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GASTROENTEROLOGY

# 6-methylmercaptopurine-induced leukocytopenia during thiopurine therapy in inflammatory bowel disease patients

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## Abstract

**Background and Aim:** Thiopurines have a favorable benefit–risk ratio in the treatment of inflammatory bowel disease. A feared adverse event of thiopurine therapy is myelotoxicity, mostly occurring due to toxic concentrations of the pharmacologically active metabolites 6-thioguaninenucleotides. In oncology, myelosuppression has also been associated with elevated 6-methylmercaptopurine (6-MMP). In this case series, we provide a detailed overview of 6-MMP-induced myelotoxicity in inflammatory bowel disease patients.

**Methods:** We retrospectively scrutinized pharmacological laboratory databases of five participating centers over a 5-year period. Patients with leukocytopenia at time of elevated 6-MMP levels (>5700 pmol/8 × $10^8$ red blood cells) were included for detailed chart review.

**Results:** In this case series, we describe demographic, clinical, and pharmacological aspects of 24 cases of 6-MMP-induced myelotoxicity on weight-based thiopurine therapy with a median steady-state 6-MMP level of 14 500 pmol/8 × $10^8$ red blood cells (range 6600–48 000). All patients developed leukocytopenia (white blood cell count 2.7 ± 0.9 × $10^9$/L) after a median period of 11 weeks after initiation of thiopurine therapy (interquartile range 6–46 weeks). Eighteen patients (75%) developed concurrent anemia (median hemoglobin concentration 6.9 × $10^9$/L), and four patients developed concurrent thrombocytopenia (median platelet count 104 × $10^9$/L). Leukocytopenia resolved in 20 patients (83%) within 4 weeks upon altered thiopurine treatment regimen, and white blood cell count was increasing, but not yet normalized, in the remaining four patients.

**Conclusion:** We observed that thiopurine-induced myelotoxicity also occurs because of (extremely) high 6-MMP concentrations in patients with a skewed thiopurine metabolism. Continued treatment with adapted thiopurine therapy was successful in almost all patients.

## Introduction

Thiopurines (i.e. azathioprine and mercaptopurine) are immunosuppressive drugs that play an indispensable therapeutic role in maintaining remission in the majority of patients with inflammatory bowel disease (IBD) (primarily Crohn's disease and ulcerative colitis).[1–3] The complex metabolism of thiopurines has been largely unraveled over the years, leading to the observation that thiopurine S-methyl transferase (TPMT) plays a pivotal role in the bioavailability of the pharmacologically active end-metabolites: 6-thioguanine nucleotides (6-TGN, therapeutic window 230–450 pmol/8 × $10^8$ red blood cells [RBC]) and 6-methylmercaptopurine (6-MMP, normal value < 5700 pmol/8 × $10^8$ RBC).[4,5] Hepatotoxicity induced by thiopurines is largely associated with the 6-MMP metabolites, whereas myelotoxicity is mostly ascribed to high concentrations of 6-TGN, leading to apoptosis and direct cytotoxicity due to DNA strand breakage.[6–8] However, when 6-MMP concentrations are (extremely) high, as seen in high-dose thiopurine therapy in oncological patients, or in IBD-patients with a skewed

thiopurine metabolism (e.g. but not solely, caused by high TPMT activity), 6-MMP can inhibit *de novo* purine synthesis, thus causing subsequent myelotoxicity.[4,9,10] Monitoring of thiopurine metabolites (6-TGN and 6-MMP) in RBC and/or TPMT mutation analysis is becoming integrated in general IBD practice to optimize efficacy and to minimize thiopurine toxicity. Furthermore, when starting thiopurine therapy, it is advised to monitor laboratory parameters on a regular basis for early detection of toxicity.[2,3] Myelotoxicity caused by high 6-MMP levels is believed to be an uncommon adverse event in IBD patients.[11–13] Here, we present 24 cases with 6-MMP-induced leukocytopenia and describe subsequently applied strategies to optimize thiopurine metabolism by preventing excessive 6-MMP generation.

