Usefulness of thiopurine methyltransferase and thiopurine metabolite analysis in clinical practice in patients with inflammatory bowel diseases

O. Dewit

St. Luc University Hospital, Department of Gastroenterology, Brussels, Belgium.

Abstract

Thiopurines (TP) are widely used in the management of inflammatory bowel diseases. Side-effects and inefficacy are a major concern as they lead to withdrawal of the drug. Tools investigating TP metabolism are useful to avoid inadequate cessation of TP therapy. TP metabolism is complex and many enzymes are involved. Among them, Thiopurine methyltransferase (TPMT) is the only one routinely measured by pheno- or genotyping. In this review, the rationale for TPMT and thiopurine metabolites, 6-thioguanine nucleotides and 6-methylmercaptopurine, determination in clinical practice is discussed, specifically in case of thiopurine failure and recommendations are given about their interpretation and potential dose optimization of TP drugs. (Acta gastroenterolog. belg., **2010**, 73, **331-335**).

Key words : azathioprine, 6-thioguanine nucleotides (6-TGN), 6-methylmercaptopurine (6-MMP), thiopurine metabolism, thiopurine side effects

Introduction

Over the last decades, the use of Thiopurine drugs (TP) has considerably increased in inflammatory bowel disease (IBD) and nowadays more than half of the IBD patients are treated with azathioprine (AZA) or 6-mercaptopurine (MP) (1). Nevertheless, many questions remain concerning their mode of action, metabolism, efficacy, side effects or practical use. Side effects are found in 9 to 34 % of cases (2) ; including allergic reactions, bone marrow suppression, pancreatitis, hepatitis, nodular regenerative hyperplasia, gastrointestinal symptoms, infections and malignancies. As a consequence, TP drugs have to be reduced or even discontinued in up to 1/3 of patients. Given the lifelong course of IBD, it seems particularly important to optimize each therapeutic agent before admitting treatment failure and introducing an alternate therapy.

Determination of Thiopurine Methyltransferase (TPMT) activity

TPMT is the most studied enzyme of TP metabolism and the only one usually tested for in routine clinic. Thiopurine metabolism is complex and depicted in figure 1. TPMT status can be checked for based on phenotype or genotype tests. There is ongoing controversy as to whether TPMT analysis should be performed before TP initiation and whether one should opt for phenotyping or genotyping in order to assess TMPT activity.

TMPT phenotyping

TPMT phenotype reflects the enzyme activity which can be measured in vitro by the conversion of MP to 6-methyl-mercaptopurine (6-MMP). Dosage of TPMT activity is difficult with considerable inter and intra-individual variability. Various assays are available using different units complicating interpretation of results in daily practice. One of the advantages of phenotyping is to determine more precisely the TPMT activity which can vary among individuals with the same genotype. Moreover, it allows detection of patients with a very high methylation activity. On the other hand, phenotyping can be influenced by a possible drug interaction whereas genotyping cannot. Indeed, studies have described interaction between aminosalicylates (3-5), diuretics (thiazidic and furosemide) (6,7), NSAIDs (8), AZA and TPMT activity. So far, the mechanisms of these interactions remain unknown as does their potential clinical impact.

In particular, allopurinol presents a clinically important toxic drug-drug interaction with TP by inhibiting xanthine-oxydase activity leading to a shift of TP metabolism towards active but potentially toxic compounds. This has been shown in various studies with myelosuppression occurring within weeks of allopurinol addition (9, 10). As a consequence the concomitant use of allopurinol and AZA or MP is not recommended. If the association is mandatory, a dose reduction of TP treatment is recommended to 25-33% of the normal dose, as generally about 2/3 of thiopurine is normally inactivated by the xanthine oxidase/dehydrogenase (XOD) pathway (10). Some authors took advantage of this drug-drug interaction for it could avoid some thiopurine resistance (by increasing 6-TGN) or hepatotoxicity (by decreasing 6-MMP) (11,12).This association should only be considered in well selected cases and, if a co-therapy is decided, careful full blood and TP metabolites monitoring is required.

