CME

Role of Thiopurine Metabolite Testing and Thiopurine Methyltransferase Determination in Pediatric IBD

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ABSTRACT

Thiopurines have been used in inflammatory bowel disease (IBD) for >30 years, and measurements of both thiopurine methyltransferase (TPMT) and thiopurine (TP) metabolites, 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP), have been readily available. The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) Committee on Inflammatory Bowel Disease thought it appropriate to review the present indications for use of TPMT and TP metabolite testing. Substantial evidence demonstrates that TP therapy is useful for both Crohn disease and ulcerative colitis. Review of the existing data yielded the following recommendations. TPMT testing is recommended before initiation of TPs to identify individuals who are homozygote recessive or have extremely low TPMT activity, with the latter having more reliability than the former. Individuals who are homozygous recessive or have extremely low TPMT activity should avoid the use of TPs because of concerns for significant leukopenia. TMPT testing does not predict all cases of leukopenia and has no value to predict hypersensitivity adverse effects such as pancreatitis. Any potential value to reduce the risk of malignancy has not been studied. All individuals taking TPs should have routine monitoring with complete blood cell count and white blood cell count differential to evaluate for leukopenia regardless of TPMT testing results. Metabolite testing can be used to determine adherence with TP therapy. Metabolite testing can be used to guide dose increases or modifications in patients with active disease. Consideration would include either increasing the dose, changing therapy or for those with elevated transaminases or an elevated 6-MMP, using adjunctive allopurinol to help raise 6-thioguanine metabolites and suppress formation of 6-MMP. Routine and repetitive metabolite testing has little or no role in patients who are doing well and taking an acceptable dose of a TP.

Key Words: azathioprine, Crohn disease, inflammatory bowel disease, 6-mercaptopurine, 6-methylmercaptopurine, 6-thioguanine nucleotides, thiopurine, thiopurine methyltransferase, ulcerative colitis

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Thiopurines (TPs) have been used in inflammatory bowel disease (IBD) for >30 years (1). Concerns regarding TP toxicity with regard to the treatment of leukemia emerged approximately 25 years ago prompting the measurements of both thiopurine methyltransferase (TPMT) and TP metabolites, 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP) (2). Measurement of TPMT and TP metabolites in patients with IBD was not investigated until 15 years ago (3), and it has only been roughly 10 years that they have become part of clinical care. With the ever-evolving therapeutic recommendations for IBD (4), the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) Committee on Inflammatory Bowel Disease thought it appropriate to review the present indications for use of TPMT and TP metabolite testing.

SUPPORT OF THE EFFICACY OF THIOPURINES IN IBD

Substantial evidence demonstrates that TP therapy is useful for both Crohn disease (CD) and ulcerative colitis (UC), although few controlled studies have been performed. Many of the existing studies have methodologic issues, including inadequate dosing and insufficient length of treatment. Despite these shortcomings, Cochrane reviews have concluded that azathioprine (AZA) and 6-mercaptopurine (6-MP) are effective in inducing remission in active CD (5) and in maintaining remission afterward (6). In active CD, the overall response rate has been reported as 113 of 209 (54%; 95% CI [confidence interval] 47–61) for treatment compared with 72 of 216 (33%; 95% CI 27–40) for placebo. Subanalysis is unable to draw a conclusion with respect to length of therapy required to achieve a meaningful effect; however, 17 weeks has been suggested as a minimum period for an adequate trial. In the review of maintenance therapy for CD (6), the overall remission rate is reported as 147 of 208 (71%; 95% CI 64–77) for AZA and 24 of 47 (51%; 95% CI 36–66) for 6-MP compared with 141 of 255 (55%; 95% CI 49–61) for placebo, showing significant benefit from AZA but not from 6-MP compared with placebo. Successful maintenance of remission did correlated with higher doses of TPs (6). In the only randomized placebo controlled trial in pediatrics (7), 55 children with CD were randomized to 6-MP ($1.5\ mg \cdot kg^{-1} \cdot day^{-1}$) or placebo after induction with prednisone. At 18 months, only 9% of the 6-MP group relapsed clinically compared with 47% of the controls.

Little data are available on the use of AZA/6-MP for maintenance of remission in UC. A Cochrane analysis (8) deemed the methodologic quality of 4 of the 6 studies unsatisfactory, and all of the studies are small (maximum number of 80 patients) (6). Nevertheless, in the pooled analysis, AZA has been superior to placebo for maintenance of remission in UC (OR [odds ratio] 0.41; 95% CI 0.24–0.70). The reviewers concluded that AZA may be effective treatment for patients with UC who have failed or cannot

tolerate maintenance therapy with mesalamine or who require repeated doses of corticosteroids to induce remission.

