Toxicity profile of thiopurines in inflammatory bowel disease: a retrospective cohort analysis

Toxicité des thiopurines au cours des maladies inflammatoires chroniques de l'intestin : étude de cohorte rétrospective analytique

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RÉSUMÉ

Introduction : Les thiopurines ont prouvé leur efficacité au cours des maladies inflammatoires chroniques de l'intestin. Cependant, leur utilisation est limitée chez un sous-groupe de patients par les effets indésirables.

Objectifs : L'objectif de cette étude est d'évaluer la toxicité des thiopurines au cours des maladies inflammatoires chroniques de l'intestin et de déterminer ses facteurs prédictifs.

Méthodes : Une étude rétrospective longitudinale a été menée chez les patients ayant une maladie inflammatoire chronique de l'intestin et traités par thiopurines. Une régression logistique multiple a été réalisée afin de déterminer les facteurs de risque de survenue d'effets indésirables.

Résultats : Deux cent dix patients ont été inclus dans l'étude. L'âge moyen au moment du diagnostic était de 29,8±11,4 ans. Cent soixante-neuf (169) patients avaient une maladie de Crohn, 29 avaient une rectocolite hémorragique et 12 avaient une colite indéterminée. Durant une période moyenne de suivi de 28,5 ± 20 mois, 56 patients avaient eu des effets indésirables à type d'intolérance digestive (n=14; 6,6%), réactions immuno-allergiques (n=8; 3,8%), myélotoxicité (n=25; 11,9%) et hépatotoxicité (n=8; 3,8%). L'arrêt du traitement a été rapporté chez 19 patients (9%). La cortico-dépendance était le seul facteur prédictif indépendant de survenue d'effets indésirables (OR= 3,96; CI 95%: 1,07- 14,53; p= 0,038).

Conclusions : Environ un quart des patients ayant une maladie inflammatoire chronique de l'intestin et traités par thiopurines développent des effets indésirables. Ces effets indésirables mènent à l'arrêt du traitement chez environ 9% des patients soit en monothérapie soit en association avec les biothérapies. Les patients corticodépendants avaient un risque significativement élevé de développer une toxicité liée aux thiopurines.

Mots-clés: maladie de Crohn ; rectocolite hémorragique ; azathioprine ; 6-mercaptopurine

SUMMARY

Background: Thiopurines have proven efficacy in inflammatory bowel disease. However, their use is limited by adverse effects in a subset of patients.

Aims: The present study aimed to evaluate toxicity profile and identify clinical predictive factors of thiopurine adverse effects in inflammatory bowel disease patients.

Methods: A retrospective longitudinal study was conducted among inflammatory bowel disease patients treated with thiopurines. Multiple logistic regression was used to identify risk factors for thiopurine adverse effects.

Results : A total of 210 patients were enrolled in the study. Mean age at disease onset was 29.8±11.4 years. One hundred sixty-nine (169) patients had Crohn's disease, 29 had ulcerative colitis and 12 had indeterminate colitis. During a median follow-up of 28.5 ± 20 months, 56 patients (26.6%) had thiopurine-related adverse effects including digestive intolerance (n=14; 6.6%), immunoallergic reactions (n=8; 3.8%), myelotoxicity (n=25; 11.9%) and hepatotoxicity (n=8; 3.8%). Treatment withdrawal was reported in 19 patients (9%). The only independent predictive factor for thiopurine adverse effects found in this study was steroid-dependence (OR= 3.96; 95% CI: 1.07- 14.53; p= 0.038).

Conclusions : Almost a quarter of inflammatory bowel disease patients treated with thiopurines developed adverse effects. These adverse effects lead to drug withdrawal in almost 9% of patients either as monotherapy or as in combination with biologic therapies. Steroid-dependent patients were significantly at higher risk for thiopurine-related toxicity.