Corresponding author : Olivier Dewit, MD, Department of Gastroenterology, St. Luc University Hospital, Av. Hippocrate 10, 1200 Brussels, Belgium. Fax : +32-2-7648927. E-mail : Olivier.Dewit@uclouvain.be

Submission date : 12/08/10
Acceptance date : 14/08/10

TMPT genotyping

TPMT genotyping consists in detecting single nucleotide polymorphism responsible for TPMT inactivation. The human TPMT gene is located on chromosome 6 and contains 10 exons. To date, 27 alleles responsible for possible TPMT activity deficiency have been described : *2,*3A,*3B,*3C,*3D,*4 to *27 (13-15). A good correlation exists between TPMT activity and TPMT genotyping and varies from 76 to 99 % as described in different studies (16). Mutation *3A contains 2 single nucleotide polymorphisms that are also found separately in mutation *3B and *3C (exon 7 and exon 10, respectively). One explanation for the low activity is the modification of the TPMT protein structure by these mutations, which leads to protein instability and decreased enzymatic activity. This is reflected by TPMT half-life which decreases from 18 hours in the TPMT wild-type genotype to 15 minutes in genotypes *2 and *3A (17). TPMT *3A, *2 and *3C are the most frequent mutations found in the Caucasian population and account for 95 % of all mutations (18,19). In the African and Asian population, TPMT *3C is the most frequently encountered genotype (19).

Based on TPMT genetic polymorphism the general population can be divided in 3 groups : wild-type homozygous TPMT with high methylation activity (88%), heterozygous for a deficient TPMT allele with intermediate activity (11%) and homozygous for deficient TPMT allele with a low methylation activity (0.3%) (20).

Impact of TPMT activities on clinical management

TMPT deficiency

It is generally admitted that a TP dose adaptation is necessary in case of TPMT deficiency. If a patient has a low or intermediate TPMT activity and takes TP, the drug metabolism will be shifted towards an increased production of active compounds, responsible for the therapeutic effect of the drug but also for myelotoxicity. In case of intermediate TPMT activity, reduction to 33 to 50% of the usual dose, thus 1 to 1.25 mg/kg for AZA and 0.5 to 0.75 mg/kg for MP, is advocated (21) whereas in total TPMT deficiency (low methylator), it is usually recommended to avoid the use of TP, or unless its use is still necessary, to decrease dosage to 10% of the normal dose. Careful follow up and very frequent blood monitoring is mandatory in such patients (22).

Although a TPMT deficient genotype has the potential to predict leucopenia (18) and prospective studies have shown the usefulness of adapted TP dose regimens to avoid leucopenia (19,20), the strategy of determining TPMT status prior to the start of TP therapy (and to adapt eventually the dose) in order to minimize the risk of myelotoxicity is still controversial (23-25). The results of various pharmacoeconomic models favor testing of TMPT before the initiation of thiopurine treatment (26-29). In a recent study, the strategy of systematic determination of TPMT activity was cost-effective taking into account both the cost of TPMT determination and the cost due to leucopenia and associated infections (30). However, studies have shown that only 25 to 27% of myelosuppression events in IBD patients taking azathioprine could be explained by a TPMT deficiency related to known mutations in the TPMT genotype (31, 32) suggesting that all possible mutations have not been discovered yet and that other factors contribute to the development of leucopenia (33). In addition, the mutations that have been found were restricted to *3A,*3C and *2, implying that a test limited to those 3 mutations might be sufficient (34).