Generally, it is thought that the TPs have a steroid-sparing effect, and the Cochrane (6) review identified 5 studies reporting data on reduction of steroid consumption (9–12). Among them, 76 of 117 (65%; 95% CI 56–74) patients with active disease who received AZA/6-MP reduced their steroid consumption, compared with 39 of 109 (35%; 95% CI 27–45) of those receiving placebo, indicating a significant steroid-sparing effect.

POTENTIAL ADVERSE EFFECTS OF THIOPURINES

The potential adverse effects of TPs need to be discussed, as patients with IBD are subject to various complications, and immunosuppressive medications add their own associated risks (13,14). Common adverse effects of TPs include myelosuppression, pancreatitis, and elevated transaminases. The 2 potential adverse effects that seem to gather the most attention are malignancy and susceptibility to infection (15). Two similar studies each with approximately 400 patients with IBD treated for 18 years or more (16,17), found that 7.4% to 14% of patients had an infectious complication, none of which were fatal. Cytomegalovirus (CMV) infection can be responsible for colitis in patients with IBD whose disease appears to be refractory to medical therapy. One study (17) suggested that TPs do not increase risk of this infection by reporting that <1% of a large group of patients with IBD (3/410) treated with 6-MP had CMV, and 2 of these 3 patients were also receiving cyclosporine. In contrast a smaller study concluded that the incidence rate of CMV in patients with IBD was higher in those treated with AZA and/or corticosteroids (18). The incidence of Epstein-Barr virus (EBV) in patients receiving TPs is not known. EBV infections can be more severe if not recognized early, and the risk of hemophagocytic lymphohistiocytosis is increased with the use of TPs (19).

Lymphoma is one of the most significant concerns in the use of any immunosuppressive therapy. It would be extremely appealing to think that TP metabolite testing could predict who is susceptible, but evidence to support this is lacking. Although beyond the scope of this review, the risk of lymphoma could preclude the use of TPs. Studies vary, but the incident risk of non-Hodgkin lymphoma is probably increased up to 4-fold (20). It is difficult to determine whether TPs, severe chronic inflammation, or a combination of the 2 is the cause of the observed increased risk of lymphoma. A 30-year retrospective, single-center study in Boston reported the risk of lymphoma in children to be roughly 4.5 cases per 10,000 patient-years, comparable to adults (21). The risk of the much rarer hepatosplenic T-cell lymphoma is more prominent in younger males (22), although children and younger adults do not appear to be at any greater risk for non-Hodgkin lymphoma than older adults (21). Although hard to determine, the overall risk-benefit of these agents has been shown to favor their usage in a theoretical model (23).

RATIONALE FOR TPMT TESTING

The rationale for TPMT testing before TP therapy is to minimize adverse effects related to TP therapy and to maximize clinical response. TPMT can be measured either by the enzyme activity in circulating red blood cells (RBCs; phenotype) or through genetic analysis for polymorphisms in leukocyte DNA (genotype). Despite some initial controversy about the utility of TPMT testing, it is recommended by the Food and Drug Administration (package insert Prometheus 2005) and supported by some cost-effective analyses (24,25). It is included in published clinical practice guidelines (15), and based on this it will be discussed before the topic of TP metabolite levels. Individuals may metabolize drugs differently based on genetically determined enzymatic activities as well as multiple drug interactions. Individuals with TPMT deficiency who receive standard TP dosing are at significant risk for toxicity, especially bone marrow suppression. In this setting, avoiding TP entirely or using extremely low dosing with close monitoring for potential toxic adverse effects should be considered. TPMT testing may also allow the use of higher therapeutic doses in patients with normal or high enzyme activity to avoid suboptimal treatment and delayed disease remission.

TP metabolism can be evaluated by measuring TPMT activity in circulating RBCs (phenotype), which has been shown to correlate with hepatic TPMT activity, or through genetic analysis for polymorphisms in leukocyte DNA (genotype). The genotype is then used to predict enzyme activity. The TPMT gene is located on chromosome 6p22. The allele for normal activity (wild type) has been designated TPMT∗1. More than 20 different variant TPMT alleles have been described so far, 16 of which have been shown to result in deficient TPMT activity (designated: TPMT∗2, ∗3A, ∗3B, ∗3C, ∗3D, ∗4, ∗5, ∗6, ∗11–16, ∗21, and ∗25). Other genetic polymorphisms also exist that are suspected of causing a deficiency of enzyme activity (26–35). Ethnicity is also an important factor in genotypic variations (29).

