Journal of Crohn's and Colitis, 2017, 175–184
doi:10.1093/ecco-jcc/jjw130
Advance Access publication July 9, 2016
Original Article

Original Article

Early Assessment of Thiopurine Metabolites Identifies Patients at Risk of Thiopurine-induced Leukopenia in Inflammatory Bowel Disease

Dennis R. Wong[a], Marieke J.H. Coenen[b,*], Sita H. Vermeulen[b,c,*], Luc J.J. Derijks[d], Corine J. van Marrewijk[b], Olaf H. Klungel[e], Hans Scheffer[b], Barbara Franke[b,f], Henk-Jan Guchelaar[g], Dirk J. de Jong[h], Leopold G.J.B. Engels[i], André L.M. Verbeek[c], Piet M. Hooymans[a], On behalf of the TOPIC recruitment team

[a]Department of Clinical Pharmacy, Pharmacology and Toxicology, Zuyderland Medical Center, Sittard-Geleen, The Netherlands [b]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands [c]Department for Health Evidence, Radboud University Medical Center, Nijmegen, The Netherlands [d]Department of Clinical Pharmacy, Máxima Medical Centre, Veldhoven, The Netherlands [e]Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, The Netherlands [f]Department of Psychiatry, Radboud University Medical Center, Nijmegen, The Netherlands [g]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Centre, Leiden, The Netherlands [h]Department of Gastroenterology, Radboud University Medical Center, Nijmegen, The Netherlands [i]Department of Gastroenterology, Zuyderland Medical Center, Sittard-Geleen, The Netherlands

*Equal contribution

Corresponding author: D.R. Wong, PharmD PhD, Department of Clinical Pharmacy, Pharmacology and Toxicology, Zuyderland Medical Center, Sittard-Geleen, PO Box 5500, Sittard-Geleen, 6130 MB, The Netherlands. Tel: +31-88-459-7709; Fax: +31-88-459-7971; E-mail: d.wong@zuyderland.nl

Abstract

Background and Aims: Only a quarter of thiopurine-induced myelotoxicity in inflammatory bowel disease [IBD] patients is related to thiopurine S-methyltransferase deficiency. We determined the predictive value of 6-thioguanine nucleotide [6-TGN] and 6-methylmercaptopurine ribonucleotide [6-MMPR] concentrations 1 week after initiation [*T1*] for development of leukopenia during the first 8 weeks of thiopurine treatment.

Methods: The study was performed in IBD patients starting thiopurine therapy as part of the Dutch randomized controlled TOPIC trial [ClinicalTrials.gov NCT00521950]. Blood samples for metabolite measurement were collected at *T1*. Leukopenia was defined by leukocyte counts of $<3.0 \times 10^9$/L. For comparison, patients without leukopenia who completed the 8 weeks on the stable dose were selected from the first 272 patients of the TOPIC trial.

Results: Thirty-two patients with, and 162 patients without leukopenia were analysed. *T1* threshold 6-TGN concentrations of 213 pmol/8×10^8 erythrocytes and 3525 pmol/8×10^8 erythrocytes for 6-MMPR were defined: patients exceeding these values were at increased leukopenia risk (odds ratio [OR] 6.2 [95% CI: 2.8–13.8] and 5.9 [95% CI: 2.7–13.3], respectively). Leukopenia rates were higher in

Abbreviations: ADA, adalimumab; 5-ASA, mesalazine, 5-aminosalicylic acid; AZA, azathioprine; AUC, area under the curve; CD, Crohn's disease; 95% CI, 95% confidence interval; 6-MP, mercaptopurine; 6-TGN, 6-thioguanine nucleotides; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 6-MTIMP, 6-methylthioinosine monophosphate; IBD, inflammatory bowel disease; IFX, infliximab; OR, odds ratio; PDNS, purine de novo synthesis; RBCs, red blood cells; ROC, receiver operating characteristics; TPMT, thiopurine S-methyltransferase; UC, ulcerative colitis.

patients treated with mercaptopurine, compared with azathioprine (OR 7.3 [95% CI: 3.1–17.0]), and concurrent anti-TNF therapy (OR 5.1 [95% CI: 1.6–16.4]). Logistic regression analysis of thiopurine type, threshold concentrations, and concurrent anti-tumour necrosis factor [TNF] therapy revealed that elevations of both *T1* 6-TGN and 6-MMPR resulted in the highest risk for leukopenia, followed by exceeding only the *T1* 6-MMPR or 6-TGN threshold concentration (area under the curve 0.84 [95% CI: 0.76–0.92]).

Conclusions: In ~80% of patients, leukopenia could be explained by *T1* 6-TGN and/or 6-MMPR elevations. Validation of the predictive model is needed before implementing in clinical practice.

Key Words: Thiopurines; toxicity; inflammatory bowel disease

1. Introduction

For both the immunomodulating thiopurine drugs azathioprine [AZA] and mercaptopurine [6-MP] therapeutic efficacy has been demonstrated with respect to induction and maintenance of remission in patients with inflammatory bowel diseases (IBD: Crohn's disease [CD] and ulcerative colitis (UC]).[1–3] Unfortunately, up to 25% of IBD patients discontinue thiopurine therapy during the first months of treatment due to adverse events.[4–6] The most important and potentially lethal adverse reaction to AZA/6-MP treatment is myelosuppression. A review of thiopurine-induced myelotoxicity in patients with IBD, including data from 66 trials [8302 patients], reported a 7% cumulative incidence of AZA/6-MP–induced myelotoxicity that predominantly occurred within the first weeks or months of treatment.[7]

Azathioprine and 6-MP undergo extensive intracellular metabolic transformations to active metabolites, of which 6-thioguanine nucleotides [6-TGN] and 6-methylmercaptopurine ribonucleotides [6-MMPR] are considered to be the most important [Figure 1]. Interindividual differences in therapeutic response and toxicity to thiopurines can be explained by variation in the formation of these metabolites, which is partly related to genetic variants in the genes encoding for crucial enzymes in the thiopurine metabolism.[8]

One of the key enzymes in the delicate balance between 6-MMPR and 6-TGN metabolite formation is thiopurine S-methyltransferase [TPMT].[8] Eleven per cent of the Caucasian population is a heterozygous carrier of an inactive *TPMT* allele and 0.3% is homozygous for a *TPMT* variant, resulting in an intermediate and low/absent TPMT activity, respectively. Humans carrying two active alleles [~89%] show normal TPMT enzyme activity. Decreased TPMT enzyme activity shifts thiopurine metabolism towards increased formation of active cytotoxic 6-TGN metabolites.[9] Prior-to-treatment *TPMT* genotype or phenotype assessment may help to identify patients at risk of developing severe myelotoxicity.[8,10]

As in less than a quarter of the patients myelosuppression could be related to TPMT deficiency, the clinical usefulness of these assessments to avoid bone marrow toxicity seems to be limited.[11,12]

Recent results of the prospective randomized controlled TOPIC trial on pre-treatment TPMT screening demonstrated a 10-fold reduction in myelotoxic events among heterozygote variant carriers who were identified and treated with a reduced AZA or 6-MP dose, when compared with variant carriers treated with a standard dose. However, genotyping of the three most common genetic variants in *TPMT* [*2, *3A and *3C] only explained 16% of the cases of leukopenia.[13]

Steady-state 6-TGN and 6-MMPR metabolite concentrations are generally reached after ~4–8 weeks of therapy.[14] The therapeutic range has been defined as a steady-state 6-TGN concentration between 235 and 490 pmol/8 × 10^8 red blood cells [RBCs].[15,16] The risk of leukopenia increases for 6-TGN steady-state concentrations higher than 490 pmol/8 × 10^8 RBCs, whereas 6-MMPR concentrations higher than 5700 pmol/8 × 10^8 RBCs are associated with hepatotoxicity.[14,15,17] In addition, extremely elevated 6-MMPR metabolites have also been associated with severe myelosuppression.[18–20]

Therapeutic drug monitoring of thiopurine metabolites is a helpful tool in strategies to reduce toxicity.[21] Since myelosuppression is linked to toxic steady-state 6-TGN and 6-MMPR metabolite concentrations, we hypothesize that elevations of these metabolites may already be detectable before steady-state levels are reached.[14,15,22,23] The aim of the present study was to evaluate the predictive value of 6-TGN and 6-MMPR metabolite concentrations [assessed 1 week after initiating thiopurine therapy] for the development of leukopenia during the first 8 weeks of thiopurine treatment.