Key-words: Crohn's disease; ulcerative colitis; azathioprine; 6-mercaptopurine

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INTRODUCTION

Thiopurines are widely prescribed drugs in patients with inflammatory bowel disease (IBD). They are mainly used for maintenance of clinical remission (1-4). The precise mechanisms responsible for their effects are still unclear but the main one might be an anti-proliferative action against antigen specific T-cells (5). They have proven their efficacy in both Crohn's disease (CD) and ulcerative colitis (UC) either as monotherapy or as combination with biologic therapies. However, their use is limited by serious adverse effects (AE) leading to drug withdrawal in 9 to 25% of patients (6). Thiopurines AE are classified into "dose-independent" (i.e. allergic or idiosyncratic reactions) such as pancreatitis or hepatitis and pharmacologically explainable "dose-dependent" side effects such as myelotoxicity, hepatitis, cancer and opportunistic infections (7). The best-known risk factor for the development of most thiopurine AE is the presence of genetic variants in the thiopurine S-methyltransferase (TPMT) gene. A low TPMT activity may lead to excessive cytotoxic 6-thioguanine nucleotide (6-TGN) metabolite formation resulting mainly in hematologic toxicities. However, only up to 25% of these adverse reactions can be explained by variants in TPMT, which suggests that environmental or clinical factors may also play a role in the development of AE (8). Therefore we analysed a cohort of thiopurine-treated IBD patients in order to assess further potential risk factors for thiopurine toxicity regardless TPMT phenotype.

METHODS

We conducted a retrospective longitudinal single center study that included all patients treated with azathioprine (AZA) or 6-mercaptopurine (6-MP) between January 2006 and December 2012. Eligible patients had a previous diagnosis of IBD with a minimum follow-up of 6 months after the start of thiopurine treatment. Exclusion criteria were previous diagnosis of hematologic or liver diseases. Diagnosis of Crohn's disease (CD) and ulcerative colitis (UC) was based on clinical, biochemical, pathological and endoscopic findings. Phenotyping for disease location, extent and behavior were based on the Montreal Classification of IBD (9). Diagnosis of indeterminate colitis (IC) was made if there were no clear clinical, endoscopic or pathological features of definitive diagnosis of either CD or UC. Demographic data, disease duration, disease location, disease behavior, history of medical and/or surgical treatment and follow up were abstracted from medical records. All patients started at full dose of azathioprine (2-2.5 mg/kg/day). 6-mercaptopurine (0.75-1.5 mg/kg) was introduced in patients with intolerance to azathioprine. Full blood count and liver function tests were performed prior to treatment and at 1, 2, 4, 12 weeks after treatment and then quarterly. There was no assessment of TPMT activity prior to thiopurine treatment. Imputability of thiopurine AE was based on chronological and clinical criteria.

Definitions

Digestive intolerance consists in symptoms of nausea, vomiting or non-specific abdominal pain in the absence of any other cause (10).

Myelotoxicity was defined as either anemia (hemoglobin level <10 g/dL) and/or neutropenia (absolute neutrophil count <1,500 /mm3 and/or lymphopenia (absolute lymphocyte count <1,500/mm3) and/or thrombocytopenia (platelet count <100,000) resolving after withdrawal of treatment or dose reduction. Severe myelotoxicity was defined as: absolute neutrophil count < 500/mm3 and/or lymphocyte count < 500/ mm3 and/or hemoglobin level <5g/dL and/or platelet count <75,000/mm3 (10-12).

Hepatotoxicity was defined as an increase in serum alanine transaminase and/or aspartate transaminase and/or alkaline phosphatase and/or gamma glutamate transpeptidase greater than twice the upper normal limit or clinical jaundice and as resolution after withdrawal of treatment or reduction in the dosage. Patients who developed jaundice and/or liver function tests elevation greater than 5 times the ULN were considered as having **severe** hepatotoxicity (13).

The diagnosis of acute pancreatitis was made if two of the following three criteria are present: characteristic upper abdominal pain; amylase and/or lipase three-times the upper limit of normal; and imaging findings consistent with acute pancreatitis (14).

