidelines or standards on the issue of TPMT screening within the specialty, we sought to determine the local practice pattern by surveying all provincial rheumatologists. As a corollary we conducted a preliminary or pilot investigation into the frequency with which deficient or low TPMT enzymatic activity levels were detected in Saskatchewan rheumatology patients.

## METHODS

All Saskatchewan rheumatologists (eleven currently practicing) were surveyed by questionnaire. No rheumatologists declined to participate. The questionnaire included: TPMT screening pre-azathioprine initiation routine, response to abnormal test results, monitoring frequencies, starting dosages, and attitudes towards potential consensus guidelines or practice standards.

A retrospective chart review was conducted with a single university based clinical practice (RTG). It is the routine in this practice that all patients being considered for azathioprine therapy have TPMT activity measured prior to medication initiation. Patients, who had undergone TPMT activity measurements at the request of that rheumatologist, were identified through the health region laboratory database. Results were extracted from the medical record and categorized. The institutional research ethics board approved this study.

TPMT activity was measured in red blood cells (RBC) in a single laboratory (Biochemical

Genetics laboratory, Mayo Clinic, Rochester, MN, USA). The enzyme activity at this laboratory is measured utilizing liquid chromatography tandem mass spectrometry. Reference ranges are categorized as consistent with TPMT deficiency, TPMT carrier status, or normal when activity is: <6.3 U/ml RBC, between 6.3–15.0 U/ml RBC, or greater than 15.1 U/ml RBC, respectively.<sup>11</sup>

## RESULTS

There are eleven rheumatologists practicing in the province of Saskatchewan. Of these, 45.5% (5/11) reported in a recent all inclusive poll, that they do not utilize pre-initiation TPMT screening whereas the remainder (6/11) routinely pre-screen. No rheumatologist declined to be surveyed. Of those who routinely pre-screen, the majority (83.3%; 5/6) await the test results before initiation of azathioprine, and also avoid azathioprine should a result indicating TPMT deficiency be received. In the event of low but not deficient (intermediate) TPMT activity test results, half those who routinely pre-screen would still avoid future use of azathioprine, only 1/6 reported they would use their standard starting dosage.

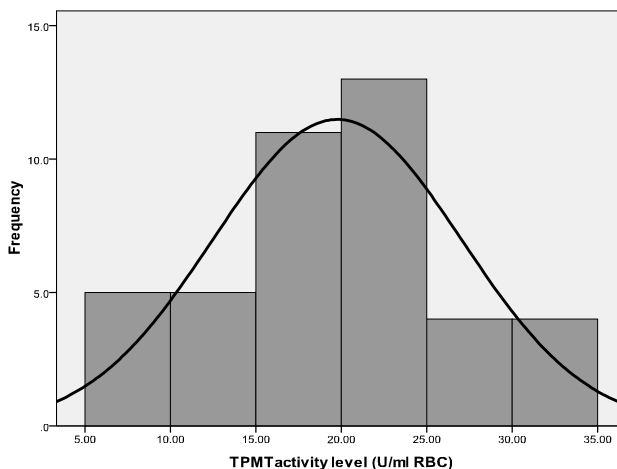
In terms of monitoring of CBCs while on azathioprine, 54.5% (6/11) of respondents would use a weekly or every two week testing schedule for the first one to two months. Beyond this initial time-period, 45.5% (5/11) reported monthly laboratory testing, and the remainder indicated

longer testing intervals. When asked whether the frequency of laboratory monitoring would change in the event of known deficient or low TPMT activity, 54.5% (6/11) of respondents indicated yes, they would make a change in frequency, 18.2% (2/11) replied ‘no’, and 27.3% (3/11) were undecided. The usual starting dosage for azathioprine was reported as 25 mg/day by 18.2% (2/11), 50 mg/day by 54.5% (6/11) and 100mg/day by 27.3% (3/11) of respondents. There was no relationship between starting azathioprine dose and choice to pre-screen or not.