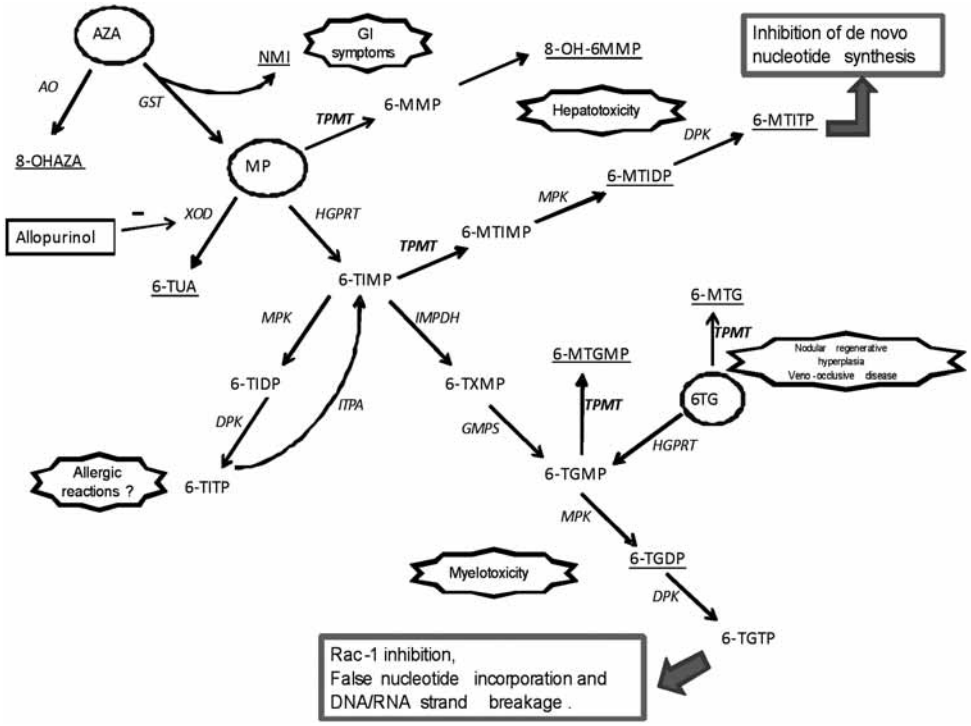
Ultra-high TMPT activities

Ultra-high enzyme activity status is found in 1 to 2% of the population. It is easily detected by a phenotypic test, and has recently been attributed to genetic modifications, i.e. a trinucleotide (GCC) polymorphism in the promoter gene of TPMT (35) referring to a modification in the number of GCC sequences (normally 6). Very high TPMT activity will shift the drug metabolism towards a more inactive compound such as 6-MMP, thereby lowering active 6-TGN levels and increasing the risk of clinical “drug resistance” as shown in several studies where clinical response rates were stratified according to baseline TMPT activity (36-38). Moreover, patients with an ultra-high TMPT activity will produce higher amounts of methylated derivates responsible for liver toxicity.

Table 1. — **Adaptation of doses in relation to TPMT activity**

TPMT activity	AZA recommended dose	Major active metabolite	Mechanism of action	Therapeutic 6-TGN levels	Potential myelotoxicity
Very high (> 26.1 U/ml)	3.0 mg/kg/day	methylated ribonucleotides	Antimetabolic	Low	Delayed
High (> 18.1-26 U/ml)	2.5 mg/kg/day	methylated ribonucleotides	Antimetabolic	Low	Delayed
Intermediate (13.8-18 U/ml)	1.5 mg/kg/day				
Low (5.1-13.7 U/ml)	0.5 mg/kg/day	6-Thioguanine nucleotides	Apoptotic	High	Acute
Very Low (< 5 U/ml)	0.125 mg/kg/day	6-Thioguanine nucleotides	Apoptotic	High	Acute

(adapted from Cara ; ref : 50)



AZA : azathioprine, MP : 6-mercaptopurine, 6TG : 6-thioguanine, 8-OHAZA :8-hydroxy-azathioprine, NMI : nitromethylimidazole, 6-TUA : 6-thiouric acid, 6-MMP : 6-methylmercaptopurine, 8-OH-6-MMP : 8-hydroxy-6methylmercaptopurine, 6-TIMP, 6-TIDP and 6-TITP : 6-thioinosine mono, di and triphosphate, 6-MTIMP,6MTIDP and 6-MTITP : 6-methylthionosine mono, di and triphosphate, 6-TXMP : 6-thioxanthosine monophosphate, 6-TGMP, 6-TGDP and 6-TGTP : 6-thioguanine mono, di and triphosphate, 6-MTGMP : 6-methylthioguanine monophosphate, 6-MTG : 6-methylthioguanine, AO : aldehyde oxydase, GST : gluthation-S-transferase, XOD :xanthine oxidase/dehydrogenase, TPMT : thiopurinemethyltransferase, HGPRT : hypoxanthine guanine phosphoribosyl transferase, MPK : monophosphate kinase, DPK : diphosphate kinase, ITPA : inosine triphosphate pyrophosphatase, IMPDH : inosine monophosphate dehydrogenase, GMPS : guanosine monophosphate synthetase.