Thiopurine-induced cutaneous rash was considered if patient had a new onset of rash after starting thiopurines that resolved after drug withdrawal (15).

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Science (SPSS) software version 21.0 (IBM Corp., Armonk. New York, USA). Data were summarized as mean and percentage. The comparison of qualitative variables was carried out by the chi-square test and in case

of non-validity by the Fischer test. The Mann-Whitney U test was used to compare quantitative variables. Univariate logistic regression analysis was conducted to investigate the association between patients' characteristics and the occurrence of thiopurine AE. Variables that had a p-value of less than or equal to 0.2 through the univariate logistic regression were selected to perform a multiple logistic regression analysis. A p-value ≤ 0.05 was deemed to denote statistical significance.

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association (16) and was approved by the local ethics committee.

RESULTS

Our study included 210 patients (98 males and 112 females) with a mean age at disease onset of 29.8±11.4 years. One hundred sixty-nine (169) patients had CD, 29 had UC and 12 had indeterminate colitis. The baseline characteristics of the retained patients are reported in Table 1. Thiopurines were prescribed simultaneously with another drug in 171 patients (81%). Concomitant drugs were corticosteroids in 133 patients (63%), aminosalicylates in 21 patients (10%), infliximab in 12 patients (6%) and cyclosporine in 5 patients (2%). Indications for thiopurines were a young age (<20 years old) at disease onset of CD in 20 patients (9.5%), corticosteroid-dependent IBD in 42 patients (20%), extensive ileal lesions of CD in 28 patients (13.3%), as maintenance therapy after severe acute colitis in 79 patients (37.6%), prevention of postoperative recurrence of CD in 51 patients (24.4%), complex perianal fistulas in 12 patients (5.7%), and upper gastrointestinal tract involvement in 8 patients (3.8%).

During a median follow-up of 28.5 ± 20 months, 56 patients (26.6%) had AZA-related AE including digestive intolerance (n=14; 6.6%), immunoallergic reactions (n=8; 3.8%), myelotoxicity (n=25; 11.9%) and hepatotoxicity (n=8; 3.8%). AZA was switched successfully to 6-MP in patients who terminated therapy due to digestive intolerance (Table 2). Severe AE consisted in acute pancreatitis (n=5), immunoallergic cutaneous rash (n=3), severe myelotoxicity (n=7) and severe hepatic toxicity (n=4). They occurred with AZT in 15 patients and 6MP in 4 patients. All these patients had favorable outcome after treatment withdrawal.

Table 1: Baseline characteristics of the study population

	N=210
Male (n) (%)	98 (46.7)
CD Patients (n) (%)	169 (80.5)
UC Patients (n) (%)	29 (13.8)
IC Patients (n) (%)	12 (5.7)
Smoking (n) (%)	57 (27.1)
Montreal classification of UC patients (localization)	
E1	4 (13.8)
E2	15 (51.7)
E3	10 (34.5)
Montreal classification of CD patients	21 (12.4)
A1	120 (71)
A2	28 (16.6)
A3	81 (47.9)
B1	40 (23.6)
B2	48 (28.4)
B3	65 (38.5)
P	54 (32)
L1	58 (34.3)
L2	57 (33.7)
L3	15 (8.8)
L4	
Median duration of IBD at start thiopurine (months)	12 [0 – 240]
Small bowel surgery (n) (%)	73 (34.7)

CD= Crohn's disease; UC=ulcerative colitis; IC= indeterminate colitis; IBD=inflammatory bowel disease