## Methods

***Study design.*** The pharmacological laboratory databases of three tertiary referral centers in the Netherlands (VU University Medical Center, Amsterdam; Erasmus Medical Center, Rotterdam; and Maastricht University Medical Center, Maastricht) and two large teaching hospitals (Zuyderland Medical Center, Heerlen-Sittard-Geleen and Máxima Medical Center, Veldhoven) were scrutinized by an automated search over a time period of 5 years (January 1, 2011–December 31, 2015) for this retrospective study. Furthermore, these databases were cross-checked with IBD databases on site.

***Patient selection.*** The pharmacological reports of all patients using thiopurines and having at least one metabolite measurement in the selected time period were analyzed. We included all IBD patients with 6-MMP concentrations above 5700 pmol/8 × $10^8$ RBC for detailed chart review. Leukocytopenia was defined as white blood cell count below 4.0 × $10^9$/L. Exclusion criteria were the absence of leukocytopenia (i.e. white blood cell count ≥ 4.0 × $10^9$/L), 6-TGN concentrations (6-TGN) above normal limits (i.e. > 450 pmol/8 × $10^8$ RBC), the absence of a skewed metabolism (i.e. 6-MMP/6-TGN ratio < 20) or the lack of laboratory measurements within 3 days prior to or after metabolite measurement.

***Demographic characteristics.*** At time of leukocytopenia, we collected the following data on patient characteristics: sex, age, weight, type of IBD, Montreal classification,[14] specific thiopurine derivative, dosage and duration of thiopurine therapy, and concomitant medication, in particular drugs known to induce myelosuppression by itself (e.g. allopurinol,[15] ACE-inhibitors,[6] ribavirin,[16] and mesalazine[17,18]) or interfere with thiopurine metabolism. Treatment strategies following (allegedly) 6-MMP-induced leukocytopenia (i.e. discontinuation, dose-reduction, allopurinol co-administration, or switch to thioguanine) were evaluated. When patients were admitted to the hospital with fever at time of diagnosed leukocytopenia, routine blood cultures and virologic tests were assessed to rule out other causes (e.g. viral infection or sepsis).

***Laboratory tests.*** We collected the following hematologic parameters from all included patients at time of diagnosed leukocytopenia and 2–6 weeks after application of thiopurine optimization strategy: white blood cell count (WBC; normal range 4.0–10.0 × $10^9$/L), hemoglobin concentration (Hb; normal range male 8.5–11.0 × $10^9$/L, female 7.5–10.0 × $10^9$/L), mean corpuscular volume (normal range 80–100 fL), platelet count (normal range 150–400 × $10^9$/L), aspartate aminotransferase (reference value male ≤ 35 U/L, female ≤ 40 U/L), and alanine aminotransferase (reference value ≤ 55 U/L). Differentials of WBC were collected when determined within 3 days after diagnosed leukocytopenia.

Furthermore, we collected thiopurine metabolites ([6-MMP] and [6-TGN]) at time of myelotoxicity (within 3 days prior to or after diagnosed leukocytopenia) and during follow-up (i.e. 2–6 weeks after optimizing therapy), when available.

Concentrations of metabolites were measured using a previously described method by Dervieux *et al.*[19] or Lennard *et al.*[20] The Lennard method has found the greatest application in clinical studies yet and has served as the basis for the establishment of treatment-related therapeutic ranges for thiopurine therapy.[21]

Laboratory measurements of the Maastricht University Medical Center and the Máxima Medical Center were performed in the Zuyderland Medical Center. In the Zuyderland Medical Center, concentrations of metabolites were measured using the method described by Lennard until April 2013. From April 2013, the method by Dervieux was applied. Concentrations of metabolites in the other two centers were measured using the method described by Dervieux. In these centers, concentrations of 6-TGN were divided by a factor of 2.6 to make them comparable to those determined by the Lennard method.[22,23] Concentrations of 6-MMP are similar in both assays.[21]

***Data analysis.*** All data are given descriptively or tabulated. Data are expressed as median with interquartile range (IQR) or range, or as mean with standard deviation according to distribution. Metabolite concentrations at baseline and after applying treatment optimization strategies were compared using the Wilcoxon signed-rank test. Correlations between nonparametric values were measured using the Spearman's rank order correlation test.