Figure 1. — Simplified scheme of thiopurine metabolism

Measurements of thiopurine metabolites 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP)

AZA and MP are both inactive prodrugs that are metabolized via 3 main enzymatic pathways to produce the nucleotide metabolites 6-thioguanine (6-TGN), 6-methylmercaptopurine (6-MMP), and 6-thiouracil (6-TU) (Figure 1). Three thioguanine nucleotides are known (6-thioguanine monophosphate [6-TGMP], 6-thioguanine diphosphate [6-TGDP], and 6-thioguanine triphosphate [6-TGTP]) that are distinguished by the number of phosphate residues attached during the anabolic conversion of 6-thioxanthosine monophosphate. Conversion of 6-TGDP to 6-TGTP is supposed to be catalysed by nucleoside diphosphate kinase (DPK), an enzyme presenting inter-individual variability with unknown impact on 6–thioguanine phosphates levels.

6-thioguanine nucleotides (6-TGN)

6-TGN appear to be some of the active metabolites responsible for therapeutic efficacy. Conflicting results about the clinical interest of 6-TGN levels in TP treated patients have been reported. Several studies showed a relationship between the therapeutic response and erythrocyte 6-TGN metabolite levels greater than 250 pmol/8×10^8 RBC (37,39) whereas others reported no relationship at all (40-42). Moreover, a recent meta-analysis (43) showed that the sensitivity of 6-TGN threshold levels for clinical response is only 62% and with a specificity of only 72%. However, the meta-analysis presented a statistically significant heterogeneity (p = 0.003). This apparent discordance between studies concerning the clinical interest of 6-TGN levels might be explained by the use of different biochemical tests that profoundly modify 6-TGN measurements. In addition, conflicting results might also be related to significant intra and inter-individual variability.

Nevertheless, in steroid-dependent IBD, AZA dose escalation guided by metabolite monitoring significantly increases the likelihood of attaining steroid-free remission (44). In addition, a subgroup of patients with high 6-TGN and increased 6-TGDP levels showed a worse outcome with lower responses rates than patients with high 6-TGN and predominantly detectable 6-TGTP

levels suggesting that 6-TGDP levels of more than 15% of total 6-TGN levels predict poor response in AZA-treated patients (45).

6-methylmercaptopurine (6-MMP)

MP is either degraded intracellularly into an inactive metabolite, 6 methylmercaptopurine (6-MMP), or transformed into the active metabolites, 6-thioguanine nucleotides (6-TGNs) and 6-methylmercaptopurineribonucleotides (6-MMPRs). Overproduction of 6–MMP might predispose patients to hepato- and myelotoxicity (39,46).

With the purpose of providing guidance to adequate use of these different metabolites, Sparrow *et al.* proposed to identify four groups of patients (Table 2) (47).

Table 2. — **Indications for Thiopurine Metabolite Measurements in IBD**

Metabolite measurements are indicated in patients not responding or experiencing adverse events to adequate weight-bases doses of TP

Group 1	Group 2	Group 3	Group 4
Low/Absent 6-TGN and Low/Absent 6-MMP = **Non-Adherence** ≈ **Education**	Low 6-TGN and Low 6-MMP = **Under-dosing** ≈ **Increase TP dosage**	Low 6-TGN and High 6-MMP = **Thiopurine resistance** ≈ **Add Allopurinol ?**	High 6-TGN and High 6-MMP = **Thiopurine refractory** ≈ **Change to another drug**

(Adapted from M Sparrow, UEGW 2009 task force of thiopurine).