Table 2: Thiopurine related adverse effects

Type of AE	Median time-to-onset*	Management of AE
Digestive intolerance (n=14; 6.6%)	5 months	Switch to 6MP (0.75 – 1.5 mg/Kg/day) with a favorable outcome
Allergic/ idiosyncratic reactions (n=8; 3.8%) Acute pancreatitis (n=5; 2.3%) Cutaneous rash (n=3; 1.5%)	2 weeks	Treatment withdrawal
Myelotoxicity (n=25; 11.9%): Lymphopenia (n=19; 9%) Neutropenia (n=11; 5.2%) Thrombocytopenia (n=11; 5.2%)	20 months	- No severe cytopenia : surveillance / dose reduction with favorable outcome - Severe myelotoxicity (n=7): treatment withdrawal
Hepatotoxicity (n=8; 3.8%): Cholestasis (n=3; 1.5%) Cytolysis (n=4; 1.9%) Regenerative nodular hyperplasia (n=1; 0.4%)	7 months	- Dose reduction (n=4) - Treatment withdrawal (n=4)

*time from start of thiopurine administration to onset of reaction;
AE=adverse effects; 6-MP=6-mercaptopurine;

In univariate analysis, factors associated with thiopurine related AE were corticosteroid-dependence (overall AE and myelotoxicity), smoking (digestive intolerance and immunoallergic reactions), concomitant anti-TNF use (immunoallergic reactions), male sex (myelotoxicity) and maximum azathioprine doses (overall AE, digestive intolerance, immunoallergic reactions and hepatotoxicity). However, patients who had late introduction of thiopurines seem to be at lower risk of hepatotoxicity (Tables 3, 4 and 5). In multivariate analysis, only corticosteroid dependence was a predictive factor of thiopurine related toxicity (OR= 3.96; 5% CI: 1.07- 14.53; p= 0.038).

Table 3: Univariate analysis to explore factors associated with the development of thiopurine-related overall adverse effects

All adverse effects			
	Yes	**No**	**P value**
Age at disease onset mean ± SD	31.4 ± 11.9	29.3±11.9	0.771
Sex, male; %	48	46.3	0.829
Types of IBD; %			
CD	82	80	0.755
UC	14	12.5	0.782
IC	4	6.3	0.735
Smoking; %	32	25.6	0.310
Indications for thiopurines; %			
Young age at disease onset	4.8	9.4	1.000
Corticosteroid-dependence	30	13.8	**0.008**
Corticosteroid-resistance	32	39.4	0.347
Extensive ileal lesions	14	13.1	0.874
Prevention of POR of CD	30.6	22.5	0.247
Complex perianal fistulas	6.1	5.6	0.896
Upper GI tract involvement	2	4.4	0.683
EIMs	2	0	0.238
Concomitant drugs; %			
Anti-TNF	14	10	0.429
Cyclosporine	8	5	0.425
Corticosteroids	86	81.3	0.442
Aminosalycilates	54	47.5	0.422
Disease duration before thiopurine introduction months, mean ± SD	38.2±54	29.8±43	0.144
Azathioprine dosage, mg/Kg/day, mean±SD	2.40±0.1	2.30±0.2	**0.001**

SD: standard deviation; IBD=inflammatory bowel disease; CD=Crohn's disease; UC=ulcerative colitis; IC=indeterminate colitis; POR=post-operative recurrence; GI=gastrointestinal; EIMs= extra-intestinal manifestations

DISCUSSION

In the current study, thiopurine toxicity was seen in 56 patients (26.6%). These AE included digestive intolerance (6.6%), immunoallergic reactions (3.8%), hematological toxicity (11.9%) and hepatotoxicity (3.8%). In multivariate regression analysis, corticosteroid-dependent patients were at increased risk of thiopurine related toxicity.

However, the main limitation of our study was related to its retrospective design resulting in a great deal of missed data. This could have generated discrepancy between our findings and those of previous research.

These drugs are known to play a pivotal role in maintaining remission in corticosteroid-refractory or corticosteroid-dependent IBD (17). Indeed, roughly 55% to 70% of IBD patients respond to thiopurines. However, these molecules are withdrawn in approximately 40% to 50% of such patients. The reason for discontinuation of thiopurine therapy is usually the occurrence of AE which is reported in 7.6% to 30% of the IBD patients using thiopurines (13, 18, 19). In our study, 26.6% of patients under thiopurines developed AE, which is in accordance with the prevalence reported in different studies (20, 21). This wide range of AE prevalence among different series is mainly due to the lack of a standard definition for each side effect, particularly myelotoxicity and hepatotoxicity.