On the basis of TPMT enzyme activity, patients can be classified as having normal/high activity (with 2 functional alleles of the active gene), intermediate activity (heterozygous, with 1 variant allele), and no/low activity (homozygous, with 2 variant alleles). These 3 distinct phenotypes are thought to occur at 89%, 11%, and 0.3% in the population, respectively (36). More recent studies suggest that the incidence of TPMT deficiency may be higher than previously reported. In a prospective study of the TPMT activity of 1000 individuals, 86.1% was found to have normal activity, 13.3% had low activity, and 0.6% was deficient (37). In a retrospective study reviewing TPMT activity in 3291 individuals, approximately 80% had normal activity, 9% had above-normal activity level, 10% had low activity, and 0.45% was deficient (38).

Advantage of TPMT Testing

The main advantages of testing for TMPT are preventing pancytopenia, specifically avoiding leukopenia, and being able to use aggressive dosing. Leukopenia is the most common and most serious toxic effect on the bone marrow during TP therapy. This can be unpredictable, may lead to sepsis, and could even be fatal. In a meta-analysis of 7 studies, including 2223 patients with IBD treated with TPs, leukopenia (defined differently between studies) occurred in 71 (3.2%) with a total of 2 deaths (0.09%) reported (24). This meta-analysis included 1 study of 396 adult patients with IBD (2), 8 (2%) of which developed severe leukopenia (defined as white blood cell [WBC] count $<2.5 \times 10^9$/L). Seven of these patients developed fever, with 3 having positive bacterial blood cultures and 1 diagnosed as having CMV. One patient had associated erythrocyte aplasia, and another had hemolysis along with leukopenia. None had isolated thrombocytopenia. All of the hematologic abnormalities resolved with drug withdrawal with no mortality. In another large retrospective study of 739 patients (39) included in the same meta-analysis, 37 (5%) individuals showed leukopenia (defined as WBC count $< 3.0 \times 10^9$/L) and/or thrombocytopenia (defined as platelet count $<100{,}000 \times 10^6$/L), requiring drug withdrawal or dose reduction. Thirty-two patients remained asymptomatic. Leukopenia occurred in 28 (3.8%) patients, with 9 having severe leukopenia (defined as WBC count $<2.0 \times 10^9$/L). Of these 9 leukopenic patients, 3 were also pancytopenic, including 2 (0.3%) who died from sepsis.

Documenting that a given patient has adequate TPMT activity allows for aggressive dosing and having relatively high but safe levels of the metabolites may be beneficial. It has been reported that patients with below-average TPMT activity have a more favorable response to AZA (40). Those with higher TPMT activity may be less likely to respond and may benefit from more aggressive dosing; however, those with high TPMT enzyme activity shunt TP metabolism from 6-TGN toward 6-6-methylmercaptopurine riboside (MMPR), a metabolite may be associated with hepatotoxicity (41). This has not been tested in a prospective, randomized fashion. Patients with deficient TPMT activity are typically offered other therapies; however, a few reports have supported cautious use of extremely low AZA dosing. One study describing 3 adult patients with IBD with genotypic homozygotic deficiency demonstrated safety in treatment with AZA doses of 0.25 to 0.29 $mg \cdot kg^{-1} \cdot day^{-1}$ (42).

Potential Disadvantages of TPMT Testing

The potential disadvantages of TPMT test are subjective but should be discussed. These include the cost of the test and the potential for overinterpretation of the results. The cost of TPMT enzyme testing is billed at roughly $400 per test at the most commonly used commercial laboratory. The cost of genotype testing, which is available at many laboratories, is also about the same. Universal testing has been recommended on all newly diagnosed patients, would result in an annual cost of $30 million in the United States, assuming an incidence of 25 cases per 100,000. The value of this as health policy can be discussed elsewhere but is dependent on the perceived benefits.