Group 1 : Non compliant patients. This subgroup of patients might represent 20% of TP treated patients : they have negligible levels of both 6-TGN and 6-MMP or methyl-metabolites.

Group 2 : Patients insufficiently treated. They have subtherapeutic levels of both metabolites. TP dose escalation seems necessary to reach clinical remission.

Group 3 : Patients pharmacologically resistant to TP. The metabolite profile is defined by low levels of 6-TGN and high levels of methyl metabolites. This might be explained by ultra high TPMT activity and eventually counteracted by allopurinol administration (48).

Group 4 : Patients refractory to TP treatment. They reach therapeutic levels of both 6-TGN and 6-MMP without clinical efficacy. Continuation of TP therapy in this scenario seems futile (49) and another class of therapeutic agents is required.

Conclusions

TPMT and thiopurine metabolites use in clinical practice is still debated. TPMT analysis does not predict all TP side-effects but determines a higher risk group which could benefit from an adapted dose as proposed by Cara in relation to TPMT activity (Table 1) (50) resulting in significant therapeutic benefits. TPMT genotype or phenotype testing performed prior-to treatment can identify a proportion of patients at risk of developing myelotoxicity, a life-threatening condition, and helps in making dosage choices. It is likely that other enzymes involved in TP metabolism like XOD, inosine triphosphate pyrophosphatase (ITPA) and glutathion-S-transferase (GST) will emerge in the future but data about their clinical interest are currently too scarce to recommend testing for these enzymes in daily practice. Thiopurine metabolites determinations should not be performed in all patients taking antipurine therapy, but only in a situation of suspected TP failure allowing to adapt the treatment strategy and to avoid inadequate cessation of TP therapy, which remain a keystone of IBD treatment. Enzyme determination or TP metabolite measurements cannot be used as a substitute for the current practice of regular monitoring of the blood cells count and liver tests.

References

1. COSNES J, NION-LARMURIER I, BEAUGERIE L, AFCHAIN P, TIRET E, GENDRE JP. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. *Gut.*, 2005, **54** : 237-41.
2. HINDORF U, LINDQVIST M, HILDEBRAND H, FAGERBERG U, ALMER S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.*, 2006, **24** : 331-42.
3. DEWIT O, VANHEUVERZWYN R, DESAGER JP, HORSMANS Y. Interaction between azathioprine and aminosalicylates : an in vivo study in patients with Crohn's disease. *Aliment. Pharmacol. Ther*., 2002, **16** : 79-85.
4. GILISSEN LP, BIERAU J, DERIJKS LJ, BOS LP, HOOYMANS PM, VAN GENNIP A, STOCKBRÜGGER RW, ENGELS LG. The pharmacokinetic effect of discontinuation of mesalazine on mercaptopurine metabolite levels in inflammatory bowel disease patients. *Aliment. Pharmacol. Ther*., 2005, **22** : 605-11.
5. LOWRY PW, FRANKLIN CL, WEAVER AL, SZUMLANSKI CL, MAYS DC, LOFTUS EV, TREMAINE WJ, LIPSKY JJ, WEINSHILBOUM RM, SANDBORN WJ. Leucopenia resulting from a drug interaction between azathioprine or 6-mercaptopurine and mesalamine, sulphasalazine, or balsalazide. *Gut*., 2001, **49** : 656-64.
6. LYSAA RA, GIVERHAUG T, WOLD HL, AARBAKKE J. Inhibition of human thiopurine methyltransferase by furosemide, bendroflumethiazide and trichlormethiazide. *Eur. J. Clin. Pharmacol*., 1996, **49** : 393-6.
7. XIN HW, FISCHER C, SCHWAB M, KLOTZ U. Thiopurine S-methyltransferase as a target for drug interactions. *Eur. J. Clin. Pharmacol*., 2005, **61** : 395-8.
8. OSELIN K, ANIER K. Inhibition of human thiopurine S-methyltransferase by various nonsteroidal anti-inflammatory drugs in vitro : a mechanism for possible drug interactions. *Drug. Metab. Dispos*., 2007, **35** : 1452-4.
9. SERRE-DEBEAUVAIS F, BAYLE F, AMIROU M, BECHTEL Y, BOUJET C, VIALTEL P, *et al.* [Hematotoxicity caused by azathioprine genetically determined and aggravated by xanthine oxidase deficiency in a patient following renal transplantation]. *Presse Med*., 1995, **24** : 987-8.
10. KENNEDY DT, HAYNEY MS, LAKE KD. Azathioprine and allopurinol : the price of an avoidable drug interaction. *Ann. Pharmacother*, 1996, **30** : 951-4.
11. SPARROW MP, HANDE SA, FRIEDMAN S, CAO D, HANAUER SB. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine. *Clin. Gastroenterol. Hepatol*., 2007, **5** : 209-14.
12. ANSARI A, PATEL N, SANDERSON J, O'DONOHUE J, DULEY JA, FLORIN TH. Low-dose azathioprine or mercaptopurine in combination with allopurinol can bypass many adverse drug reactions in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2010, **31** : 640-7.
13. DERIJKS LJ, WONG DR. Pharmacogenetics of thiopurines in inflammatory bowel disease. *Curr. Pharm. Des*., 2010, **16** : 145-54.
14. KHAM SK, SOH CK, AW DC, YEOH AE. TPMT*26 (208F—>L), a novel mutation detected in a Chinese. Br. J. Clin. Pharmacol., 2009, 68 : 120-3.