2. Materials and Methods

2.1. Study design and outcome definition

The study was conducted in a subset of the patients from the TOPIC [Thiopurine response Optimization by Pharmacogenetics testing in Inflammatory bowel disease Clinics] trial, a randomized multicentre trial, including 769 IBD patients, that evaluated the effectiveness of pre-treatment *TPMT* genotyping. The TOPIC trial was approved by the Medical Ethics Committees of the participating centres. Written informed consent was obtained from all participating patients. The study is registered at clinicaltrials.gov [NCT00521950].

For a detailed description of the study design and patient selection of the TOPIC trial we refer to Coenen *et al.*[13]

Patients were enrolled by the TOPIC recruitment team [see Acknowledgments section for a list of TOPIC collaborators]. Patients meeting the inclusion criteria were randomly assigned to either standard thiopurine treatment [AZA 2–2.5 mg/kg/day or 6-MP 1–1.5 mg/kg/day] or a dose regimen based on the pre-treatment revealed *TPMT* genotype. Patients of the intervention group carrying a genetic variant were treated with 50% [heterozygous *TPMT*] or 0–10% [homozygous *TPMT*] of the standard thiopurine dose, according to the guidelines of the Dutch Pharmacogenetics Working Group.[24]

All patients were clinically followed for 20 weeks after treatment initiation. Safety was evaluated by measurement of haematological and biochemical parameters 1 week before initiation and at least at weeks 1, 2, 4, 6, 8 and 20 after initiation of thiopurine therapy.

For the present study, blood samples for 6-TGN and 6-MMPR assessment were collected 1 week [i.e. ~7 ± 1 days] after thiopurine initiation [*T1*].

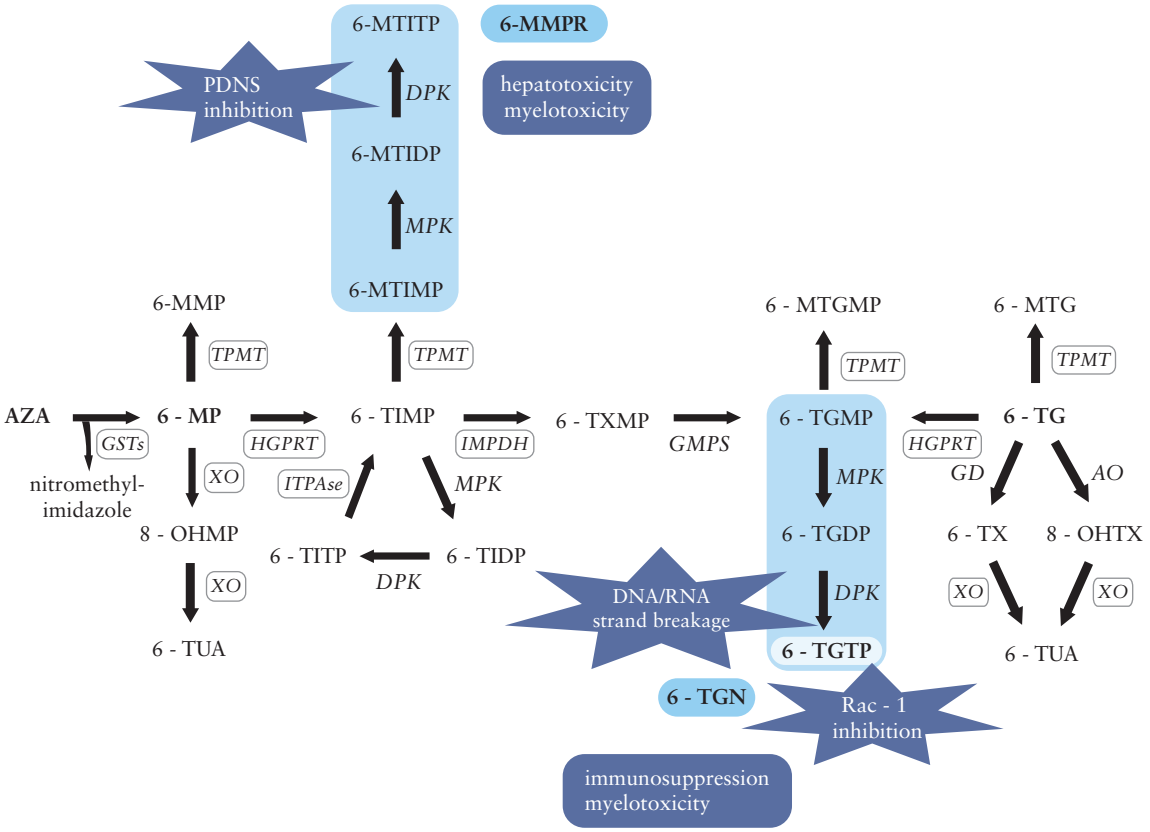

Figure 1. Proposed thiopurine metabolism. AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-MMP, 6-methylmercaptopurine; 8-OHMP, 8-hydroxy-6-mercaptopurine; 6-TUA, 6-thiouric acid; 6-MTIMP, 6-methylthioinosine monophosphate; 6-MTIDP, 6-methylthioinosine diphosphate; 6-MTITP, 6-methylthioinosine triphosphate; 6-TIMP, 6-thioinosine monophosphate; 6-TIDP, 6-thioinosine diphosphate; 6-TITP, 6-thioinosine triphosphate; 6-TXMP, 6-thioxanthosine monophosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGDP, 6-thioguanine diphosphate; 6-TGTP, 6-thioguanine triphosphate; 6-MTGMP, 6-methylthioguanine monophosphate; XO, xanthine oxidase; GST, glutathione S-transferase; TPMT, thiopurine S-methyl transferase; HGPRT, hypoxanthine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; GMPS, guanosine monophosphate synthetase; MPK, monophosphate kinase; DPK, diphosphate kinase; ITPase, inosine triphosphate pyrophosphatase; 6-TG, 6-thioguanine; 6-MTG, 6-methylthioguanine; 6-TX, 6-thioxanthine; 8-OHTX, 8-hydroxy-6-thioxanthine; GD, guanine deaminase; AO, aldehyde oxidase; PDNS, purine *de novo* synthesis; DNA, deoxyribonucleic acid; RNA, ribonucleic acid. 6-MTIMP, 6-MTIDP and 6-MTITP together form the 6-methylmercaptopurine ribonucleotides [6-MMPR]. 6-TGMP, 6-TGDP and 6-TGTP together form the 6-thioguaninenucleotides [6-TGN]. Enzymes encoded by genes that are subject to known genetic polymorphisms are circled in grey.

Leukopenia was defined by leukocyte counts of <3.0 × 10^9/L. Thiopurine dose adjustments were defined as thiopurine dose escalation or reduction. Any decision regarding dose adjustments or discontinuation of thiopurine therapy was made by the responsible physician and was recorded.

For comparison, patients of the first consecutive 272 patients of the TOPIC trial who completed a follow-up period of 8 weeks without leukopenia on stable thiopurine dose were eligible. Patients with thiopurine dose adjustments or thiopurine discontinuation during the first 8 weeks that were not related to leukopenia [a leukocyte count of <3.0 × 10^9/L] were excluded from the analysis.

Thiopurine metabolite concentrations were assessed in the first 272 consecutive included IBD patients and in the remaining patients included in the TOPIC trial who developed leukopenia in the first 8 weeks after treatment initiation.