Regarding treatment withdrawal, 9% of our patients had to stop the treatment because of AE. Our data are in the lower part of the range of studies from northern European areas where treatment withdrawal was indicated in 28 to 60% of patients (22-24). The main AE leading to treatment withdrawal among our patients were allergic/ idiosyncratic reactions (n=8), myeolotoxicity (n=7) and hepatotoxicity (n=4).

Immunoallergic or idiosyncratic reactions are potentially life-threatening reactions. They occurred in 8 patients in our series within the first weeks of treatment. Acute pancreatitis occurred in 2.3% of cases, which is in accordance with other studies where prevalence of acute pancreatitis varies from 1 to 5% (13, 18, 19, 25-28). Although anecdotal evidence has shown successful and safe reintroduction of thiopurines following a previous episode of suspected pancreatitis, thiopurines are considered to be contraindicated in such patients (29). In the current study, smoking, concomitant anti-TNF use and maximum azathioprine doses (2.5 mg/Kg/day) were significantly associated with development of immunoallergic reactions.

Table 4: Univariate analysis to explore factors associated with the development of thiopurine-related digestive intolerance and immunoallergic reactions

	Digestive intolerance			Immunoallergic reactions		
	Yes	No	P value	Yes	No	P value
Age at disease onset mean ± SD	29±10.7	29±11.8	0.981	27±6.2	30±11.6	0.681
Sex, male; %	50	53	0.796	25	54	0.150
Types of IBD; %						
CD	92.9	79.6	0.226	75	80.7	0.655
UC	7.1	13.3	0.439	12.5	12.9	0.726
IC	0	6.1	0.427	12.5	5.4	0.381
Smoking; %	50	25.5	**0.05**	62.5	25.7	**0.036**
Indications for thiopurines, %						
Young age at disease onset	7.1	9.7	0.606	12.5	9.4	0.577
Corticosteroid-dependence	28.6	16.8	0.278	37.5	16.8	0.149
Corticosteroid-resistance	21.4	38.8	0.196	37.5	37.6	1.000
Extensive ileal lesions	7.1	13.8	0.699	25	12.9	0.289
Prevention of POR of CD	35.7	23.6	0.337	12.5	24.9	0.683
Complex perianal fistulas	7.1	5.6	0.575	0	6	1.000
Upper GI tract involvement	0	4.1	0.570	12.5	3.5	0.271
EIMs	7.1	0	0.067	0	0.5	1.000
Concomitant drugs; %						
Anti-TNF	7.1	11.2	0.531	37.5	9.9	**0.045**
Cyclosporine	7.1	5.6	0.573	25	5	0.069
Corticosteroids	92.9	81.6	0.472	75	82.7	0.633
Aminosalycilates	64.3	48	0.238	62.5	48.5	0.492
Disease duration before thiopurine introduction months, mean ± SD	44.7±50	30.8±45	0.469	41.1±47	31.4±46	0.779
Azathioprine dosage, mg/Kg/day, mean±SD	2.40±0.1	2.30±0.2	**0.001**	2.50±0	2.40±0.2	**0.001**

SD: standard deviation; IBD=inflammatory bowel disease; CD=Crohn's disease; UC=ulcerative colitis; IC=indeterminate colitis; POR=post-op; GI=gastrointestinal; EIMs= extra-intestinal manifestations

Table 5. Univariate analysis to explore factors associated with the development of thiopurine-related myelotoxicity and hepatotoxicity