15. DE BEAUMAIS TA, FAKHOURY M, PIGNEUR B, VIOLA S, MEDARD Y, BROLY F, JACQZ-AIGRAIN E. Characterization of a novel TPMT mutation associated with azathioprine induced myelosuppression. *Br. J. Clin. Pharmacol*., 2009, **68** : 770-2.
16. SCHAEFFELER E, FISCHER C, BROCKMEIER D, WERNET D, MOERIKE K, EICHELBAUM M, ZANGER UM, SCHWAB M. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics*, 2004, **14** : 407-17.
17. TAI HL, KRYNETSKI EY, SCHUETZ EG, YANISHEVSKI Y, EVANS WE. Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2) : mechanisms for the genetic polymorphism of TPMT activity. *Proc. Natl. Acad. Sci. USA*, 1997, **94** : 6444-9.
18. EVANS WE. Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. *Ther. Drug. Monit*., 2004, **26** : 186-91.
19. SAHASRANAMAN S, HOWARD D, ROY S. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur. J. Clin. Pharmacol*., 2008, **64** : 753-67.
20. WEINSHILBOUM RM, SLADEK SL. Mercaptopurine pharmacogenetics : monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am. J. Hum. Genet*., 1980, **32** : 651-62.
21. SANDERSON J, ANSARI A, MARINAKI T, DULEY J. Thiopurine methyltransferase : should it be measured before commencing thiopurine drug therapy ? *Ann. Clin. Biochem*., 2004, **41** : 294-302.
22. KASKAS BA, LOUIS E, HINDORF U, SCHAEFFELER E, DEFLANDRE J, GRAEPLER F, SCHMIEGELOW K, GREGOR M, ZANGER UM, EICHELBAUM M, SCHWAB M. Safe treatment of thiopurine S-methyltransferase deficient Crohn's disease patients with azathioprine. *Gut*., 2003, **52** : 140-2.
23. GISBERT JP, NINO P, RODRIGO L, CARA C, GUIJARRO LG. Thiopurine methyltransferase (TPMT) activity and adverse effects of azathioprine in inflammatory bowel disease : long-term follow-up study of 394 patients. *Am. J. Gastroenterol*., 2006, **101** : 2769-76.
24. GISBERT JP, LUNA M, MATE J, GONZALEZ-GUIJARRO L, CARA C, PAJARES JM. Choice of azathioprine or 6-mercaptopurine dose based on thiopurine methyltransferase (TPMT) activity to avoid myelosuppression. A prospective study. *Hepatogastroenterology*, 2006, **53** : 399-404.
25. GISBERT JP, GOMOLLON F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease : a review. *Am. J. Gastroenterol*., 2008, **103** : 1783-800.
26. PRIEST VL, BEGG EJ, GARDINER SJ, FRAMPTON CM, GEARRY RB, BARCLAY ML, CLARK DW, HANSEN P. Pharmacoeconomic analyses of azathioprine, methotrexate and prospective pharmacogenetic testing for the management of inflammatory bowel disease. *Pharmacoeconomics*, 2006, **24** : 767-81.
27. WINTER J, WALKER A, SHAPIRO D, GAFFNEY D, SPOONER RJ, MILLS PR. Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2004, **20** : 593-9.
28. KITIYAKARA T, HAYAT J, MCINTYRE AS. Cost-effectiveness of thiopurine methyltransferase genotype screening in IBD. *Aliment. Pharmacol. Ther*., 2005, **21** : 790-1, author reply 791-2.