In this study, the association between 6-TGN and 6-MMPR metabolite concentrations and leukopenia was evaluated; therefore, dose reduction based on prior-to-treatment *TPMT* genotyping in the course of the TOPIC trial did not interfere with the primary outcome.

The design of the study and the flow chart for the patient selection are depicted in Figure 2.

2.2. Measurement of 6-TGN and 6-MMPR metabolite concentrations

The 6-TGN and 6-MMPR metabolite concentrations were measured after the follow-up period of the TOPIC trial. The thiopurine metabolite concentrations were determined by the modified high performance liquid chromatography [HPLC] method of Lennard and Singleton, as published previously.[14] The lower limits of quantification for 6-TGN and 6-MMPR metabolite levels were 40 pmol/8 × 10^8 RBCs and 300 pmol/8 × 10^8 RBCs, respectively. To secure metabolite stability, blood samples were immediately stored in the refrigerator [2–8°C] and subsequently sent to the laboratory of the Department of Clinical Pharmacy and Toxicology of the Orbis Medical Center [currently Zuyderland Medical Center, Sittard-Geleen, The Netherlands], where the erythrocytes were washed, counted and stored at –20°C until required.

2.3. Data analysis

Descriptive data analysis was in the form of percentages for categorical variables, and in the form of median values and ranges for continuous characteristics. Box-and-whisker plots were used in graphical representations. Differences in patient characteristics were evaluated using Pearson's chi-square or Fisher's exact tests; for

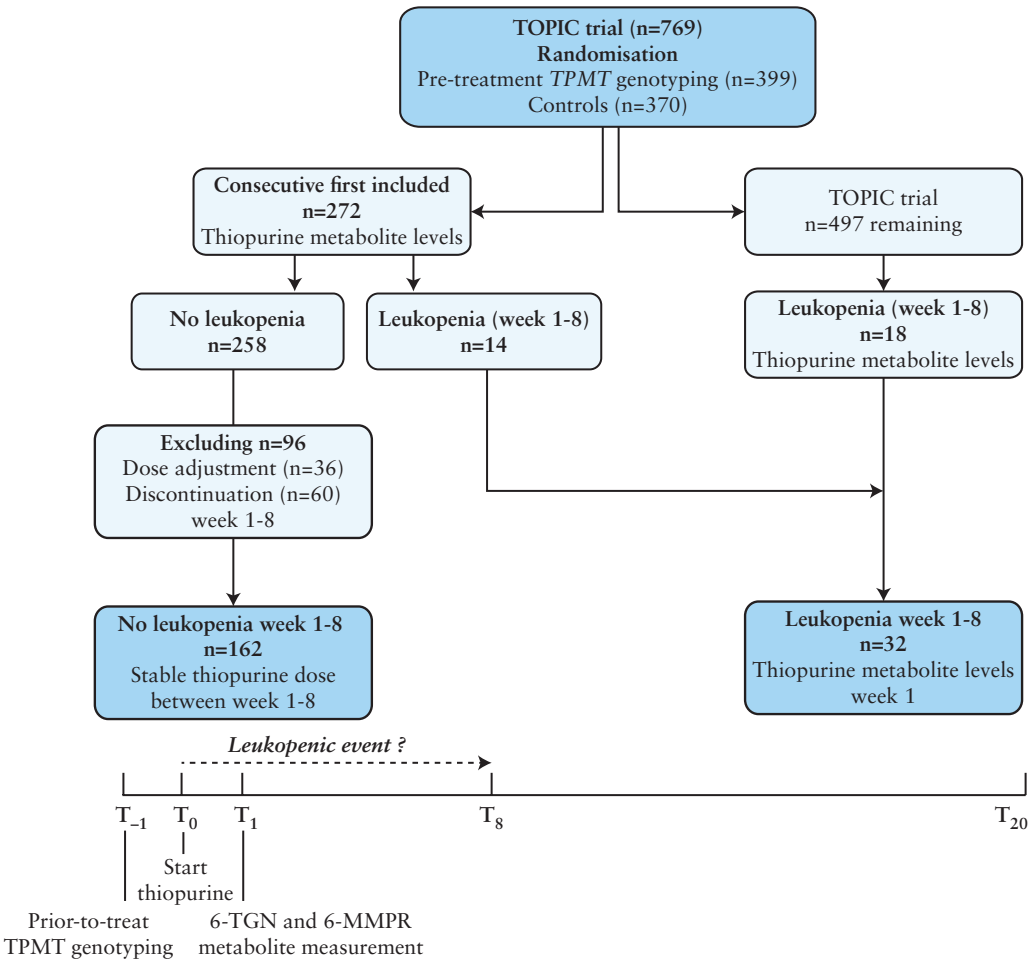

Figure 2. Study design and time-line within the TOPIC-trial.

differences in metabolite concentrations, the Mann-Whitney U test or Kolmogorov–Smirnov Z test were applied, when appropriate. A *p*-value of <0.05 [two-sided] was considered to be statistically significant. SPSS for Windows version 20.0 was used for all data analysis.

To study the discriminative power of 6-TGN and 6-MMPR concentrations regarding leukopenia, Receiver Operating Characteristic [ROC] curves were generated, which plot sensitivity rates and false positive rates [1 – specificity] regarding the occurrence of leukopenia. As a summary measure, the area under the ROC curves [AUC] and 95% confidence interval [95% CI] were calculated.

The predictive power of the metabolites for the development of leukopenia was studied by logistic regression modelling taking covariates into account such as age, gender, disease type [CD/UC], and medication [thiopurine type, thiopurine dose and concurrent use of known potential myelotoxic drugs or drugs interfering with thiopurine metabolism, e.g. mesalazine [5-ASA], or anti-TNF therapy (i.e. infliximab [IFX], adalimumab [ADA]).

The results are expressed as odds ratios [ORs] with 95% confidence intervals.

2.4. Ethics approval

The TOPIC study protocol was approved by the Institutional Review Board of the Radboud University Medical Center [Commissie Mensgebonden Onderzoek Regio Arnhem Nijmegen, protocol number: 13171]; approval for inclusion of patients in other institutes was obtained from the institutional ethics committee.

3. Results

3.1. Baseline cohort analysis

Fourteen [5%] of the first 272 consecutive IBD patients included in the TOPIC trial developed leukopenia during the first 8 weeks. Ninety-six [37%] of the 258 patients without a leukopenic event were excluded from the analysis due to thiopurine dose adjustments [*n* = 36, 14%] or treatment discontinuation [*n* = 60, 23%] during the first 8 weeks, leaving 162 patients without leukopenia for evaluation. Eighteen patients of the remaining 497 patients [4%] included in the TOPIC trial developed leukopenia during the first 8 weeks of thiopurine therapy and were additionally included, resulting in a total number of 32 patients with leukopenia [Figure 2].

The baseline characteristics of the leukopenic and non-leukopenic patient groups are given in Table 1.

Overall, gender, age, IBD type, and thiopurine dose were similar in both patient groups.

The median time to onset of leukopenia was 4.8 weeks [range 1.0–8.3] after thiopurine initiation.

Leukopenia rates were higher in patients treated with 6-MP, when compared with rates in those treated with AZA (OR 7.3 [95% CI: 3.1–17.0]). Patients concomitantly treated with anti-TNF therapy at baseline developed leukopenia significantly more often, when compared with patients without that treatment (OR 5.1 [95% CI: 1.6–16.4]).

The 6-TGN and 6-MMPR concentrations at week 1 were higher in patients treated with 6-MP, compared with the concentrations

Table 1. Baseline characteristics of the study population.