***Ethical approval.*** This study was approved by the Medical Ethics Review Committee of the VU University Medical Center with file-number 2016-824.

## Results

***Patient characteristics.*** A total of 24 patients (50% male, 50% female) were included with a mean age at initiation of thiopurine therapy of 44 ± 18 years. Crohn's disease and ulcerative colitis were diagnosed in nine (38%) and 15 (62%) patients, respectively. Median duration of thiopurine therapy until development of myelotoxicity was 11 weeks (IQR 6–46). All patient characteristics are summarized in Table 1.

***Development of leukocytopenia.*** After a median period of 11 weeks after initiation of thiopurine therapy, leukocytopenia developed with a mean WBC of 2.7 ± 0.9 × $10^9$/L. In 18 patients (75%), hemoglobin decreased under the lower reference limit to a median of 6.9 × $10^9$/L (range 3.2–8.4) simultaneously. Concurrent

**Table 1** Demographics of included patients

| Nos | Sex | Age (years) | D | Montreal | Drug | Dose (mg/day) | Weight (kg) | Dose (mg/kg) | Relevant co-meds | Week of leukocytopenia[†] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 55 | CD | A2L2B1 | MP | 50 | 64 | 0.8 | none | 1000 |
| 2 | F | 80 | CD | A3L1B2 | MP | 75 | 67 | 1.1 | none | 220[‡] |
| 3 | M | 33 | CD | A2L3B1p | MP | 75 | 60 | 1.3 | none | 9 |
| 4 | F | 24 | CD | A2L1B2 | MP | 75 | 56 | 1.3 | none | 6 |
| 5 | F | 34 | CD | A2L3B1 | MP | 100 | 87 | 1.1 | none | 110 |
| 6 | M | 18 | CD | A2L1B1 | MP | 100 | 68 | 1.5 | none | 6 |
| 7 | F | 43 | CD | A2L3B1 | MP | 100 | 65 | 1.5 | mesalazine | 20 |
| 8 | M | 21 | CD | A1L3B1 | MP | 100 | 56 | 1.8 | none | 6 |
| 9 | F | 62 | CD | A3L2B1 | AZA | 150 | 72 | 2.1 | lisinopril | 4 |
| 10 | F | 26 | CD | A2L1B1 | AZA | 175 | 76 | 2.3 | none | 46 |
| 11 | M | 74 | UC | E3 | MP | 75 | 71 | 1.1 | mesalazine | 12 |
| 12 | M | 23 | UC | E3 | MP | 75 | 65 | 1.2 | mesalazine | 12 |
| 13 | F | 49 | UC | E3 | MP | 75 | 65 | 1.2 | mesalazine | 6 |
| 14 | M | 50 | UC | E3 | MP | 100 | 78 | 1.3 | none | 104 |
| 15 | F | 67 | UC | E2 | MP | 100 | 75 | 1.3 | mesalazine | 6 |
| 16 | F | 75 | UC | E2 | MP | 100 | 75 | 1.3 | none | 45 |
| 17 | F | 34 | UC | E3 | MP | 100 | 57 | 1.8 | none | 5 |
| 18 | M | 31 | UC | E2 | MP | 125 | 89 | 1.4 | mesalazine | 6 |
| 19 | F | 32 | UC | E3 | MP | 150 | 92 | 1.6 | none | 12 |
| 20 | M | 51 | UC | E3 | MP | 150 | 86 | 1.7 | mesalazine | 4 |
| 21 | M | 35 | UC | E3 | MP | 150 | 84 | 1.8 | none | 5 |
| 22 | M | 63 | UC | E1 | AZA | 125 | 67 | 1.9 | none | 156 |
| 23 | M | 51 | UC | E2 | AZA | 150 | 60 | 2.5 | mesalazine | 45 |
| 24 | M | 34 | UC | E3 | AZA | 200 | 87 | 2.3 | mesalazine | 9 |

[†]week of diagnosed leukocytopenia

[‡]Leukocytopenia developed 4 weeks after dose increase.

