

Thiopurine Methyltransferase, Red Blood Cell

FOR DETECTING PATIENTS WHO MAY BE AT RISK FOR SEVERE MYELOSUPPRESSION IF TREATED WITH STANDARD DOSING OF THIOPURINE DRUGS.

Test Highlights

- Evaluates phenotype for thiopurine methyltransferase (TPMT).
- Used to personalize dosing of thiopurine drugs.

Disease Overview

- Thiopurine drugs, such as azathioprine (Imuran®), 6-mercaptopurine (Purinethol®, 6-MP), and 6-thioguanine (Tabloid®), are antimetabolites used to treat acute lymphoblastic leukemia, autoimmune diseases, and inflammatory bowel disease. They are also used to prevent rejection after solid organ transplant.
- Thiopurine drugs are prodrugs that must be metabolized to 6-thioguanine nucleotides for activity.
- The proportion of active 6-thioguanine nucleotides is regulated by the balance between activation and inactivation mechanisms. A primary metabolic route for inactivation of thiopurine drugs is catalyzed by TPMT. When TPMT activity is low, proportionately more 6-MP may be converted into the active (cytotoxic) 6-thioguanine nucleotides. Because TPMT also catalyzes metabolism of 6-thioguanine nucleotides, the active/cytotoxic metabolites will accumulate when TPMT activity is low.
- Excess 6-thioguanine in the bone marrow inhibits purine synthesis, thus inhibiting cell proliferation, and contributes to excess myelosuppression.
- Individuals with low TPMT activity may be at risk for 6-thioguanine mediated toxicity, but may be treated successfully with low doses of thiopurine drugs.
- Dose reductions for TPMT-deficient individuals have been proposed.

Epidemiology

- Approximately one in 300 Caucasian individuals is deficient in TPMT enzyme activity and is at risk for bone-marrow toxicity if treated with a standard dose of thiopurine drugs.
- Intermediate TPMT activity is expected in approximately 11 percent of the Caucasian population.
- The frequency and clinical significance of high TPMT activity is not currently known.

Indications for Ordering

Plans for thiopurine drug therapy; this test should be performed with blood collected prior to thiopurine drug administration.

Interpretation

- A reference interval study was performed with 145 healthy volunteers [71 males, 74 females; 93 percent Caucasian; aged 19–70 years], to generate interpretive guidelines.

Phenotype	TPMT Activity (U/mL)
Normal	40–65
Low (deficient)	<20
Intermediate	20–39.9
High	>65

- No statistically significant difference was observed based on age or gender.
- Normal TPMT activity (40–65 U/mL) represents the 20th–99th percentiles and is associated with low risk of bone marrow toxicity; no dose adjustment based on TPMT activity is recommended.
- Low TPMT activity (<20 U/mL) is associated with high risk of bone marrow toxicity; a dramatic dose reduction (80–90 percent) may be required. Therapeutic drug monitoring may help optimize dose.
- Intermediate TPMT activity (20–39.9 U/mL) is associated with increased risk of life-threatening bone marrow toxicity; a moderate dose reduction (20–50 percent) may be required. Therapeutic drug monitoring may help optimize dose.
- High TPMT activity (>65 U/mL) is not well characterized but may be associated with therapeutic failure; higher than standard dosing may be required.

Limitations

- TPMT phenotype testing does not replace the need to provide clinical monitoring of patients treated with thiopurine drugs.
- Drugs or processes that inhibit xanthine oxidase, another important enzyme involved in thiopurine metabolism (e.g., allopurinol [Aloprim™, Zylloprim®]), are not detected in this test.
- Genotype for TPMT cannot be inferred from TPMT activity (phenotype).
- The TPMT enzyme is unstable. Blood specimens must be processed within three days of collection, and blood must be kept refrigerated.

- The TPMT enzyme can be inhibited by several common drugs—naproxen (Aleve[®], Midol[®]), ibuprofen (Advil[®], Motrin[®]), ketoprofen (Orudis[®]), furosemide (Lasix[®]), sulfasalazine (Azulfidine[®]), mesalamine (Asacol[®]), olsalazine (Dipentum[®]), mefenamic acid (Ponstel[®]), thiazide diuretics, and benzoic acid inhibitors. TPMT inhibitors may contribute to falsely low results; patients should abstain from these drugs for at least 48 hours prior to blood collection intended for TPMT testing.
- This test should not be requested for patients currently treated with thiopurine drugs. The presence of thiopurine drugs (azathioprine, 6-mercaptopurine) in the blood specimen prior to testing will falsely elevate the results.
- TPMT phenotypes for patients who received a blood transfusion within 30 days prior to testing may reflect activity of the blood donor rather than the blood recipient.

Methodology

- Red blood cell (RBC) lysate is incubated with 6-MP.
- A reaction product, 6-methylmercaptopurine (6-MMP), is detected and quantified by HPLC-UV.
- The concentration of reaction products is expressed in U/mL: nanomoles of 6-MMP generated per mL of packed RBCs per one hour incubation at 37°C.

References

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2. Oselin K, et al. Determination of thiopurine S-methyltransferase (TPMT) activity by comparing various normalization factors: Reference values for Estonian population using HPLC-UV assay. *J Chromatography* 2006;834:77–83.
3. Dervieux T and Boulieu R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 1998;44: 551–555.
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5. Menor C, et al. Determination of thiopurine methyltransferase activity in human erythrocytes by HPLC: Comparison with the radiochemical method. *Therapeutic Drug Monitoring* 2003; 23:536–541.

Test Information

0092066 Thiopurine Methyltransferase, RBC

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult[®] at www.arupconsult.com.