	All patients	Leukopenia	No leukopenia	*p*-value
Patients [*n*]	194	32	162	
Age in years [median, range]	38 [19–81]	43 [19–76]	40 [19–81]	0.160
Gender [male/female]	86/108	14/18	72/90	0.942
Bodyweight [median, kg]	70 [40–160]	65 [50–160]	71 [40–100]	0.411
IBD				
Crohn's disease*	112	16 [50%]	96 [59%]	
Ulcerative colitis*	81	16 [50%]	65 [40%]	0.313*
Indeterminate colitis	1	0	1	
Disease duration in years [median; range] [missing n = 1/1]	2.1 [0–50]	4.1 [0–50]	1.7 [0–42]	0.171
Medication				
Azathioprine/6-mercaptopurine	129/65	9/23	120/42	<0.000001
Azathioprine [*n*]	129 [66%]	9 [28%]	120 [74%]	
median AZA dose in mg [range]	150 [50–250]	150 [50–225]	150 [50–250]	0.723
median AZA dose in mg/kg [range]	2.2 [0.8–3.1]	2.2 [1.3–2.3]	2.2 [0.8–3.1]	0.781
6-mercaptopurine [*n*]	65 [34%]	23 [72%]	42 [26%]	
median 6-MP dose in mg [range]	75 [25–150]	75 [25–150]	75 [50–125]	0.782
median 6-MP dose in mg/kg [range]	1.2 [0.4–1.5]	1.2 [0.4–1.5]	1.2 [0.6–1.4]	0.351
Concurrent IBD medication [*n*]				
5-ASA	110 [57%]	16 [50%]	94 [58%]	0.402
Corticosteroids	160 [82%]	25 [78%]	135 [83%]	0.479
Anti-TNF-α [i.e. IFX, ADA]	13 [7%]	6 [19%]	7 [4%]	0.006

* The asterisk of the p-value correspond to the patients of Crohn's disease and ulcerative colitis; (the one patient with Indeterminate colitis was excluded).

in those treated with AZA. In addition, 6-MMPR concentrations were higher in women at week 1, compared with those in men. Concomitant treatment with systemic corticosteroids, 5-ASA, or anti-TNF-α treatment did not influence the 6-TGN or 6-MMPR concentrations at week 1 [Table 2].

3.2. Thiopurine metabolite concentrations at week 1 in relation to leukopenia

At week 1, the median 6-TGN concentration in patients who developed leukopenia within the first 8 weeks [*n* = 32] was higher than in patients who did not [*n* = 162]: 240 [83–641] and 151 [40–564] pmol/8 × 10^8 RBCs, respectively [$p < 0.0001$, Figure 3a]. The 6-MMPR levels were also higher in patients who developed leukopenia (4051 [300–13 576] versus 1834 [300–7996] pmol/8 × 10^8 RBCs, $p < 0.0001$, Figure 3b]. The median values of the 6-MMPR/6-TGN ratio did not differ between the leukopenic and non-leukopenic patients: 17 [1–117] and 14 [1–64], respectively [$p = 0.190$].

As concomitant anti-TNF therapy was associated with the development of leukopenia, we also performed an analysis excluding 13 patients treated with IFX or ADA. This revealed median 6-TGN levels of 273 [83–641] pmol/8 × 10^8 RBCs in leukopenic patients [*n* = 26] and 151 [40–564] pmol/8 × 10^8 RBCs in non-leukopenic patients [*n* = 155] [$p < 0.0001$]. The median 6-MMPR concentrations were 3725 [430–13 348] and 1849 [300–7996] pmol/8 × 10^8 RBCs, respectively [$p = 0.001$].

The area under the ROC curves of the continuous 6-TGN concentration for the occurrence of leukopenia was 0.73 [95% CI: 0.63–0.84], and 0.72 [95% CI: 0.61–0.83] for 6-MMPR [Figure 4]. Excluding patients with concurrent anti-TNF therapy at baseline resulted in areas under the ROC curves of 0.76 [95% CI: 0.64–0.87] for 6-TGN and 0.70 [95% CI: 0.58–0.82] for 6-MMPR.

3.3. 6-TGN and 6-MMPR threshold values

Based on contingency tables of *T1* 6-TGN and 6-MMPR concentration quartiles for the development of leukopenia, we defined threshold values for 6-TGN to be 213 pmol/8 × 10^8 RBCs and 3525 pmol/8 × 10^8 RBCs for 6-MMPR [75th percentiles]. The associated

Table 2. Thiopurine metabolite concentrations at week 1 for various patient characteristics.

	Patient number [*n*]	T1 6-TGN[a]	T1 6-MMPR[a]
All patients	194	159 [40–641]	2133 [300–13576]
Gender			
Male	86	150 [42–528]	1605 [300–13576]
Female	108	168 [40–641]	2750 [300–11705]
p-value		0.165	0.001
IBD			
Crohn's disease	112	166 [40–641]	2283 [300–13576]
Ulcerative colitis	81	147 [42–607]	1798 [300–13348]
p-value[b]		0.286	0.402
Thiopurine type			
AZA	129	139 [40–607]	1812 [300–13576]
6-MP	65	207 [76–641]	2725 [300–13348]
p-value		<0.000001	0.009
Concurrent IBD medication			
5-ASA			
Yes	110	164 [40–607]	2006 [300–11705]
No	84	156 [40–641]	2185 [300–13576]
p-value		0.259	0.398
Corticosteroids			
Yes	170	156 [40–641]	2133 [300–13576]
No	35	163 [40–607]	2171 [300–11230]
p-value		0.168	0.753
Anti-TNF-α [IFX, ADA]			
Yes	13	176 [51–459]	3241 [300–13576]
No	181	158 [40–641]	2092 [300–13348]
p-value		0.484	0.258

[a]6-TGN and 6-MMPR concentrations [pmol/8 × 10^8 RBCs] are presented as median values and ranges; [b]*n* = 1 missing [indeterminate colitis]; 6-TGN, 6-thioguanine nucleotides; 6-MMPR, 6-methylmercaptopurine ribonucleotides.

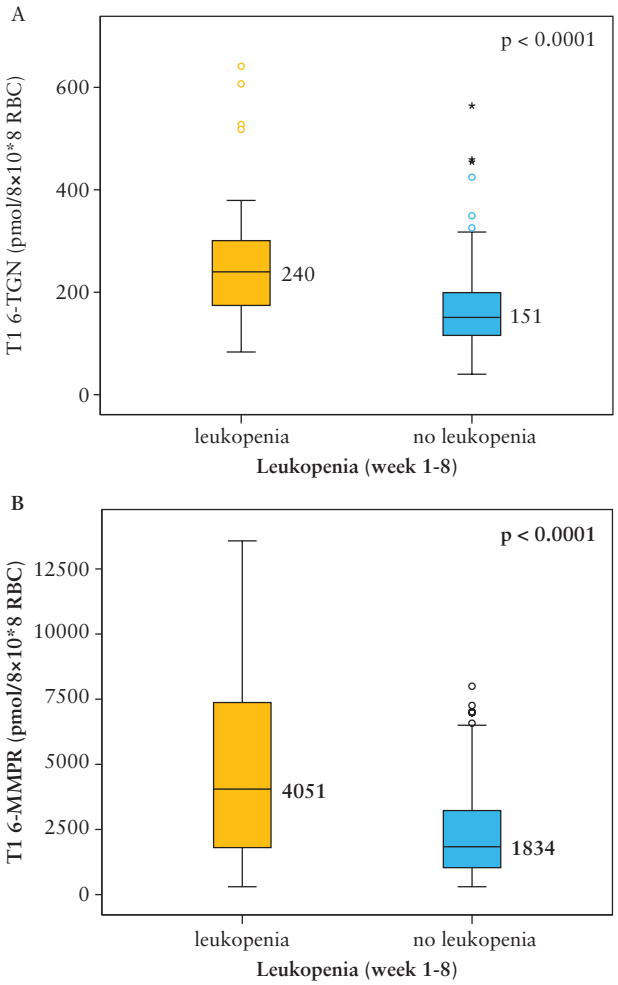

Figure 3. Box-and-whisker plots of 6-TGN [a] and 6-MMPR [b] concentrations at week 1 in patients who developed leukopenia [$n = 32$] and patients who did not develop leukopenia [$n = 162$] in weeks 1–8.

specificity and sensitivity rates of 80% and 59%, respectively, were considered as to be clinically useful. Patients with 6-TGN levels >213 pmol/8 × 10^8 RBCs were at significantly increased risk of leukopenia, OR 6.2 [95% CI: 2.8–13.8]. A similar risk was observed for patients with 6-MMPR levels >3525 pmol/8 × 10^8 RBCs, OR 5.9 [95% CI: 2.7–13.3]. The discriminative performance of 6-TGN and 6-MMPR dichotomized at the threshold measured by ROC analysis revealed an AUC = 0.70 [95% CI: 0.59–0.81] for both.