29. SAYANI FA, PROSSER C, BAILEY RJ, JACOBS P, FEDORAK RN. Thiopurine methyltransferase enzyme activity determination before treatment of inflammatory bowel disease with azathioprine : effect on cost and adverse events. Can. J. Gastroenterol., 2005, 19 : 147-51.
30. DUBINSKY MC, REYES E, OFMAN J, CHIOU CF, WADE S, SANDBORN WJ. A cost-effectiveness analysis of alternative disease management strategies in patients with Crohn's disease treated with azathioprine or 6-mercaptopurine. *Am. J. Gastroenterol.*, 2005, **100** : 2239-47.
31. COLOMBEL JF, FERRARI N, DEBUYSERE H, MARTEAU P, GENDRE JP, BONAZ B, SOULÉ JC, MODIGLIANI R, TOUZE Y, CATALA P, LIBERSA C, BROLY F. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology*, 2000, **118** : 1025-30.
32. DEWIT O, MOREELS T, BAERT F, PEETERS H, REENAERS C, DE VOS M, *et al*. Impact of an extensive Thiopurine Methyltransferase genotyping in Inflammatory Bowel Disease patients who experienced myelosuppression during antipurine therapy. *Gastroenterology*, 2010, **138** : s-671.
33. EVANS WE, HON YY, BOMGAARS L, COUTRE S, HOLDSWORTH M, JANCO R, KALWINSKY D, KELLER F, KHATIB Z, MARGOLIN J, MURRAY J, QUINN J, RAVINDRANATH Y, RITCHEY K, ROBERTS W, ROGERS ZR, SCHIFF D, STEUBER C, TUCCI F, KORNEGAY N, KRYNETSKI EY, RELLING MV. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J. Clin. Oncol*., 2001, **19** : 2293-301.
34. OENDER K, LANSCHUETZER C, LAIMER M, KLAUSEGGER A, PAULWEBER B, KOFLER B, HINTNER H, BAUER JW. Introducing a fast and simple PCR-RFLP analysis for the detection of mutant thiopurine methyltransferase alleles TPMT*3A and TPMT*3C. *J. Eur. Acad. Dermatol.Venereol.*, 2006, **20** : 396-400.
35. ROBERTS RL, GEARRY RB, BLAND MV, SIES CW, GEORGE PM, BURT M, MARINAKI AM, ARENAS M, BARCLAY ML, KENNEDY MA. Trinucleotide repeat variants in the promoter of the thiopurine S-methyltransferase gene of patients exhibiting ultra-high enzyme activity. *Pharmacogenet. Genomics*, 2008, **18** : 434-8.
36. ANSARI A, HASSAN C, DULEY J, MARINAKI A, SHOBOWALE-BAKRE E-M, SEED P, MEENAN J, YIM A, SANDERSON J. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2002, **16** : 1743-1750.
37. CUFFARI C, DASSOPOULOS T, TURNBOUGH L, THOMPSON RE, BAYLESS TM. Thiopurine methyltransferase activity influences clinical response to azathioprine in inflammatory bowel disease. *Clin. Gastroenterol. Hepatol*., 2004, **2** : 410-7.
38. ANSARI A, ARENAS M, GREENFIELD SM, MORRIS D, LINDSAY J, GILSHENAN K, SMITH M, LEWIS C, MARINAKI A, DULEY J, SANDERSON J. Prospective evaluation of the pharmacogenetics of azathioprine in the treatment of inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2008, **28** : 973-983.
