PHARMACOGENOMIC AND METABOLITE MARKERS FOR PATIENTS WITH INFLAMMATORY BOWEL DISEASE TREATED WITH AZATHIOPRINE (6-MP)

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coverage:

Genotypic analysis of the TPMT gene is considered experimental or investigational.

Analysis of the metabolite markers of azathioprine and 6-mercaptopurine, including 6-MMP and 6-TG, is considered experimental or investigational.

DESCRIPTION:

Azathioprine, which is a derivative of 6-mercaptopurine (6-MP), is considered an effective immunosuppressive treatment of inflammatory bowel disease, particularly in patients with steroid resistant disease. For example, in the course of 1 year, 50% of patients with Crohn’s disease will require steroids for its treatment; of these 50% will either be steroid resistant or steroid dependent, and thus candidates for immunosuppressive therapy. Azathioprine therapy eliminates the need for corticosteroids in about 75% of patients; azathioprine is also considered an effective therapy for fistulizing disease. Results of a recent randomized clinical trial of children with Crohn’s disease suggest that compared to prednisone alone, inclusion of azathioprine with prednisone at the time of initial diagnosis is associated with improved maintenance of remission while simultaneously decreasing the dose of prednisone.

However, the use of azathioprine is limited by both its long onset of action (3-4 months) and drug toxicities, which include hepatotoxicity, bone marrow suppression, pancreatitis, and allergic reactions. Long-term drug use has been associated with neoplasia. Due to these side effects it is estimated that less than 5% of patients with Crohn’s disease ever receive azathioprine.

Pharmacogenomics

Azathioprine is converted to 6-mercaptopurine in vivo, where it is subsequently metabolized to 2 active metabolites; either 6-thioguanine nucleotides (6-TG) by the enzyme IMPDH, or to 6-methylmercaptopurine ribonucleotides (6-MMRP) by the enzyme TPMT. TPMT also converts 6-MP to an inactive metabolite 6-methyl-mercaptopurine (6-MMP). 6-TG is considered cytotoxic and thus is associated with bone marrow suppression, while 6-MMRP is associated with hepatotoxicity. In population studies the activity of the enzyme TPMT has been shown to be trimodal with 90% of subjects having high activity, 10% intermediate activity, and 0.3% with low or no activity. In patients with intermediate to low activity, the metabolism of 6-MP is shunted toward the IMPDH pathway with greater accumulation of 6-TG.
nucleotides; these patients are considered to be at risk for bone marrow suppression.

This variation in TPMT activity has been related to 3 distinct TPMT mutations and has permitted the development of TPMT genotyping based on a polymerase chain reaction (PCR). For example, patients with high TPMT activity are found to have 2 normal (wild-type) alleles for TPMT, those with intermediate activity are heterozygous (i.e., have a mutation on 1 chromosome), while those with low TPMT activity are homozygous for TPMT mutations (i.e., a mutation is found on both chromosomes.) Genetic analysis has been explored as a technique to proactively identify patients at risk for bone marrow suppression; those with intermediate TPMT activity may be initially treated with lower doses of azathioprine, while those with low TPMT activity may not be good candidates for azathioprine therapy.

Metabolite Markers

Monitoring of azathioprine therapy has been based on clinical assessment of response in addition to monitoring blood counts, liver function, and pancreatic function tests. However, recently there has been interest in monitoring intracellular levels of azathioprine metabolites to predict response and complications, with the ultimate aim of tailoring drug therapy to each individual patient. While genotyping of TPMT would only be performed once, metabolite markers might be tested at multiple times during the course of the disease.

Prometheus is a commercial laboratory that offers pharmacogenomic and metabolite testing for those undergoing azathioprine therapy. The tests are referred to as Pro-Predict Rx TPMT and Pro-Predict Rx 6MP, respectively.

RATIONALE:

As with any diagnostic technology, there are 3 steps in the technology assessment process; evaluation of the technical feasibility, a determination of how the information will be used in the management of the patient, and finally whether the change in management results in an improved outcome. These outcomes are discussed below, both for pharmacogenomics and metabolite markers.

Technical Performance:

Pharmacogenomics

The genotypic analysis of the TPMT gene is based on well-established polymerase chain technology (PCR) to detect 3 distinct mutations.
Metabolite Markers

Metabolite markers have been assessed using HPLC (i.e., high performance liquid chromatography) technology. It would be optimal to assess metabolite markers in peripheral leukocytes, since they reflect the status of bone marrow precursors. However, it is technically easier to measure metabolites in red blood cells (RBC) instead of leukocytes. Cuffari and colleagues reported that RBC and leukocyte 6-TG levels were directly correlated.