Bone marrow toxicity leading to leukopenia is usually an early event and may occur in 3% of patients treated with TPs. The majority of cases of myelosuppression cannot be attributed to deficiency of TPMT enzyme activity. A prospective study of 130 patients screening with both TPMT activity and genotype, although neither dose or TP metabolite level was stated. Adverse effects during AZA therapy were seen in 44 (34%) with 4 (3%) developing severe myelosuppression. Other adverse effects necessitating treatment discontinuation included gastrointestinal upset, hepatotoxicity, flu-like symptoms, rash, and pancreatitis. Seventeen of the patients had intermediate or low enzyme activity, and only 11 had the matching heterozygote genotype yielding 65% sensitivity for the genotype alone. No association was found between intermediate TPMT deficiency and any adverse effect (24). A retrospective study assessed TPMT genotype in 41 patients with CD who developed myelosuppression while receiving AZA therapy. Only 27% of patients had mutant alleles associated with enzyme deficiency (43). The high degree of variability in TPMT activity among different ethnic groups, as mentioned above, plays an important role. Some patients with a heterozygous genotype demonstrate high TPMT activity, whereas some homozygous wild-type subjects show intermediate activity (44). Other unrelated factors may also cause myelosuppression, including infections or concomitant use of other drugs such as allopurinol, metronidazole, captopril, trimethoprim-sulfamethoxazole, and mesalamine (44,45). These data suggest that continued monitoring of blood cell counts remains mandatory while receiving treatment with AZA.

The availability of TPMT genotyping and enzyme activity analysis may give families a false sense of security because the majority of instances of leukopenia are independent of TPMT enzyme activity and are thus far unpredictable. Also, TPMT genotype or enzyme activity does not correlate with development of hepatotoxicity or pancreatitis. TPMT enzyme activity does not predict allergic or non–dose-dependent adverse events (16). TPMT testing may delay the initiating the drug. Clinicians must weigh the risk of starting TP without knowing the TPMT genotype or phenotype status against the potential benefit of using the drug, especially because the therapeutic effect of AZA requires many weeks to become evident (46).

The cost-related benefit of TMPT testing may be offset by increased treatment effectiveness, higher quality of life, and reduced adverse effects. By reducing AZA dose administered to heterozygotes and entirely avoiding TPs in homozygote-deficient individuals to limit leukopenia and other complications, some experts have suggested routine TMPT screening before AZA therapy. A study assessing different treatment models suggested that TPMT activity testing alone helped achieve comparative treatment effectiveness at lower cost at 1 year, compared with only TP metabolite monitoring, combined TPMT and TP metabolite monitoring, and community care. Several of the assumptions in this model were questionable, possibly underestimating treatment efficacy and overestimating the cost of complications (25). Another study evaluating the cost-effectiveness of TPMT genotype testing in patients with IBD accounted for the direct costs associated with morbidity (leukopenia, infection-related complications) and mortality (life-year saved). The use of screening genotype testing was found to be favorable and was comparable to other health care technologies such as use of statins for secondary prevention in coronary heart disease. This study did not evaluate the impact of genotype testing on treatment efficacy (24). Model assumptions included reduced mortality related to leukopenia in TPMT-deficient patients, but this remains unproven. The potential advantages of screening for TPMT deficiency may also be inflated as some patients may be identified by careful hematologic monitoring without prior knowledge of TPMT status, possibly without an alteration in prognosis.

Use of Genotype Versus Phenotype

Positive aspects of phenotype testing include the low long-term and circadian intraindividual variability in RBC TPMT activity, at least as documented in healthy subjects (47). Compared with genotype testing, phenotype evaluation can provide better determination of the present metabolic status; however, this can be altered by several factors through drug interactions, recent blood transfusions, and uremia. Sulfasalazine and 5-aminosalicylic acid have been shown to inhibit TPMT enzyme activity in in vitro studies (48,49). Genotype testing, unlike phenotyping, is not affected by drug interactions or recent blood transfusions. Several alleles are associated with low levels of TPMT activity (29,43); however, an appreciable subset of patients with low activity are not accounted for by these known alleles as seen in the previously mentioned study by Colombel et al (43). Subsequently, a normal genotype cannot exclude the possibility of TPMT deficiency and development of myelosuppression in all patients, as other rare or yet unknown mutations may exist. The cost of genotype testing and enzyme testing are extremely comparable but using both has not become standard. In view of the fact that some genotypes may be missed and that the enzyme testing has more functionality, although with some potential to be affected by medication and transfusion, the use of the phenotypic test is preferable, although the genotypic test can be an alternative at times.