Six of 32 patients [19%] with leukopenia showed 6-TGN levels >213 pmol/8 × 10^8 RBCs, and an additional 6 patients [19%] showed 6-MMPR >3525 pmol/8 × 10^8 RBCs at week 1. Thirteen patients [41%] showed both elevated 6-TGN and 6-MMPR levels. Thus, in 25 of 32 patients [78%], leukopenia can be explained by elevated 6-TGN and/or 6-MMPR metabolites at week 1.

The predictive power for three cut-off values of 6-TGN and 6-MMPR concentrations at *T1*, set at specificity rates of 80, 90, and 95%, are presented in Table 3. Odds ratios serve as an overall measure, whereas positive and negative likelihood ratios [true positive versus false positive and false negative versus true negative] facilitate medical practice. The ranges of OR, positive and negative likelihood ratios do not reveal preference for a specific threshold value.

3.4. Prediction model for leukopenia, including thiopurine type and concurrent anti-TNF treatment

A multivariable logistic regression analysis [$n = 194$] was performed based on the 6-TGN and 6-MMPR threshold parameters, thiopurine type [6-MP or AZA], and concomitant anti-TNF treatment to predict the development of leukopenia. Patients treated with AZA monotherapy and both 6-TGN and 6-MMPR levels below the defined threshold values were used as the reference group.

Patients treated with 6-MP monotherapy exceeding either the 6-MMPR or 6-TGN threshold concentration were at increased risk of leukopenia (OR 10.2 [95% CI: 2.0–51.6] and OR 11.7 [95% CI: 2.5–55.2], respectively]. Patients with both elevated 6-TGN and 6-MMPR concentrations were at highly increased risk of developing leukopenia: OR 65.3 [95% CI: 11.1–386] and OR 28.0 [95% CI: 2.9–271.9] for 6-MP and AZA therapy, respectively. The risk of

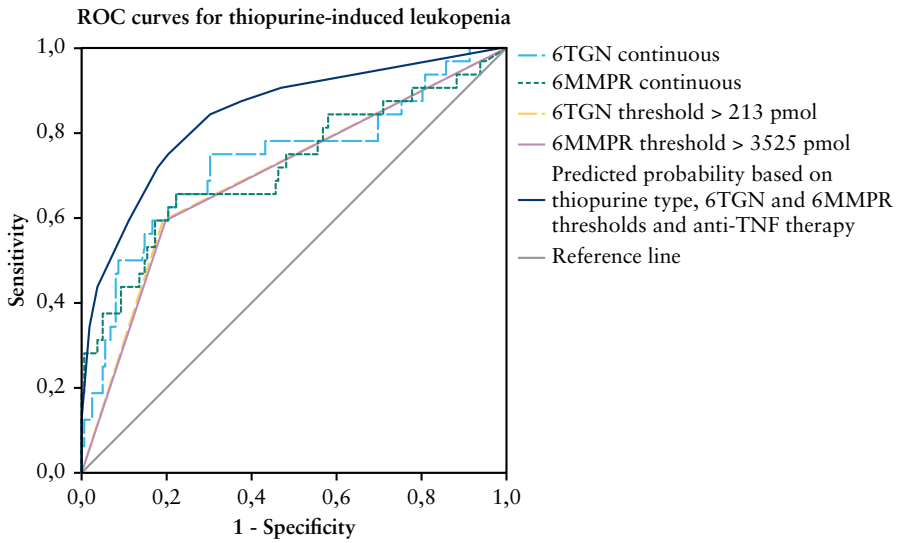

Figure 4. Receiver operating characteristic [ROC] curves for 6-TGN and 6-MMPR metabolite concentrations at week 1 with the threshold parameters, thiopurine type and concomitant anti-TNF therapy.

Table 3. Predictive power for three cut-off threshold values of 6-TGN and 6-MMPR concentrations set on specificity rates of 80%, 90% and 95% for thiopurine-induced leukopenia.

Threshold value[a]	Specificity [%]	Sensitivity [%]	Odds ratio [95% CI]	Positive likelihood ratio	Negative likelihood ratio
6-TGN					
213	80	59	6.2	3	0.5
245	90	50	9.1	5	0.6
301	95	25	6.4	5	0.8
6-MMPR					
3525	80	59	5.9	3	0.5
5220	90	44	7.1	4	0.6
6270	95	38	11.6	8	0.7

[a]Thiopurine metabolite concentrations are given in pmol/8×10^8 RBC; 6-TGN, 6-thioguanine nucleotides; 6-MMPR, 6-methylmercaptopurine ribonucleotides.

leukopenia was highest among patients concomitantly treated with anti-TNF therapy with elevated 6-TGN and 6-MMPR concentrations, as all patients [$n = 4$] treated with 6-MP developed leukopenia. In this category there were no patients treated with AZA.

The area under the ROC curve for the obtained predicted probabilities based on the four parameters was 0.84 [95% CI: 0.76–0.92] [Figure 4].

Validation of the predictive algorithm among the first 272 included patients, who were on stable thiopurine dose during the first 8 weeks [$n = 176$, 14 patients with and 162 patients without leukopenia, respectively] resulted in an area under the ROC curve of 0.79 [95% CI: 0.65–0.93].

4. Discussion

In this prospective study we showed for the first time that elevated 6-TGN and 6-MMPR metabolite levels assessed 1 week after initiation are highly predictive for thiopurine-induced leukopenia during the first 8 weeks of thiopurine therapy in IBD patients. Almost 80% of the leukopenia cases were explained by elevated 6-TGN and/or 6-MMPR metabolite concentrations at week 1.

In previous studies the relation between thiopurine metabolites and leukopenia has been studied during steady-state maintenance therapy; however, leukopenia mostly occurs in the early stage of therapy, before steady-state concentrations are reached.[15,20] Therefore, in clinical practice steady-state thiopurine metabolite concentrations are less useful for identifying patients at risk of developing leukopenia during the first weeks of treatment.

ROC analyses of the *T1* 6-TGN and 6-MMPR concentrations predicting leukopenia revealed an area under the ROC curve of 0.73 and 0.72, respectively, indicating a proper discriminative performance for both thiopurine metabolites.

Until now, myelotoxicity has mainly been attributed to elevated 6-TGN concentrations.[15,17] Surprisingly, we observed that the predictive value for the occurrence of leukopenia of the 6-MMPR metabolites at week 1 is similar to that of the 6-TGN metabolites. Only a limited number of studies related the development of leukopenia to the methylated thiopurine metabolites. Hindorf *et al.* reported that the first methylated metabolite of the 6-MMPR, 6-methylthioinosine monophosphate [6-MTIMP], early in the steady-state phase was associated with myelotoxicity in IBD patients following a dose escalation schedule for AZA and 6-MP.[20] In addition, two case reports also indicated that extremely elevated 6-MMPR levels with subtherapeutic 6-TGN levels can cause severe myelosuppression.[18,19]

The interpretation of the findings from previous studies on the relation between thiopurine metabolites and myelotoxicity is complex because different definitions of leukopenia and myelosuppression were often used, and the association was mostly studied during various phases of thiopurine therapy. In addition, different thiopurine metabolites were evaluated, and they were determined by different analytical HPLC methods in a relatively small number of pat ients.[14,15,20,25,26] These factors make the data hard to compare and to apply in clinical practice.