In the event of development of national consensus guidelines or practice standards for rheumatology, 81.8% (9/11) of Saskatchewan rheumatologists indicated they would adjust their practice to conform to national guidelines, 18.2% (2/11) felt their own practice pattern to be the most appropriate.

Health Region Laboratory database retrieval identified 42 patients who had undergone TPMT phenotype testing within a single rheumatology practice. We undertook a chart review of all 42 of these rheumatology patients. Diagnoses included systemic lupus erythematosus (SLE), anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis, and rheumatoid arthritis. Within this group, TPMT activity was found to be deficient in 2.4% (1/42), in the carrier range in 21.4% (9/42), and normal in 76.2% (32/42). Figure 1 illustrates the distribution of enzyme activity levels.

**FIG. 1** Frequency Distribution of TPMT Activity Levels



## DISCUSSION

The question of azathioprine related haematologic toxicity is of particular concern to those involved in the care of patients with autoimmune inflammatory disease. Azathioprine is used as a disease modifying, immunomodulating therapy for a number of systemic inflammatory disorders.<sup>10</sup> Awareness of the association between TPMT deficiency and haematologic toxicity is particularly relevant to clinical practice. Further, other medications prescribed by rheumatologists, such as xanthine oxidase inhibitors or sulphasalazine may be associated with modulation in thiopurine metabolism.<sup>12,13</sup> Conversely, high levels of TPMT activity have been associated with a poorer response to therapy, potentially requiring higher doses of azathioprine for therapeutic effect.<sup>14</sup>

Variation in screening practices for TPMT deficiency exist between and within medical specialty groups.<sup>15,16</sup> A 2004 survey of UK specialists reported pre-azathioprine TPMT screening was performed by 94% of dermatologists, 60% of gastroenterologists and 47% of rheumatologist respondents.<sup>16</sup>

In this provincially based survey study, we determined that approximately half of rheumatologists report TPMT screening prior to initiation of azathioprine. Those who do pre-screen, report responding to diminished TPMT activity phenotype results through reduced initial dosage or drug avoidance. Interestingly, half of those who pre-screen indicated they would avoid use of azathioprine rather than use lower dosages not only in patients deficient in TPMT but also in those with low or intermediate TPMT activity. This raises the concern that measurement of TPMT levels may result in potentially inappropriate underutilization of azathioprine.

This divide in screening utilization amongst rheumatologists in the province of Saskatchewan likely reflects ongoing uncertainty and controversy in the wider rheumatology community on this practice. Relling et al and others have advocated for routine use of pre-azathioprine TPMT screening<sup>17</sup>; however, the effectiveness of this recommended practice is not clearly established.<sup>7</sup> The majority (81.8%) of participants in this survey indicated a willingness to modify their practice pattern in response to

establishment of consensus guidelines or practice standards in rheumatology clinical care.

Our second objective was to obtain preliminary or pilot data on the frequency of deficient or low enzyme activity levels in provincial rheumatology patients. In comparison with the previously reported trimodal distribution of TPMT activity, we found a higher frequency of deficient or diminished TPMT activity in our patient group; 23.8% were deficient or had low enzyme activity, compared to 11.3% in the Minnesota population.<sup>5</sup> This difference may well be attributable to the size of the study population; however, this university-based clinic services a northern referral catchment area and approximately half our clinic patient population are aboriginal Canadians. As the population sample for this pilot study was small, interpretation is limited, however the results do raise speculation as to the frequency of variant alleles in this demographic which has not been hitherto established.

Evaluation of the effectiveness of pre-azathioprine TPMT screening was outside the scope of this study. There have been several cost-effectiveness analyses evaluating TPMT pre-testing in different populations. An evaluation in a Canadian rheumatology population has suggested that in select populations it may be cost-effective.<sup>18</sup> The cost-effectiveness may be impacted by the frequency of variant alleles in the population. In this small patient group, 2.4% were deficient in TPMT activity, and 21.4% had intermediate enzyme activity. Further larger studies to determine variant allele frequency in Canadian populations would aid in cost-benefit determinations.

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