Use of Thiopurine Metabolites

Metabolism of Thiopurines

The TPs, AZA and 6-mercaptopurine (6-MP), are considered prodrugs. The AZA molecule is composed of 2 portions, mercaptopurine and an imidazole derivative (50). After ingestion and absorption, the prodrug AZA undergoes approximately 90% conversion to 6-MP through nonenzymatic attack by sulfhydryl-containing compounds such as glutathione or cysteine. 6-MP can be

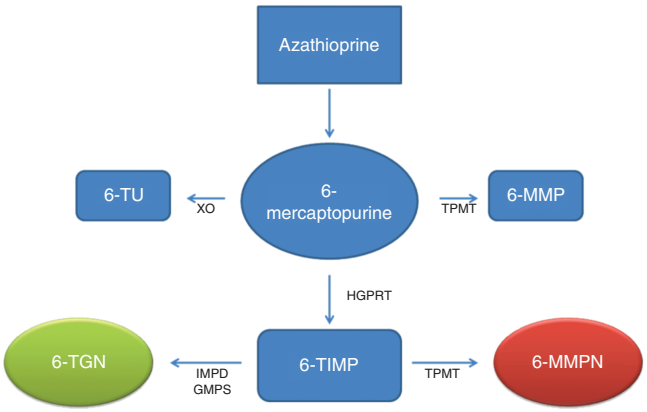

FIGURE 1. Schematic diagram of thiopurine metabolism. GMPS = guanosine 5′-monophosphate synthetase; HGPRT = hypoxanthine phosphoribosyltransferase; IMPD = inosine 5′-monophosphate dehydrogenase; 6-MMP = 6-methylmercaptopurine; 6-MMPN = 6-methyl-mercaptopurine nucleotides; 6-TGN = 6-thioguanine nucleotide; 6-TIMP = 6-thioinosine-5′-monophosphate; TPMT = thiopurine methyltransferase; 6-TU = 6-thiouracil; XO = xanthine oxidase.

considered a prodrug also, because it undergoes further complex metabolism via 3 main metabolic pathways: phosphorylation to 6-TGNs with inosine monophosphate dehydrogenase, which is presumed to be a rate-limiting enzyme; methylation by the polymorphic enzyme, TPMT; and catabolism to thiouric acid by xanthine oxidase (Fig. 1).

The precise mechanism of action of the TPs is not clear. The main focus of TP metabolism has centered on 6-TGN which, through incorporation into the DNA of lymphocytes, produces the cytotoxic actions (50), although other metabolites may have roles. 6-TGN results from the phosphorylation process: 6-MP forms the intermediary metabolite thioinosine monophosphate and then via inosine monophosphate dehydrogenase produces 6-TGN. Some of this particular intermediary metabolite, thioinosine monophosphate, gets transformed by the same TPMT, leading to the production of *S*-methyl-thioinosine monophosphate, a strong inhibitor of purine de novo synthesis (PDNS) (51). The inhibition of PDNS is thought to be the mechanism of immunosuppression, which blocks proliferation of various types of lymphocyte lines. TPs increase apoptosis of activated T-lymphocytes. 6-TGN accumulates within lymphocytes and in the presence of T-cell activation, as occurs in immunologically driven diseases; 6-TGN blocks the expression of TRAIL, TNFRS7, and α4-integrin, all effects that functionally decrease inflammation (52).

Genetic polymorphisms have been identified in the gene regulating inosine triphosphate pyrophosphatase (ITPase) enzyme. This enzyme phosphorylates the intermediary metabolite TIMP to 6-thioinosine triphosphate, which may compete with or have similar proapoptotic properties as 6-GTP (53). As stated above, 6-MP is activated through a 6-thio-IMP intermediate, and, in ITPase-deficient patients, potentially toxic 6-thio-ITP is predicted to accumulate (54). These other polymorphisms may account for non–TPMT-related adverse effects from AZA/6-MP therapy such as influenza-like symptoms, rash, and pancreatitis.

Availability of Thiopurine Metabolite Testing

TP metabolites are measured in most laboratories by determining their concentration in extracts of erythrocytes. High-performance liquid chromatography (HPLC) is used for the measurements, with some methodologic differences between laboratories in the extraction procedure. TGN and the methylated thioinosine derivatives are not measured directly. Rather, the 2 compounds actually measured are 6-thioguanine (TG) for TGN and 4-amino-5-(methylthio)carbonyl imidazole (AMTCI) for methylated thioinosine derivatives (55). 6-TG levels actually combine 6-TG, 6-thioguanosine (TGR), and TGN, and AMTCI levels include 6-MMP, MMPR, and 6-methylthioinosine nucleotides (meTIN). For both 6-TG and AMTCI, the measured levels include both nucleotides and deoxynucleotides. These deoxynucleotides are present at low concentrations, but there is the possibility that these minor deoxynucleotides may indeed play a larger role in the efficacy and adverse effects of the medication.