The present study results show that both 6-TGN and 6-MMPR are independently correlated with leukopenia and that the cytotoxic effect of one is presumably enhanced by the other. The cytotoxic effect of the 6-TGN has been ascribed to them being incorporated into DNA, acting as fraudulent bases, resulting in strand breakage.[27,28] In addition, the 6-MMPR, in particular the cytotoxic metabolite 6-MTIMP, have been shown to inhibit purine *de novo* synthesis [PDNS], leading to inhibition of DNA formation, decreased cell proliferation, and cytotoxic effects.[28,29] AZA and 6-MP may mainly exert their cytotoxic effect through PDNS inhibition by 6-MTIMP, independently of 6-TGN formation.[28] The latter may explain, at least for a part, the fact that thiopurine-induced leukopenia is not always related to elevated 6-TGN concentrations due to low or absent TPMT enzyme activity.

Myelosuppression is often multifactorial or caused by other factors, such as viral infections [e.g. Parvovirus B19, *Varicella zoster*], medication causing myelosuppression by itself [e.g. captopril, metronidazole or trimethotrim-sulfamethoxazol], or medication interfering with thiopurine metabolism [i.e. allopurinol, mesalazine].[11,30] Viral infections have not been evaluated in our study, due to lack of data. We did evaluate concomitant IBD medication [mesalazine and anti-TNF therapy] in association with the occurrence of leukopenia. Concurrent treatment of 5-ASA has been demonstrated to result in elevated 6-TGN concentrations, which may lead to an increased risk for development of myelotoxicity.[31–33] In the present study we could not confirm this for metabolite concentrations at week 1 and the occurrence of leukopenia during the first 8 weeks of treatment. However, anti-TNF agents appeared to be an independent risk factor for leukopenia in patients concomitantly treated with thiopurines, while thiopurine metabolite concentrations at week 1 were not affected. Consequently, this finding indicates that the myelotoxic properties of thiopurines may be potentiated by additional myelotoxic effects of anti-TNF agents, as we have previously described for concurrent ADA therapy in a small CD patient group showing mainly low-therapeutic 6-TGN concentrations during thiopurine maintenance therapy.[34] This finding is clinically relevant because

combined anti-TNF and thiopurine therapy seems to have beneficial therapeutic effects in the treatment of moderate-to-severe active IBD[35–37] and is recommended for minimizing the immunogenicity of anti-TNF monoclonal antibodies to prevent loss of response.

Some remarks should be made concerning our study design and patient selection. First, leukopenia was defined by a leukocyte count of $<3.0 \times 10^9$/L in order to study the relation between thiopurine metabolites and moderate-to-severe leukopenia. Unfortunately, we were unable to evaluate the 6-TGN and 6-MMPR metabolite concentrations during any leukopenic event. Second, patients for whom the thiopurine dose was adjusted by the clinician due to any adverse event not related to leukopenia were excluded from analysis. Consequently, patients whose dose was reduced due to a mild leukopenia [leukocyte count $3.0–4.0 \times 10^9$/L] were not evaluated, possibly explaining the moderate sensitivity [both 59%] of the defined predictive 6-TGN and 6-MMPR threshold values for developing leukopenia. The moderate sensitivity rates may also be explained by the fact that the metabolite concentrations were assessed in erythrocytes, as a surrogate marker for the actual target cells, the leukocytes. Furthermore, patients who developed leukopenia after the follow-up period of 8 weeks of treatment were not evaluated. Third, in the present study patients treated with 6-MP were at higher risk of leukopenia, compared with patients treated with AZA. Although AZA and 6-MP in all patients were per protocol equivalently dosed [AZA 2–2.5 mg/kg/day and 6-MP 1–1.5 mg/kg/day], this observation may be partly explained by the fact that the concentrations of 6-TGN and 6-MMPR were both higher in patients treated with 6-MP. No difference in 6-MP dose [mg/kg] was observed between patients who did, or did not, develop leukopenia, which indicates that individual thiopurine metabolism is more important than the dose.[14]

The formation of 6-TGN and 6-MMPR metabolites is often inversely related within each individual, so that the predictive value of one may be affected by the other. In addition, patients treated with 6-MP and concurrent anti-TNF therapy were more likely to develop leukopenia. Multivariable regression analysis of these determinants revealed that patients with both 6-TGN and 6-MMPR above the predictive threshold concentrations had a highly increased risk of leukopenia, followed by that of patients with either 6-TGN or 6-MMPR levels above the defined thresholds at week 1. Patients on concurrent anti-TNF therapy with elevated *T1* 6-TGN and 6-MMPR levels all developed leukopenia. Consequently, based on different combinations of the aforementioned parameters, patients can be classified using this accurate predictive algorithm [AUC = 0.84] to assess the degree of risk for the development of thiopurine-induced leukopenia.

Early identification of patients at increased risk of leukopenia is important because it may reduce morbidity and hospitalization, or even mortality.

Over the past decade, the usefulness of pre-treatment *TPMT* geno- or phenotyping in daily practice has frequently been debated. Since *TPMT* genotyping is the more reliable test, especially in *TPMT* heterozygotes, it has been suggested that genotyping should be considered the primary choice for pre-treatment *TPMT* screening.[38]

Prior-to-treatment *TPMT* genotype-based dosing results in a significant reduction in leukopenia in IBD patients carrying a genetic *TPMT* variant, importantly without loss of treatment efficacy.[13] Diagnostic test results should generally be obtained within 5–10 working days by using proper logistic arrangements. Furthermore, the cost-effectiveness has repeatedly been stated.[39]

On the other hand, *TPMT* testing only explains a small part of thiopurine-induced myelotoxicity.[11–13] Thiopurine metabolism is complex and unpredictable due to the involvement of the great variety of metabolic enzymes, of which TPMT is only one.[8] In the present study we demonstrated that thiopurine-induced leukopenia also strongly correlated with elevated 6-MMPR metabolites. *TPMT* screening only gives the clinician information about a possible excessive 6-TGN formation, but nothing about the rate of 6-MMPR metabolite formation.[40]

Patients who show excessive 6-MMPR formation resulting from a skewed metabolism are also at risk for early thiopurine failure due to intolerable adverse events or refractoriness.[41] In these cases, precautionary measures can be taken by reducing the thiopurine dose to 25–30% in combination with 100 mg allopurinol per day[42] or by a switch to 6-thioguanine [0.3 mg/kg bodyweight per day, max. 25 mg per day; Figure 1], which has been proposed as an alternative thiopurine in IBD patients failing to tolerate or to respond to conventional thiopurine therapy.[43]

Assessment of thiopurine metabolites at week 1 is an attractive way to reveal a patient's 'ultimate phenotype', displayed by the most important active metabolites, 6-TGN and 6-MMPR.

In future research, the proposed algorithm should be validated in a prospective randomized controlled intervention study evaluating whether optimizing strategies based on the *T1* metabolite profile will prevent the occurrence of leukopenia. Until then, complementary to pre-treatment *TPMT* testing, *T1* thiopurine metabolite assessment may be helpful for identifying patients at risk of developing leukopenia due to high cytotoxic 6-TGN and 6-MMPR concentrations resulting from causes other than a TPMT deficiency, such as overdosing or a skewed thiopurine metabolism. Intensive safety monitoring and adequate thiopurine dose reduction is then advocated.

Therapeutic drug monitoring during steady state can be used to evaluate pharmacological action, for dose optimization purposes or to evaluate patient compliance.

In conclusion, both 6-TGN and 6-MMPR metabolites accurately predict the occurrence of leukopenia during the first 8 weeks of therapy. Assessment of 6-TGN and 6-MMPR metabolite concentrations 1 week after thiopurine initiation helps to identify patients at risk of developing thiopurine-induced leukopenia.

Funding

This work was supported by The Netherlands Organization for Health Research and Development [grant number 94507606] and the participating institutes.

Conflict of Interest

None. The funding organization had no role in the design or conduct of the study, collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Acknowledgments

We thank the patients for participation in the study. We thank Marlies Naber, Johanne Groothuismink, Marielle Maas, Miet Fiddelaers, Milevis Reitsma, Leonie Peters, and Jean Cilissen for their skilful technical assistance, and Debbie Heinen, Marjolein van Donkelaar, Fresteh Golestani, Marlies de Vos, Angelien Heister, Domenique Nijsten, Mascha Schijvenaars, and Martine Cranen for their support in data-management.