AZA, azathioprine, CD, Crohn's disease, D, diagnosis, F, female, M, male, MP, mercaptopurine, UC, ulcerative colitis.

Relevant co-medications were defined as mesalazine, sulfasalazine, ace-inhibitors, trimethoprim, indomethacin, and ribavirin.

Montreal classification:[14] A: age at diagnosis—1: below 17 years, 2: 17–40 years, 3: above 40 years.L: location—1: ileal, 2: colonic, 3: ileocolic.B: behavior—1: non-stricturing non-penetrating, 2: stricturing, 3: penetrating, p: perianal involvement.E: extent—1: proctitis, 2: left-sided colitis, 3: extensive colitis.

thrombocytopenia occurred in four patients (17%) with a median of 104 × $10^9$/L (range 79–132). Three patients developed pancytopenia.

Median 6-MMP was 14 500 pmol/8 × $10^8$ RBC (range 6600–48 000) with therapeutic 6-TGN in nine patients (38%; mean concentration 196 ± 98 pmol/8 × $10^8$ RBC) In the other 15 patients, 6-TGN concentrations were lower than the therapeutic cut-off level (i.e. < 235 pmol/8 × $10^8$ RBC). The median 6-MMP/6-TGN ratio was 102 (range 24–327). The 6-MMP/6-TGN ratio was not correlated to WBC ($P = 0.23$), but there seemed to be a trend towards lower WBC in patients with higher 6-MMP concentrations ($r = -0.30$, $P = 0.08$). An overview of these results is depicted in Tables 2 and 3.

Four patients (nos 6, 15, 20, and 21) were admitted to the hospital because of complicated myelotoxicity combined with fever, deep anemia, and/or worsening of IBD. Of these patients, three patients (nos 6, 15, and 21) were febrile and treated with intravenous antibiotics per local protocol. One patient (no. 20) was admitted for blood transfusion (Hb 3.2 × $10^9$/L) and received three units of erythrocytes concentrate, after which the anemia resolved. Patient nos 15 and 20 were also admitted because of worsening of IBD course and received an induction course of prednisolone. In these patients, thiopurine treatment was immediately ceased.

***White blood cell count differentials.*** As depicted in Table 3, WBC differentials were available in 9/24 patients (38%). In four patients, there was an isolated absolute neutropenia, and in one patient, there was an isolated absolute lymphopenia. In the other four patients, both neutrophil count and lymphocyte count were decreased.

***Alternative optimizing treatment strategies after 6-MMP induced leukocytopenia.*** Of all patients developing leukocytopenia on thiopurine therapy, 11 patients (45%) received subsequent allopurinol 100 mg/day combined with the original thiopurine in a reduced dose (25–33% of original dose), leading to a normalization of 6-MMP to a median of 220 pmol/8 × $10^8$ RBC (IQR 100–288; $P < 0.01$) in all patients. Concentrations of 6-TGN did neither differ from pre-treatment (6-TGN; median 206 *vs* 192 pmol/8 × $10^8$ RBC, $P = 0.54$) in this subgroup nor in the total group (median 188 *vs* 193 pmol/8 × $10^8$ RBC, $P = 0.95$), but 6-MMP/6-TGN ratios decreased from a median of 102 to 1.3 ($P < 0.001$) in the total group.

In five patients (21%), thiopurine therapy was switched into the alternative thiopurine derivative thioguanine, which undergoes a less complex metabolism without the formation of 6-MMP.