When specific methodology is used to obtain accurate determinations of thioguanosine monophosphate (TGMP), thioguanosine diphosphate (TGDP), and thioguanosine triphosphate (TGTP), and methylthioinosine monophosphate (meTIMP) are done, the correlation between these phosphorylated forms and conventional 6-TGN values was poor. The concentration of meTIMP obtained by the routine HPLC method compared with the specific method also was not optimal, suggesting that other unmeasured metabolites may exert some of the therapeutic benefits as well as the adverse effects (56).

Several authors have highlighted significant problems with the methodology of present TP metabolite determinations and the reproducibility of the assays. Great variations occur depending on how specimens are shipped and whether they are refrigerated. For example, median 6-TGN concentrations on day 7 decreased significantly to 53% of baseline when kept at ambient temperature, compared with a smaller decrease to 90% of baseline when refrigerated. A similar pattern was observed for 6-MMP measurements (57). Different acids used to hydrolyze the nucleotides before HPLC will cause variation in the concentrations of the metabolites, and many are of the opinion that specific metabolites and nucleotides should be determined individually. There is also the consideration that the concentration of the drug within the erythrocyte may not fully correlate with the more important concentrations within mononuclear cells (58). It is not certain that the measure concentration of the metabolites in erythrocytes correlate with the concentration in mononuclear cells.

Devising Target Levels

Before the advent of TP metabolite testing, the use of TPs was monitored only with routine complete blood counts (CBCs) and aminotransferase measurements. Understanding that high levels of 6-TGN could potentiate leukopenia and lymphopenia, and that high levels of 6-MMPN could result in abnormal elevations in aminotransferases, specifically alanine transaminase and aspartate aminotransferase, led to the generally accepted practice of monitoring these indices; however, no direct relationship has been established to predict an individual patient's response to a specific dose of TP.

The advent of TP metabolite testing commercially now provides the ability to monitor 6-TGN levels in relation to AZA or 6-MP dosing. This testing is presently proprietary intellectual property of one particular laboratory, and although widely available, it is performed by a single laboratory. The turnaround time is manageable, and a relatively small amount of blood is required (5 mL of whole blood) with a moderate fee, of $270.

TP metabolite testing performed by HPLC yields 2 results: 6-TGN and 6-MMPN levels. The results are reported in pmol/RBC and offer a reference range and comment on how this may be interpreted by the clinician. The reported reference range for 6-TGN is 230 to 400 pmol/RBC, and a value of 230 to 400 is

identified as a higher likelihood of response. A value <230 is reported as lower likelihood of response, and a value above 400 is associated with a higher risk for leukopenia. The reported reference range for 6-MMPN is <5700, and a level >5700 is thought to be associated with a higher chance of hepatotoxicity. As will be seen below, no direct one-to-one correlation has been established, and even the official report eschews the practice that metabolite testing could serve as a replacement for routine laboratory measures.

The target levels as described on the metabolite report were based on several studies that reported on the frequency of remission at given metabolite levels (3,59–61). The above accepted reference ranges were based on the study by Dubinsky et al (60) evaluating a pediatric population of 92 patients. This was a prospective trial in which patients received 1.25 mg/kg of 6-MP. Responders had an average 6-TGN level of 312 compared with nonresponders having an average 6-TGN level of 199, with poor correlation between dose and 6-TGN levels. By comparison, a study by Cuffari et al (59) evaluated 82 adult patients in which treatment efficacy correlated with 6-TGN levels >250 in patients with colonic and fistulizing CD but not in patients with ileocolonic disease. This study demonstrated the utility of metabolites levels as a tool to monitor and maximize therapy. Twenty-two patients who were not responding to initial therapy were given increased doses while monitoring metabolite levels. The average 6-TGN levels increased from 194 to 330 with no evidence of leukopenia, and 18 of these 22 patients responded to the dose escalation (59,62). Roblin et al (63) evaluated 106 patients and found that patients with a 6-TGN level >250 were more likely to be in remission, with an OR of 11.2. No patient with a level below 250 was in remission in this study. Metabolite testing might be particularly useful in young children with IBD wherein typically higher doses need to be used to maintain therapeutic effect and actual metabolite levels (64).