We are grateful to Wilbert H.M. Peters and René H.M. te Morsche [Department of Gastroenterology, Radboud University Medical Center, Nijmegen, The Netherlands] for the enzyme activity measurements.

We want to thank Prof. Dr Ad A.M. Masclee, Prof. Dr Kees Neef, and Dr Ad A. van Bodegraven for their helpful advice regarding intellectual content.

The TOPIC recruitment team was responsible for patient recruitment and collection of clinical data. Compensation was given to the members of the recruitment team for additional biochemical measurements and examinations that had to be performed for the TOPIC study. TOPIC recruitment team members are: *Department of Gastroenterology, Academisch Ziekenhuis Maastricht, Maastricht, The Netherlands*—AAM Masclee, MD PhD; M Pierik, MD PhD; W Mares, MD; W Hameeteman, MD PhD; *Department of Gastroenterology, Rijnstate Ziekenhuis Arnhem, Arnhem, The Netherlands*—PJ Wahab, MD PhD; H Seinen, MD PhD; *Department of Gastroenterology, Amphia Ziekenhuis, Breda, The Netherlands*—MCM Rijk, MD PhD; IM Harkema, MD; *Department of Gastroenterology, Atrium Medisch Centrum, Heerlen, The Netherlands*—M de Bièvre, MD; L Oostenbrug, MD PhD; CM Bakker, MD PhD; M Aquarius, MD; C van Deursen, MD PhD; AB van Nunen, MD PhD; JG Goedhard, MD PhD; M Hamacher, MD; *Department of Gastroenterology, Bernhoven Hospital, Oss, The Netherlands*—IAM Gisbertz, MD PhD; BJ Brenninkmeijer, MD PhD; *Department of Gastroenterology, Canisius Wilhelmina Ziekenhuis*, Nijmegen, *The Netherlands*—ACITL Tan, MD PhD; MN Aparicio-Pagés, MD PhD, EM Witteman, MD PhD; *Department of Gastroenterology, Diakonessenhuis, Utrecht, The Netherlands*—SAC van Tuyl, MD; R Breumelhof, MD PhD; *Department of Gastroenterology, Catharina Ziekenhuis, Eindhoven, The Netherlands*—A Stronkhorst, MD PhD; LPL Gilissen, MD PhD; EJ Schoon, MD PhD; *Department of Gastroenterology, Elkerliek Ziekenhuis, Helmond, The Netherlands*—JWM Tjhie-Wensing, MD; A Temmerman, MD*; HagaZiekenhuis, 's-Gravenhage, The Netherlands*—JJ Nicolaï, MD PhD; *Department of Gastroenterology, Gelderse Vallei Hospital, Ede, The Netherlands*—JD van Bergeijk, MD PhD; DJ Bac, MD PhD; BJM Witteman, MD PhD; N Mahmmod, MD; JJ Uil, MD PhD; H Akol, MD PhD; *Department of Gastroenterology, Ikazia Hospital, Rotterdam, The Netherlands*—RJTh Ouwendijk, MD PhD; *Department of Gastroenterology, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands*—IP van Munster, MD PhD; M Pennings, MD; AMP De Schryver, MD PhD; ThJM van Ditzhuijsen, MD PhD; RCH Scheffer, MD PhD; TEH Römkens, MD; DL Schipper, MD PhD; *Department of Gastroenterology, Laurentius Hospital, Roermond, The Netherlands*—PJ Bus, MD; *Department of Gastroenterology, Máxima Medisch Centrum, Eindhoven-Veldhoven, The Netherlands*—JWA Straathof, MD PhD; ML Verhulst, MD PhD; PJ Boekema, MD PhD; JTh Kamphuis, MD; HJ van Wijk, MD PhD; JMJL Salemans, MD PhD; *Department of Gastroenterology, Meander MC, Amersfoort, The Netherlands*—JR Vermeijden, MD; *Department of Gastroenterology, MC Haaglanden, Den Haag, The Netherlands*—SDJ van der Werf, MD PhD; RJ Verburg MD PhD; *Department of Gastroenterology, Medisch Centrum Leeuwarden, Leeuwarden, The Netherlands*—P Spoelstra, MD PhD; JML de Vree, MD PhD; K van der Linde, MD PhD; HJA Jebbink, MD PhD; M. Jansen; H. Holwerda; *Department of Gastroenterology, Medisch Spectrum Twente, Enschede, The Netherlands*—N van Bentem, MD; JJ Kolkman, MD PhD; MGVM Russel, MD PhD; GH van Olffen, MD; MJ Kerbert-Dreteler, MD; M Bargeman, MD PhD; JM Götz, MD PhD; R Schröder, MD; *Department of Gastroenterology, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands*—JM Jansen, MD; *Department of Gastroenterology, Zuyderland Medisch Centrum [formerly Orbis Medisch Centrum], Sittard-Geleen, The Netherlands*—LP Bos, MD PhD; LGJB Engels, MD PhD; MJL Romberg-Camps, MD; ETP Keulen, MD PhD; *Department of Gastroenterology, Radboud University Medical Center, Nijmegen, The Netherlands*—AAJ van Esch, MD; JPH Drenth, MD PhD; MCA van Kouwen, MD PhD; GJA Wanten, MD PhD; TJ Bisseling, MD PhD; TEH Römkens, MD; MWJ van Vugt; *Department of Gastroenterology, Slingeland Hospital, Doetinchem, The Netherlands*—PC van de Meeberg, MD PhD; SJ van den Hazel, MD PhD; *Department of Gastroenterology, St Elisabeth Ziekenhuis, Tilburg, The Netherlands*—WNHM Stuifbergen, MD PhD; MJAL Grubben, MD PhD; U de Wit, MD PhD; GAH Dodemont, MD PhD; RF Eichhorn, MD; *Department of Gastroenterology, Tergooiziekenhuizen, Blaricum-Hilversum, The Netherlands*—JMH van den Brande, MD PhD; AHJ Naber, MD PhD; EJ van Soest, MD PhD; PJ Kingma, MD PhD; *Department of Gastroenterology, TweeSteden Ziekenhuis, Tilburg, The Netherlands*—NC Talstra, MD; KF Bruin, MD PhD; FHJ Wolfhagen, MD PhD; *Department of Gastroenterology, University Medical Centre Leiden, Leiden, The Netherlands*—DW Hommes, MD PhD; PPJ van der Veek, MD PhD; JCA Hardwick, MD PhD; RJ Stuyt, MD PhD; HH Fidder, MD; *Department of Gastroenterology, University Medical Centre Utrecht, Utrecht, The Netherlands*—B Oldenburg, MD PhD; *Department of Gastroenterology, Ziekenhuisgroep Twente, Hengelo, The Netherlands*—TG Tan, MD.

Author Contributions

All authors listed were involved in the conception and design of the study. CvW, MC, and DdJ collected data. Thiopurine metabolite measurements were performed under the guidance of DW and PH. Data analysis was performed by DW, assisted by MC, SV, and AV. Interpretation of data was performed by DW, PH, MC, SV, and AV, with the intellectual input of LE, HS, OK, BF, and HJG. BF was supervisor of the TOPIC study. BF, HS, and HJG obtained funding. The manuscript was drafted by DW, assisted by MC, PH, LD, LE, and SV. Members of the TOPIC recruitment team enrolled patients and recorded clinical data. All authors revised the manuscript critically for important intellectual content and gave approval for the final version of the article, including the authorship list.

Conference presentations of the work

Dutch Society of Gastroenterology, Veldhoven, The Netherlands, March 2014, oral presentation [*'Best abstract' award*, section Inflammatory Bowel Diseases]. Dutch Society of Clinical Pharmacology & Biopharmacy, Leiden, The Netherlands; April 2014, oral presentation.9th Congress of ECCO, Copenhagen, Denmark,
February 2014; digital poster presentation, DOP035. Initiative on Crohn and Colitis [initiative of Dutch Gastroenterologists to improve patient care, education and medical science], September 2013; oral presentation of preliminary data.