How Information May Be Used in the Management of Patients:

Pharmacogenomics

Ideally one would like to have data reporting the sensitivity and specificity of genetic mutations in predicting toxicity. Several studies have correlated the presence of mutations to toxicity, however the sensitivity and specificity cannot be calculated from the data published so far. For example, Black and colleagues tested 67 consecutive patients with rheumatoid arthritis undergoing azathioprine therapy. Six of the patients were heterozygous for a mutant allele; 5 of 6 of these patients discontinued therapy within 1 month due to low leukocyte counts. The number of patients without mutations who may have discontinued therapy due to low leukocyte counts was not reported; therefore the sensitivity is not known. Colombel and colleagues tested 34 patients with Crohn’s disease receiving azathioprine therapy who had cytopenia. Since only patients with cytopenia were tested and not a broader group of patients taking azathioprine, the sensitivity of genetic testing cannot be calculated. Twelve percent of the patients had mutant alleles but the presence of other potential causes of bone marrow suppression, such as the use of other myelosuppressive drugs or concurrent infection, could explain the lack of specificity. However, the presence of mutant alleles was correlated with the delay between introduction of azathioprine and the onset of cytopenia. For example, in the 4 patients with 2 mutant alleles, cytopenia appeared between 1 and 2 months, in the 5 patients with 1 mutant allele, the cytopenia appeared between 1 and 12 months, and in the 25 patients with no mutations, the cytopenia appeared between 1 week and 84 months. Dubinsky and colleagues performed TPMT genotyping in 92 pediatric patients with inflammatory bowel disease and correlated the results to clinical response and toxicities. A total of 8 (9%) were heterozygous for the TPMT allele. Of the 13 patients with leukenopenia, only 1 was heterozygous. Overall, 35 of 36 patients with a drug-related toxicity had a normal TPMT genotype.

The following clinical applications of pharmacogenomics have been
proposed:

- **TPMT genotyping** before the initiation of therapy may identify those patients who are at higher risk for hematologic toxicity and who may benefit from more intense surveillance.

- **Patients with 2 TPMT mutations** are at highest risk for bone marrow toxicity and therefore alternative therapy may be considered.

- **In patients with 1 TPMT mutation**, azathioprine therapy may be safely initiated, but a lower (50%) dose may be considered.

- **In patients with no TPMT mutations**, azathioprine may be initiated at a higher dose.

- **Azathioprine is typically given orally**, but in some cases, it may be initially given intravenously to accelerate its onset of action. IV dosing may be considered contraindicated in patients with 2 TPMT mutations.

### Metabolite Testing

Several case series have explored the correlation between levels of 6-TG, toxicity of azathioprine and treatment effectiveness. Cuffari and colleagues measured the metabolites 6-TG and 6-MMP in 25 pediatric patients with Crohn's disease. Achievement of clinical remission was correlated with 6-TG levels, but not 6-MMP. In 1 patient, low 6-TG levels suggested noncompliance, which was subsequently confirmed on further questioning. Dubinsky and colleagues measured 6-TG and 6-MMP levels in 92 pediatric patients. Higher median levels of 6-TG were observed at points of clinical response versus non-response. Quartile analysis on all samples revealed that the best probability of treatment occurred when 6-TG levels were greater than 235 (measured in pmol/8 x 10 to the eighth). The 6-MMP levels did not correlate with disease activity, but elevated levels did correlate with hepatotoxicity, observed in 16 patients. Elevated 6-TG levels were also associated with hematologic toxicity. In contrast, Gupta and colleagues reported discordant results in a case series of 54 patients with inflammatory bowel disease being treated with azathioprine. A total of 36% of patients in relapse had 6-TG levels greater than 230, compared with 30% of those in remission. Conversely, 57% of patients with 6-TG levels less than 230 were in remission, versus 50% of patients with 6-TG levels greater than 230. These results question the 235 cut-off point suggested by Dubinsky. Similarly, Lowry and colleagues reported that the 6-TG concentration did not correlate with disease activity or leukopenia in a case series of 170 patients with...
inflammatory bowel disease.

The following clinical applications of metabolite testing have been proposed:

- Based on the Dubinsky study, patients may undergo metabolite monitoring to tailor azathioprine therapy. For example, when 6-TG levels reach a target of 235, tapering of steroids may be considered. In the past, the onset of leukopenia was considered a target point for steroid tapering. The use of 235 as a cut-off is challenged by the results of Gupta et al. and Lowry et al., reviewed above.

- Noncompliance may be suspected in patients with very low 6-TG levels.

- 6-TG levels higher than 400 are associated with an increased risk of myelosuppression; dose reduction may be suggested.

- Excessive TPMT activity may be suspected when 6-TG levels are low, while 6-MMP levels are elevated. This profile may provide an explanation for drug resistance.

Improvement in Health Outcomes:

The use of pharmacogenomics and azathioprine metabolite testing create the possibility of tailoring a drug regimen for each individual patient, with the ultimate goal of attaining disease remission and elimination of steroid therapy. However, none of the applications listed above have been tested in a prospective trial to determine whether or not their use results in improved health outcomes compared to drug therapy based on clinical assessment.

2002 Update

A review of the literature based on the MEDLINE database was performed for the period of 2000 through May 2002. No prospective studies were identified that focused on the use of pharmacogenomic testing or metabolite testing in the actual management of the patient. However, numerous cross-sectional studies (8-12) in different populations of patients continue to support the scientific basis of both tests, and to support their theoretical use in the management of the patient.

PRICING:

There are no specific CPT codes for genotypic analysis of the TPMT.
gene or metabolite markers of azathioprine and 6-mercaptopurine. However, these laboratory tests are commercially available at only 1 reference laboratory, Prometheus.

REFERENCES:

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DISCLAIMER:

State and federal law, as well as contract language, including
definitions and specific inclusions/exclusions, takes precedence over
Medical Policy and must be considered first in determining coverage.
The member’s contract benefits in effect on the date that services are
rendered must be used. Any benefits are subject to the payment of
premiums for the date on which services are rendered. Medical
technology is constantly evolving, and we reserve the right to review
and update Medical Policy periodically.
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information regarding HMO claims/reimbursement information and other
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