In contrast to these studies, several studies have found no correlation between specific 6-TGN levels and disease remission. Lowry et al (65) evaluated 170 patients in a prospective adult study and found no correlation of 6-TGN levels and disease activity defined by Inflammatory Bowel Disease Questionnaire score, although the authors omitted the more conventional Crohn's Disease Activity Index. Belaiche et al (66) reported that in 28 patients with TP metabolite levels available, there was no significant difference in mean 6-TGN levels between patients with active and quiescent disease. Gupta et al (67) evaluated metabolite levels in a prospective pediatric population. The results were not able to correlate 6-TGN levels >235 with clinical remission; in fact, 58% of patients with 6-TGN levels <235 were in clinical remission. Although no correlation was found between 6-TGN target levels and clinical response, the proposed recommendation was to use serial measurements coordinated with increased dosing as being a good strategy to optimize 6-MP therapy. In a recent study, Haines et al (68) described a retrospective adult cohort in which 6-MP metabolite levels showed great variability between patients and correlated weakly to actual dosage. In this series, 29% of patients were underdosed based on metabolite testing, using the proposed ranges, and another 11% were noncompliant with therapy. In the retrospective group, 87% of patients had improved clinical status after escalation of dosing based on metabolite testing.

The above series and additional ones were summarized in a large meta-analysis on metabolite testing by Osterman et al (69), which included the studies up to 2006 outlined above. A total of 12 studies were evaluated. Seven studies showed no correlation between drug dose and 6-TGN levels, as supported by all of the studies we reviewed. Eight studies showed a significant difference in the dose of 6-MP in patients with active disease versus those in remission. 6-TGN levels >230 to 260 were more significantly associated with disease remission. Although 6-MP metabolite levels were only 62% sensitive and 72% specific for clinical response, they do allow for dose optimization and identifying noncompliance as well as identifying nonresponders, therefore decreasing exposure to a medication dose that is ineffective. Dubinsky et al (25) support the assertion that metabolite testing is beneficial for maintaining a sustained response to 6-MP by optimizing dosage, monitoring noncompliance and early identification of nonresponders.

In a recent study by Waljee et al (70), the investigators created an algorithm using the intricacies of routine laboratory findings and compared it against the ability of metabolite testing to correlate to clinical response. The algorithm differentiated responders from nonresponders with greater accuracy than 6-TGN levels or mean cell volume/WBC ratios. In the algorithm, the most important independent variables in differentiating clinical responders from nonresponders were neutrophil count, alkaline phosphatase, red cell distribution width, age, and WBC count. These findings emphasize the potential pharmacodynamic variation among patients that lead to varying responses to 6-MP therapy.

In terms of 6-TGN metabolite level reproducibility, Cuffari et al (3,71) has shown in 2 separate studies that 6-TGN levels were reproducible with <10% variability for patients on stable dosing over a period of 2 to 24 months. It is important to note that for this study, the 6-TGN levels were done with aliquots that were immediately frozen and handled specifically.

Other drugs, most notably aminosalicylates and allopurinol, will affect TP metabolite levels and might be a reason for determination. 5-Aminosalicylates (5-ASA) are commonly used as induction and maintenance therapy of mild UC and CD because of their anti-inflammatory properties (72). The addition of 5-ASA to TP therapy results in increased 6-TGN levels (73–77) in a dose-dependent manner (75). This may be the result of aminosalicylate-induced inhibition of TPMT (48,73–76,78,79,80). Regardless of the mechanism, the consequence of increased 6-TGN levels may include adverse events such as leukopenia (48,74,75). Andrews et al (81) showed that 5-ASA use increased 6-TGN levels in patients receiving both medications. Additionally, withdrawal of 5-ASA while receiving stable 6-MP dosing led to a decrease in measured 6-TGN levels. No specific dose adjustment was indicated; however, careful observation is important for leukopenia in a patient on both 6-MP and 5-ASA, or conversely, for disease relapse in a patient who is withdrawing 5-ASA from a stable therapeutic regimen involving 6-MP.