	Myelotoxicity			Hepatotoxicity		
	Yes	No	P value	Yes	No	P value
Age at disease onset mean ± SD	33.2±13	29.3±11	0.213	36.3±14	29.6±11	0.180
Sex, male; %	72	50.8	**0.050**	66.7	46.1	0.421
Types of IBD; %						
CD	80	80.6	1.000	83.3	80.4	0.858
UC	16	12.4	0.538	16.7	12.7	0.567
IC	4	5.9	1.000	0	5.9	1.000
Smoking; %	12	29.2	0.07	16.7	27.5	1.000
Indications for thiopurines; %						
Young age at disease onset	8	9.7	1.000	16.7	9.3	0.456
Corticosteroid-dependence	32	15.7	**0.048**	16.7	17.6	1.000
Corticosteroid-resistance	32	38.4	0.537	66.7	36.8	0.201
Extensive ileal lesions	16	13	0.753	0	13.7	1.000
Prevention of POR of CD	33.3	23.2	0.279	16.7	24.6	1.000
Complex perianal fistulas	8.3	5.4	0.633	0	5.9	1.000
Upper GI tract involvement	0	4.3	0.600	0	3.9	1.000
EIMs	0	0.5	1.000	0	0.5	1.000
Concomitant drugs; %						
Anti-TNF	8	11.4	1.000	16.7	10.8	0.506
Cyclosporine	0	6.5	0.368	16.7	5.4	0.301
Corticosteroids	84	82.2	1.000	100	81.9	0.593
Aminosalycilates	48	49.2	1.000	50	49	1.000
Disease duration before thiopurine introduction months, mean ± SD	39.2±60	30.8±43	0.103	1.3±2	32.7±46	**0.012**
Azathioprine dosage, mg/Kg/day, mean±SD	2.40± 0.1	2.44±0.2	0.052	2.50±0	2.40±0.2	**0.001**

SD: standard deviation; IBD=inflammatory bowel disease; CD=Crohn's disease; UC=ulcerative colitis; IC=indeterminate colitis; POR=post-operative recurrence; GI=gastrointestinal; EIMs= extra-intestinal manifestations

Our results are in accordance with those of previous studies showing that smoking is the most important risk factor for AZA-induced pancreatitis (30, 31). In fact, experimental studies have shown that cigarette smoking may result in increased inflammatory activity and oxydative stress in the pancreas. It may also induce ischaemia in the pancreas and lead to an imbalance between digestive enzyme trypsinogen and the inhibitory antiprotease pancreatic inhibitory trypsin inhibitor (32, 33). These findings support the need to give up smoking after diagnosis of IBD, particularly in CD. With regard to concomitant anti-TNF use, our findings did not corroborate those of previous research where addition of anti-TNF agents to other treatment modalities for IBD reduced the risk for developing pancreatitis, probably due to decreased inflammation (34). Unlike our results, azathioprine dosage have not been shown previously to be associated with immunoallergic reactions since the latter are dose-independent reactions.

Myelotoxicity was the main AE reported among our patients (11.9%). It occurred after a median time-to-onset of 20 months. Actually, the prevalence of myelotoxicity in IBD patients receiving thiopurines ranges from 1.7 to 11% (25, 35-37). Although hematological disorders may develop at any time during drug treatment, they usually occur during the first months of treatment. The major concern in patients with myelotoxicity is bone marrow suppression. It remains probably the most important and potentially lethal AE of thiopurines occurring in 2–5% of the patients treated with these agents (38). None of our patients had this complication. In our series, male sex and steroid-dependence were significantly associated with myelotoxicity. To our knowledge, no previous research studies has shown a relationship between gender and thiopurine-related myelotoxicity in IBD patients. As regard with concomitant drug use, only biologics have been reported to be significantly associated with thiopurine-related toxicity (8). Moreover, steroids have been thought to prevent leucopenia caused by thiopurines. In fact, it has been shown that thiopurines without accompanying steroids may lead to marked leucopenia (39). Thus, we could not explain what the current study found with respect to the association between steroid-dependence and myelotoxicity.

Digestive intolerance was the second most common AE in our series. It occurred in 6.6% of our patients, which match the prevalence range reported in literature (4.2 to 11.4%) (15, 19). Patients under thiopurines may develop digestive intolerance usually during the first weeks of treatment, although it happened after a mean time-to-onset of 5 months in the current study.