References

1. Prefontaine E, Macdonald JK, Sutherland LR. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2009:CD000545.
2. Prefontaine E, Sutherland LR, Macdonald JK, Cepoiu M. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2009:CD000067.
3. Timmer A, McDonald JW, Macdonald JK. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2007:CD000478.
4. Schwab M, Schaffeler E, Marx C, *et al*. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. *Pharmacogenetics* 2002;**12**:429–36.
5. Jharap B, Seinen ML, de Boer NK, *et al*. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis* 2010;**16**:1541–9.
6. Chaparro M, Ordas I, Cabre E, *et al*. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis* 2013;**19**:1404–10.
7. Gisbert JP, Gomollon F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: a review. *Am J Gastroenterol* 2008;**103**:1783–800.
8. Derijks LJ, Wong DR Pharmacogenetics of thiopurines in inflammatory bowel disease. *Curr Pharm Des* 2010;**16**:145–54.
9. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980;**32**:651–62.
10. Higgs JE, Payne K, Roberts C, Newman WG. Are patients with intermediate TPMT activity at increased risk of myelosuppression when taking thiopurine medications? *Pharmacogenomics* 2010;**11**:177–88.
11. Colombel JF, Ferrari N, Debuysere H, *et al*. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology* 2000;**118**:1025–30.
12. Dewit O, Moreels T, Baert F, *et al*.; Belgian Inflammatory Bowel Disease Research Group (BIRD). Limitations of extensive TPMT genotyping in the

management of azathioprine-induced myelosuppression in IBD patients. *Clin Biochem* 2011;**44**:1062–6.
13. Coenen MJ, de Jong DJ, van Marrewijk CJ, *et al*. Identification of patients with variants in TPMT and dose reduction reduces hematologic events during thiopurine treatment of inflammatory bowel disease. *Gastroenterology* 2015;**149**:907–17.
14. Derijks LJ, Gilissen LP, Engels LG, *et al*. Pharmacokinetics of 6-mercaptopurine in patients with inflammatory bowel disease: implications for therapy. *Ther Drug Monit* 2004;**26**:311–8.
15. Dubinsky MC, Lamothe S, Yang HY, *et al*. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000;**118**:705–13.
16. Cuffari C, Theoret Y, Latour S, Seidman G. 6-Mercaptopurine metabolism in Crohn's disease: correlation with efficacy and toxicity. *Gut* 1996;**39**:401–6.
17. Seidman EG. Clinical use and practical application of TPMT enzyme and 6-mercaptopurine metabolite monitoring in IBD. *Rev Gastroenterol Disord* 2003;3 Suppl **1**:S30–8.
18. Gilissen LP, Derijks LJ, Verhoeven HM, *et al*. Pancytopenia due to high 6-methylmercaptopurine levels in a 6-mercaptopurine treated patient with Crohn's disease. *Dig Liver Dis* 2007;**39**:182–6.
19. Seinen ML, van Bodegraven AA, van Kuilenburg AB, de Boer NK. High TPMT activity as a risk factor for severe myelosuppression during thiopurine therapy. *Neth J Med* 2013;**71**:222.
20. Hindorf U, Lindqvist M, Peterson C, *et al*. Pharmacogenetics during standardised initiation of thiopurine treatment in inflammatory bowel disease. *Gut* 2006;**55**:1423–31.
21. de Jong DJ, Derijks LJ, Naber AH, Hooymans PM, Mulder CJ. Safety of thiopurines in the treatment of inflammatory bowel disease. *Scand J Gastroenterol Suppl.* **2003**:69–72.
22. Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. *Lancet* 1990;**336**:225–9.
23. Cuffari C, Seidman EG, Latour S, Theoret Y. Quantitation of 6-thioguanine in peripheral blood leukocyte DNA in Crohn's disease patients on maintenance 6-mercaptopurine therapy. *Can J Physiol Pharmacol* 1996;**74**:580–5.
24. Swen JJ, Nijenhuis M, de Boer A, *et al*. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin Pharmacol Ther* 2011;**89**:662–73.
25. Pettersson B, Almer S, Albertioni F, Soderhall S, Peterson C. Differences between children and adults in thiopurine methyltransferase activity and metabolite formation during thiopurine therapy: possible role of concomitant methotrexate. *Ther Drug Monit* 2002;**24**:351–8.
26. Armstrong VW, Shipkova M, von Ahsen N, Oellerich M. Analytic aspects of monitoring therapy with thiopurine medications. *Ther Drug Monit* 2004;**26**:220–6.
27. Lennard L. The clinical pharmacology of 6-mercaptopurine. *Eur J Clin Pharmacol* 1992;**43**:329–39.
28. Coulthard SA, Hogarth LA, Little M, *et al*. The effect of thiopurine methyltransferase expression on sensitivity to thiopurine drugs. *Mol Pharmacol* 2002;**62**:102–9.
29. Dervieux T, Blanco JG, Krynetski EY, Vanin EF, Roussel MF, Relling MV. Differing contribution of thiopurine methyltransferase to mercaptopurine versus thioguanine effects in human leukemic cells. *Cancer Res* 2001;**61**:5810–6.
30. van Asseldonk DP, Kanis BM, de Boer NK, van Bodegraven AA. Leukopenia due to parvovirus B19 in a Crohn's disease patient using azathioprine. *Digestion* 2009;**79**:211–4.
31. Lowry PW, Franklin CL, Weaver AL, *et al*. Leucopenia resulting from a drug interaction between azathioprine or 6-mercaptopurine and mesalamine, sulphasalazine, or balsalazide. *Gut* 2001;**49**:656–64.
32. de Boer NK, Wong DR, Jharap B, *et al*. Dose-dependent influence of 5-aminosalicylates on thiopurine metabolism. *Am J Gastroenterol* 2007;**102**:2747–53.
33. Gao X, Zhang FB, Ding L, *et al*. The potential influence of 5-aminosalicylic acid on the induction of myelotoxicity during thiopurine therapy in inflammatory bowel disease patients. *Eur J Gastroenterol Hepatol* 2012;**24**:958–64.
34. Wong DR, Pierik M, Seinen ML, *et al*. The pharmacokinetic effect of adalimumab on thiopurine metabolism in Crohn's disease patients. *J Crohns Colitis* 2014;**8**:120–8.
35. Colombel JF, Sandborn WJ, Reinisch W, *et al*. SONIC Study Group. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;**362**:1383–95.
36. Reenaers C, Louis E, Belaiche J, Seidel L, Keshav S, Travis S. Does cotreatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? *Aliment Pharmacol Ther* 2012;**36**:1040–8.
37. Panaccione R, Ghosh S, Middleton S, *et al*. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 2014;**146**:392–400.
38. Hindorf U, Appell ML. Genotyping should be considered the primary choice for pre-treatment evaluation of thiopurine methyltransferase function. *J Crohns Colitis* 2012;**6**:655–9.
39. Lennard L. Implementation of TPMT testing. *Br J Clin Pharmacol* 2014;**77**:704–14.
40. van Egmond R, Chin P, Zhang M, Sies CW, Barclay ML. High TPMT enzyme activity does not explain drug resistance due to preferential 6-methylmercaptopurine production in patients on thiopurine treatment. *Aliment Pharmacol Ther* 2012;**35**:1181–9.
41. Kreijne JE, Seinen ML, Wilhelm AJ, *et al*. Routinely established skewed thiopurine metabolism leads to a strikingly high rate of early therapeutic failure in patients with inflammatory bowel disease. *Ther Drug Monit* 2015;**37**:797–804.
42. 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.
43. Seinen ML, van Asseldonk DP, Mulder CJ, de Boer NK. Dosing 6-thioguanine in inflammatory bowel disease: expert-based guidelines for daily practice. *J Gastrointestin Liver Dis* 2010;**19**:291–4.