Four patients (17%) with mild myelotoxicity (nos 3, 4, 5, and 16) received a 50% dose reduction of the original thiopurine with

**Table 2** Laboratory parameters of patients developing myelotoxicity on thiopurine therapy because of high 6-methylmercaptopurine concentrations

| CASE | At time of developing myelotoxicity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WBC ($\times 10^9$/L) | Hb ($\times 10^9$/L) | MCV (fL) | PC ($\times 10^9$/L) | AST (U/L) | ALT (U/L) | 6-MMP | 6-TGN | 6-MMP/6-TGN ratio |
| 1 | 2.2 | 7.5 | 122 | 194 | 130 | 88 | 19 000 | 139 | 137 |
| 2 | 3.7 | 7.1 | 97 | 294 | 27 | 38 | 12 500 | 58 | 216 |
| 3 | 3.2 | 8.4 | 101 | 152 | - | 30 | 13 000 | 296 | 44 |
| 4 | 2.7 | 7.7 | - | 225 | - | 100 | 12 000 | 212 | 57 |
| 5 | 3.6 | 4.9 | - | 167 | - | 30 | 36 500 | 139 | 263 |
| 6 | 1.7 | 6.8 | 89 | 234 | 20 | 36 | 33 000 | 173 | 173 |
| 7 | 2.3 | 6.3 | 112 | 203 | - | - | 48 000 | 279 | 172 |
| 8 | 2.0 | 7.8 | - | 269 | - | 14 | 11 000 | 423 | 26 |
| 9 | 2.5 | 7.6 | 122 | 132 | 69 | 55 | 22 000 | 215 | 102 |
| 10 | 3.7 | 8.5 | 94 | 311 | 18 | 15 | 6600 | 85 | 78 |
| 11 | 3.5 | 6.6 | 110 | 79 | 51 | 50 | 7500 | 308 | 24 |
| 12 | 2.6 | 6.6 | 90 | 329 | 28 | 52 | 13 000 | 262 | 50 |
| 13 | 3.5 | 7.5 | - | 253 | - | 35 | 13 000 | 169 | 77 |
| 14 | 3.9 | 9.6 | 89 | 249 | - | 181 | 16 000 | 80 | 200 |
| 15 | 1.8 | 7.2 | 98 | 125 | 29 | 39 | 46 000 | 273 | 168 |
| 16 | 3.8 | 7.3 | 108 | 267 | 60 | 68 | 22 000 | 273 | 81 |
| 17 | 3.4 | 7.0 | 106 | 209 | - | 10 | 36 000 | 110 | 327 |
| 18 | 1.8 | 6.6 | 91 | 409 | - | 69 | 30 000 | 319 | 94 |
| 19 | 2.3 | 6.5 | 99 | 199 | - | 61 | 37 000 | 262 | 141 |
| 20 | 1.6 | 3.2 | 100 | 417 | 11 | 9 | 19 000 | 73 | 260 |
| 21 | 0.8 | 5.8 | - | 155 | 111 | 274 | 13 000 | 65 | 200 |
| 22 | 2.2 | 7.4 | 104 | 82 | 27 | 38 | 13 000 | 127 | 102 |
| 23 | 3.5 | 8.4 | 90 | 315 | 47 | 41 | 10 000 | 223 | 45 |
| 24 | 2.5 | 8.1 | 90 | 204 | 17 | 20 | 12 000 | 133 | 90 |

The symbol (-) means not available.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin concentration; MCV, mean corpuscular volume; PC, platelet count; WBC, white blood cell count.

6-MMP, 6-methylmercaptopurine; 6-TGN, 6-thioguaninenucleotides (Lennard). Metabolite concentrations are displayed as pmol/8 $\times 10^8$ red blood cells. All hematologic parameters were determined at the time of metabolite measurement. These values do not have to be the lowest by definition.

subsequent normalization of hematologic parameters. In three patients, 6-MMP and 6-TGN concentrations decreased (6-TGN to suboptimal levels) after dose reduction, and in the other patients, thiopurine metabolites were not measured.

In four patients (17%; nos 5, 6, 11, and 22), thiopurine therapy was discontinued with normalization of hematologic parameters shortly (respectively 21 and 30 days) after discontinuation. Thiopurine therapy was not rechallenged in these patients, based on patient's request.

