



Assessing the Risk of Azathioprine Immunosuppression: Changes to Thiopurine Methyltransferase (TPMT) Testing

KEY POINTS

- All patients should be screened for TPMT activity prior to commencing azathioprine immunosuppression
- TPMT activity is a major factor determining azathioprine metabolism
- 10% of individuals have low-absent TPMT activity
- Low-absent TPMT enzyme activity may lead to increased risk of myelosuppression with azathioprine therapy
- Dosage modifications according to TPMT activity may help prevent myelotoxicity
- No correlation between TPMT activity and azathioprine-hepatotoxicity
- TPMT genotyping will now be routinely performed as default pathway for TPMT testing

Azathioprine is a purine antimetabolite immunosuppressive agent commonly used for induction and maintenance immunosuppression in a broad range of clinical indications. These include treatment of systemic rheumatic disease, systemic vasculitis, inflammatory bowel disease, autoimmune liver disease, inflammatory skin conditions as well as prevention of allograft rejection.

Azathioprine therapy can cause severe, potentially life-threatening, acute myelosuppression. Toxicity is, in part, related to activity of the enzyme thiopurine methyltransferase (TPMT), which plays an important role in azathioprine metabolism. TPMT activity exhibits inter-individual and ethnic variability, mainly as a result of genetic polymorphism. Individuals with intermediate or deficient TPMT activity are at increased risk for myelotoxicity after receiving standard doses of azathioprine. TPMT activity does not predict azathioprine-induced hepatotoxicity or gastro-intestinal adverse effects.

Testing for TPMT activity

Phenotyping: Functional measurement of TPMT activity prior to initiation of azathioprine therapy has proven to be a useful tool in preventing toxicity and optimising immunosuppressive therapy. However, the assay for measurement of functional TPMT activity is time-consuming, labour-intensive and involves exposure to radio-isotopes. In addition, results may be falsely elevated by recent blood transfusions and falsely lowered by red cell aging or concurrent medications, e.g. xanthine oxidase inhibitors.

Genotyping: Common polymorphisms in the TPMT gene are associated with functional variation of enzyme activity. Genotyping is a safe and rapid method of detecting the most common genetic polymorphisms, including wild type (*1) and those associated with reduced enzymatic function (*2, *3A, *3B, *3C, *4). At Dorevitch Pathology, genotyping has replaced TPMT phenotyping as the first line investigation.

Dosage modifications according to the TPMT variant alleles detected are recommended in order to prevent toxicity and enhance efficacy (Table 1). Azathioprine therapy is not advisable in individuals with negligible activity. However, not all patients who develop myelosuppression have detectable TPMT gene mutations. Therefore close monitoring of the full blood count and liver function tests must be performed in all patients after initiation of azathioprine therapy.

Assessing response to azathioprine

Azathioprine is metabolised primarily through 3 major enzymes, including TPMT. The main therapeutic effects of azathioprine are through the incorporation of one of its major metabolites, 6-thioguanine nucleotides (6-TGN), into DNA. 6-TGN, together with another significant metabolite, 6-methylmercaptopurine (6-MMP), may be measured and used together with markers of disease activity to help adjust ongoing dosage and determine appropriate therapeutic dose for an individual.

Table 1

Genotype	TPMT activity	Frequency (%)	Recommendations for therapy
Homozygous wild-type alleles (*1/*1)	normal-high	90	Commence normal starting dose azathioprine Close monitoring of FBC/LFT Adjust dose based on disease-specific guidelines May require more aggressive oral dosing
Heterozygous wild-type/variant alleles (*1/*2, *3A, *3B, *3C or *4)	intermediate	10	Commence azathioprine at 30-70% target dose Close monitoring of FBC/LFT Titrate dose based on tolerance
Homozygous variant alleles (two nonfunctional alleles - *2, *3A, *3B, *3C or *4)	reduced-absent	0.3	Relative contra-indication to azathioprine therapy Consider alternative agents

How to order:

- Request: TPMT
 - If measurement of functional TPMT is required, request TPMT phenotype or functional assay
 - TPMT genotyping is Medicare rebatable.
 - **Specimen requirements:** 6.0 mL pink top EDTA tube (BD Vacutainer)
- 6-TGN and 6-MMP are now Medicare rebatable
 - **Specimen requirements:** 4.0mL EDTA tube

For any further enquiries regarding this test, please contact:

Dr Melody Caramins, Genetics Pathologist on (02) 9005 7000

References:

Black AJ, McLeod HL, Capell HA, et al. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann Intern Med.* 1998;129:716-718

Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui C-H, Yee SW, Stein CM, Carrillo M, Evans WE, Hicks JK, Schwab M, and Klein TE. Clinical Pharmacogenetics Implementation Consortium Guidelines for Thiopurine Methyltransferase Genotype and Thiopurine Dosing: 2013 Update. *Clin Pharmacol Ther.* 2013; 93: 324–325. doi:10.1038/clpt.2013.4