Allopurinol is a xanthine oxidase inhibitor, which may be used as an adjunct to TP therapy in patients who have suboptimal 6-TGN levels, high 6-MMP levels, or elevated transaminases. The addition of allopurinol, even with a reduction in TP dose, typically results in an increase in 6-TGN and decrease in 6-MMP levels (45,82–87) as well as an improvement in transaminases (45,84,86–88). Studies demonstrate that patients also clinically improve with the addition of allopurinol to TP therapy by requiring lower, or no further, doses of corticosteroids (45,82–85,87,89) and having a reduction in activity index scores (82,85). Close monitoring of the WBC count is needed when allopurinol is used to potentiate the use of a TP as well as consideration of the obtaining 6-TGN levels so as to avoid the reversible neutropenia that may occur (45,83,86,87,89).

Less frequently discussed is the role of 6-MMP levels in predicting hepatotoxicity. In a series of 173 adult patients on TPs, 8 patients (4.6%) had elevated transaminases with an average 6-MMP level of 10,537 compared a 6-MMP level of 3452 in the patients with normal transaminases; however, of patients with elevated 6-MMP >5700, 90% had normal values (90). In a smaller series of patients, 7 of 44 with abnormal transaminases had 6-MMP levels of 7836 ± 4589, an obviously wide range, suggesting great

variability (91). No study has looked at the risk of eventual hepatotoxicity with normal transaminases and an elevated 6-MMP (92). Somewhat reassuring are 3 cases of early liver failure associated with 6-MMP levels >26,000 that all resolved with discontinuation of the drug. High 6-MMP levels can correlate with hepatotoxicity; however, the >5700 cutoff without additional monitoring with aminotransferases would not be prudent. A recommended approach would correlate both aminotransferases and 6-MMP levels, especially in dose escalation secondary to poor clinical response or low 6-TG levels.

Disadvantages of Metabolite Testing

The disadvantages of TP metabolite testing should be considered, even though these tests have become extremely well established in clinical practice. Two major potential disadvantages of testing TP metabolites fall into 2 broad categories: the first is cost-versus-benefit of the test, and the second involves mistakes made because of misinterpretation or overinterpretation.

In this era of dwindling resources and focus on system-based practice, it is hard to endorse the use of tests whose exact significance is uncertain. When metabolite levels are ordered multiple times on any one patient without a specific reason, they may add little to the management except to document adherence. As discussed, the precise target levels for 6-TGN are not clear, and other metabolites may be at play that are either equally or more important. Overordering of laboratory tests is actually quite common in all areas of clinical medicine, and probably applies to at least 30% of all laboratory tests (93).

Expenses aside, probably the most detrimental outcome in the use of TP levels is the misconception by both patients and providers that these laboratories provide assurance that optimal dosing is being used to prevent complications of therapy. Despite the assumption that measuring levels will prevent adverse effects, clearly most of the adverse effects from TPs are not directly related to 6-TGN or 6-MMP levels. Clinical experience has demonstrated that infections associated with TP use do not regularly correlate with leukopenia, and elevated transaminases can occur despite normal 6-MMP levels.

It is also presently uncertain that attaining certain levels of 6-TGN will be sufficient to treat a particular patient. Clearly, the target ranges of metabolite levels are only guidelines, which likely apply to many patients, but not all patients. If a patient is not responding, consideration should be given to increasing the dose, provided there is no evidence of toxicity.

CONSENSUS RECOMMENDATIONS

Although this present review cannot draw precise conclusions, the review of the literature has generated the following recommendations:

1. TPMT testing is recommended before initiation of TPs to identify individuals who are homozygote recessive or have extremely low TPMT activity, with the latter having more reliability than the former. (HIGH).
2. Individuals who are homozygous recessive or have extremely low TPMT activity should avoid use of TPs because of concerns for significant leukopenia. (HIGH)
3. TMPT testing does not predict all cases of leukopenia and has no value to predict hypersensitivity adverse effects such as pancreatitis. Any potential value to reduce the risk of malignancy has not been studied. All individuals on TPs should have routine monitoring with CBC and WBC count differential to evaluate for leukopenia regardless of TPMT testing results. (HIGH)
4. Metabolite testing can be used to determine adherence to TP therapy. (HIGH)
5. Metabolite testing can be used to guide dose increases or modifications in patients with active disease. Consideration would include either increasing the dose, changing therapy or for those with elevated transaminases or an elevated 6-MMP, using adjunctive allopurinol to help raise 6-TG metabolites and suppress formation of 6-MMP. (MODERATE)
6. Routine and repetitive metabolite testing has little or no role in patients who are doing well and taking an acceptable dose of a TP. (MODERATE)

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