In 20 of 24 (83%) patients, leukocyte count normalized 4 weeks after changing treatment regimen. In the remaining four patients, WBC was not normalized yet but improved compared with the initial leukocytopenia (Fig. 1). Anemia at initial presentation resolved in 12/18 (67%) of patients and improved in the remaining six patients. At follow-up, four patients (17%) had thrombocytopenia. No mortality was observed in our cohort. These results are summarized in Table 4. Overall, restart with adapted thiopurine therapy was successful in 20 of 24 (83%) patients.

6-Methylmercaptopurine concentrations were neither correlated with the use of co-medication (i.e. mesalazine), nor with the dose of thiopurine therapy in the different patients. The development of leukopenia was not correlated with the use of co-medication.

Genotyping of the TPMT enzyme (i.e. wild-type *1/*1 *vs* heterozygous/homozygous polymorphisms) was performed using a polymerase chain reaction in seven patients at baseline (29%; nos 2, 5, 7, 10, 16, 23, and 24). All these patients were carriers of the wild-type TPMT genotype (*1/*1).

## Discussion

In this case series, a detailed description of 24 patients developing myelotoxicity on thiopurine therapy due to a skewed, ultramethylating thiopurine metabolism, and their follow-up is provided. In these patients, 6-MMP-induced leukocytopenia developed after a median of 11 weeks after initiation of thiopurine therapy and resolved within 4 weeks upon altered treatment regimen in 83% of the patients. One case has been published before.[11]

Over recent years, metabolism of thiopurines in IBD patients has been extensively investigated. Most dose-dependent adverse events of thiopurines in IBD patients have been ascribed to two metabolite groups, 6-MMP and 6-TGN. Thiopurine-induced myelotoxicity is almost exclusively being described in relation to grossly elevated 6-TGN levels, causing DNA strand breakage leading to direct cytotoxicity and apoptosis of activated T-lymphocytes.[4,9] High 6-TGN concentrations are associated with low TPMT activity caused by a mutant genotype, thus shifting the balance between 6-MMP and 6-TGN formation. Besides toxic 6-TGN concentrations, 6-MMP in (extremely) high concentrations can cause myelotoxicity as well, because of inhibition of *de novo* purine synthesis.[4,11,24] Purines are essential compounds in nucleic

**Table 3** Differential effects on white cell line in patients with leukocytopenia and TPMT genotyping (when available)

| CASE | WBC ($\times 10^9$/L) | Leukocyte differentiation | | | | TPMT genotype |
|---|---|---|---|---|---|---|
| | | Neutrophils ($\times 10^9$/L) [*N* 1.5–7.5] [*N* 40–75%] | Lymphocytes ($\times 10^9$/L) [*N* 1.0–3.5] [*N* 25–35%] | Eosinophils ($\times 10^9$/L) [*N* < 0.5] [*N* 0–5%] | Monocytes ($\times 10^9$/L) [*N* 0.1–1.0] [*N* 2–10%] | |
| 1 | 2.2 | - | - | - | - | - |
| 2 | 3.7 | - | - | - | - | - |
| 3 | 3.2 | 1.92 (60%) | **0.96** (30%) | 0.06 (2%) | 0.20 (6%) | - |
| 4 | 2.7 | - | - | - | - | - |
| 5 | 3.6 | - | - | - | - | *1/*1 |
| 6 | 1.7 | **0.54 (32%)** | 1.14 (68%) | 0.00 | **0.02 (1%)** | - |
| 7 | 2.3 | - | - | - | - | *1/*1 |
| 8 | 2.0 | - | - | - | - | - |
| 9 | 2.5 | **1.21** (48%) | 1.04 (42%) | 0.07 (3%) | 0.19 (7%) | - |
| 10 | 3.7 | - | - | - | - | *1/*1 |
| 11 | 3.5 | **1.43** (41%) | 1.47 (42%) | 0.19 (5%) | 0.34 (10%) | - |
| 12 | 2.6 | **1.12** (43%) | 1.20 (46%) | 0.13 (5%) | 0.14 (5%) | - |
| 13 | 3.5 | - | - | - | - | - |
| 14 | 3.9 | - | - | - | - | *1/*1 |
| 15 | 1.8 | **0.64 (36%)** | **0.97** (54%) | 0.07 (5%) | **0.09** (5%) | - |
| 16 | 3.8 | - | - | - | - | *1/*1 |
| 17 | 3.4 | - | - | - | - | - |
| 18 | 1.8 | **1.13** (63%) | **0.53** (29%) | 0.02 (1%) | **0.07** (4%) | - |
| 19 | 2.3 | - | - | - | - | - |
| 20 | 0.9[†] | **0.46** (51%) | **0.13 (14%)** | 0.22 (24%) | **0.02** (2%) | - |
| 21 | 0.8 | **0.40** (50%) | **0.34** (43%) | 0.00 | **0.06** (7%) | - |
| 22 | 2.2 | - | - | - | - | - |
| 23 | 3.5 | - | - | - | - | *1/*1 |
| 24 | 2.5 | - | - | - | - | *1/*1 |