This AE did not result in treatment withdrawal in our patients since all of them had good clinical response after switching to 6-MP. In fact, Kennedy et al showed that switching to 6-MP is a safe therapeutic strategy for over two-thirds of azathioprine-intolerant patients (40). The current study has shown that smoking and maximum azathioprine doses (2.4 mg/Kg/day) were associated with digestive intolerance. Indeed, it has been shown that dose-decreased AZA added to low dose allopurinol may reduce digestive intolerance particularly nausea, in more than 80% of patients (41). This strategy often allows to reach therapeutic levels of 6-TGN and clinical remission and reduce 6-methyl-MP (6-MMP) production (42). Other risk factors for digestive intolerance have been reported such as female sex and CD which is not in accordance with our findings (15).

In our cohort, 3.8% of patients developed hepatotoxicity. Liver injury induced by thiopurines seems more often dose dependent than idiosyncratic (42). Among dose-dependent injuries, vascular lesions such as nodular regenerative hyperplasia (NRH) have been reported in patients treated with thiopurines. Prevalence of NRH in IBD patients treated with azathioprine was estimated to be around 0.8 % (43). It was histologically diagnosed in one of our patients. It is worth noting that IBD per se is a risk factor for NRH (44).

In the current study, hepatotoxicity was significantly associated with maximum azathioprine doses (2.5 mg/Kg/day). Some authors suggested hepatotoxicity of thiopurines is correlated with 6-MMP levels, which usually occurs in a dose-dependent manner (45). Thus, similar to what we have previously mentioned regarding digestive intolerance, a reduction in the dose of the azathioprine to 25–33% of the original dose in conjunction with low dose of allopurinol has been shown to reverse the preferential metabolism towards 6-MMP with subsequent normalization of liver function tests (46). Moreover, split dosing strategies have been reported to be effective in overcoming hepatotoxicity, particularly in so-called preferential 6-MMP metabolizers (47).

Previous reports have shown an increase risk of hepatotoxicity in men and in patients co-treated with steroids (48, 49). In the present study, hepatotoxicity was

more commonly encountered in men and in patients who had concomitant steroid use although this difference did not reach statistical significance. Indeed, steroids may precipitate hepatotoxicity through exacerbating insulin resistance and/or risk factors for fatty liver disease such as obesity, diabetes and hypertriglyceridemia (50).

Univariate analysis revealed that apart from hepatotoxicity, steroid-dependence was more frequently noted in patients who developed AE (overall and specific types) with statistical significance only for overall AE. In multivariate analysis, steroid-dependence has been found to be the only significant predictive factor for thiopurine related AE. This finding could be explained mainly by the increased risk of hepatotoxicity in patients co-treated with steroids and, probably to a lesser degree, by higher incidence of digestive intolerance in such patients, as it could simply be a side effect of steroids.

Actually, almost 25% of these adverse reactions can be explained by variants in TPMT. In fact, a decreased enzyme activity in patients treated with standard doses of thiopurines could result in many AE such as myelotoxocity, gastrointestinal intolerance, pancreatitis and hypersensitivity (51). On the other hand, a high activity of TPMT may lead to high serum levels of hepatotoxic metabolites such as 6-MMP or 6-methyl-mercaptopurine-ribonucleotide (6-MMPR) (52, 53). That being said, approximately 70% of patients with AE have normal TPMT activity. Hence, the cost effectiveness of widespread TPMT activity assessment in routine clinical practice remains to be defined. In the current study, in accordance with the European Crohn's and Colitis Organization guidelines, TPMT activity was not taken into consideration prior to treatment.

In conclusion, roughly a quarter of our IBD patients treated with thiopurines developed AE leading to drug withdrawal in almost 9% of patients. The most frequent AE were myelotoxicity and digestive intolerance. We have identified steroid-dependence as the significant predictive factor of AE. Thus, stringent safety monitoring based on clinical assessment as well as blood count and hepatic tests is warranted to prevent severe toxicity and treatment discontinuation.

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