39. DUBINSKY MC, LAMOTHE S, YANG HY, TARGAN SR, SINNETT D, THÉORÊT Y, SEIDMAN EG. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology*, 2000, **118** : 705-13.
40. BELAICHE J, DESAGER JP, HORSMANS Y, LOUIS E. Therapeutic drug monitoring of azathioprine and 6-mercaptopurine metabolites in Crohn disease. *Scand. J. Gastroenterol*., 2001, **36** : 71-6.
41. REINSHAGEN M, SCHUTZ E, ARMSTRONG VW, BEHRENS C, VON TIRPITZ C, STALLMACH A, HERFARTH H, STEIN J, BIAS P, ADLER G, SHIPKOVA M, KRUIS W, OELLERICH M, VON AHSEN N. 6-thioguanine nucleotide-adapted azathioprine therapy does not lead to higher remission rates than standard therapy in chronic active crohn disease : results from a randomized, controlled, open trial. *Clin. Chem*., 2007, **53** : 1306-14.
42. LOWRY PW, FRANKLIN CL, WEAVER AL, PIKE MG, MAYS DC, TREMAINE WJ, LIPSKY JJ, SANDBORN WJ. Measurement of thiopurine methyltransferase activity and azathioprine metabolites in patients with inflammatory bowel disease. *Gut*., 2001, **49** : 665-70.
43. OSTERMAN MT, KUNDU R, LICHTENSTEIN GR, LEWIS JD. Association of 6-thioguanine nucleotide levels and inflammatory bowel disease activity : a meta-analysis. *Gastroenterology*, 2006, **130** : 1047-53.
44. ROBLIN X, SERRE-DEBEAUVAIS F, PHELIP JM, FAUCHERON JL, HARDY G, CHARTIER A, *et al*. 6-tioguanine monitoring in steroid-dependent patients with inflammatory bowel diseases receiving azathioprine. *Aliment. Pharmacol. Ther*., 2005, **21** : 829-39.
45. NEURATH MF, KIESSLICH R, TEICHGRABER U, FISCHER C, HOFMANN U, EICHELBAUM M, *et al*. 6-thioguanosine diphosphate and triphosphate levels in red blood cells and response to azathioprine therapy in Crohn's disease. *Clin. Gastroenterol. Hepatol.*, 2005, **3** : 1007-14.
46. HINDORF U, LINDQVIST M, PETERSON C, SÖDERKVIST P, STRÖM M, HJORTSWANG H,, POUSETTE A, ALMER S. Pharmacogenetics during standardised initiation of thiopurine treatment in inflammatory bowel disease. *Gut*., 2006, **55** : 1423-31.
47. SPARROW MP, IRVING PM, HANAUER SB. Optimizing conventional therapies for inflammatory bowel disease. *Curr. Gastroenterol. Rep*., 2009, **11** : 496-503.
48. ANSARI A, ELLIOTT T, BABURAJAN B, MAYHEAD P, O'DONOHUE J, CHOCAIR P, SANDERSON J, DULEY J.. Long-term outcome of using allopurinol co-therapy as a strategy for overcoming thiopurine hepatotoxicity in treating inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2008, **28** : 734-741.
49. ROBLIN X, CHARTIER A, DUCROS V. Interaction between homocystein metabolism and TPMT activity in IBD patients under azathioprine. *Gastroenterology*, 2006, **130** (Suppl 2) : A199.
50. CARA CJ, PENA AS, SANS M, RODRIGO L, GUERRERO-ESTEO M, HINOJOSA J, GARCÍA-PAREDES J, GUIJARRO LG. Reviewing the mechanism of action of thiopurine drugs : towards a new paradigm in clinical practice. *Med. Sci. Monit*., 2004, **10** : RA247-54.