[†]3 days after initial diagnosed leukocytopenia

The symbol (-) means result unavailable.

Values expressed in **bold** are lower than reference values.

TPMT, thiopurine methyl-S-transferase, WBC, white blood cell count, *1/*1, wildtype genotype.

acids, needed for the generation of DNA.[25] When thiopurines are administered in high (oncological) dosages, the median time to develop leukocytopenia is approximately ten days.[26] In IBD, dosage of thiopurine therapy is substantially lower because of another mode-of-action, because the required effect is mainly anti-apoptotic, instead of anti-metabolic.[4] In the current analysis with lower IBD dosages, in which high 6-MMP concentrations were studied as a result of a skewed thiopurine metabolism, the median time to leukocytopenia was 11 weeks.[6]

We observed that 11 (45%) patients who developed myelotoxicity because of ultramethylation benefitted from the addition of allopurinol to a reduced (25–33%) dose of the original thiopurine. Allopurinol is an inhibitor of the enzyme xanthine oxidase and also has an indirect inhibiting function on TPMT enzyme activity, thus leading to lower 6-MMP and higher 6-TGN production.[27–29] In addition, allopurinol seems to have an enhancing effect on hypoxanthine-guanine phosphoribosyltransferase (HGPRT), contributing to higher 6-TGN formation as well.[30,31] Additionally, five patients with leukocytopenia on conventional thiopurine therapy benefitted from a switch to thioguanine therapy, hereby avoiding the formation of 6-MMP.[32]

As shown in a large prospective cohort study by Chaparro and colleagues,[33] leukocytopenia is witnessed in about 4% of the patients treated with thiopurines after a median period of 7 months. This finding was underlined in a systematic review by Gisbert *et al.*[34] This effect is predominantly seen in patients with high 6-TGN concentrations (e.g. caused by heterozygote/homozygote TPMT mutations or patients with a NUDT15 mutation), and incidence is probably lower in patients with high 6-MMP levels.[35–37]

Recently, results of a prospective study showed that elevated 6-MMP and 6-TGN metabolites assessed one week after initiation were independently associated with thiopurine-induced leukopenia.[38] Furthermore, it was demonstrated that patients who show excessive 6-MMP formation are also at risk for early thiopurine failure because of intolerable adverse events or refractoriness.[39]

One of the limitations of our case series is the retrospective nature. Another possible limitation is that patients in this cohort were identified based on a skewed thiopurine metabolism, and these results were linked to leukocytopenia afterwards. Because therapeutic drug monitoring is not performed routinely in all patients of the participating centers, the total number of 6-MMP-induced leukocytopenia might be higher than suggested in our analysis. Unfortunately, differentials of WBC were available in only 9/24 patients, because this measurement is not performed per protocol in the participating centers. Only WBC differentials within 3 days after diagnosed leukocytopenia were added to our analysis. Interestingly, it is not clear why patients develop neutropenia and/or lymphopenia, as shown in our results. To our

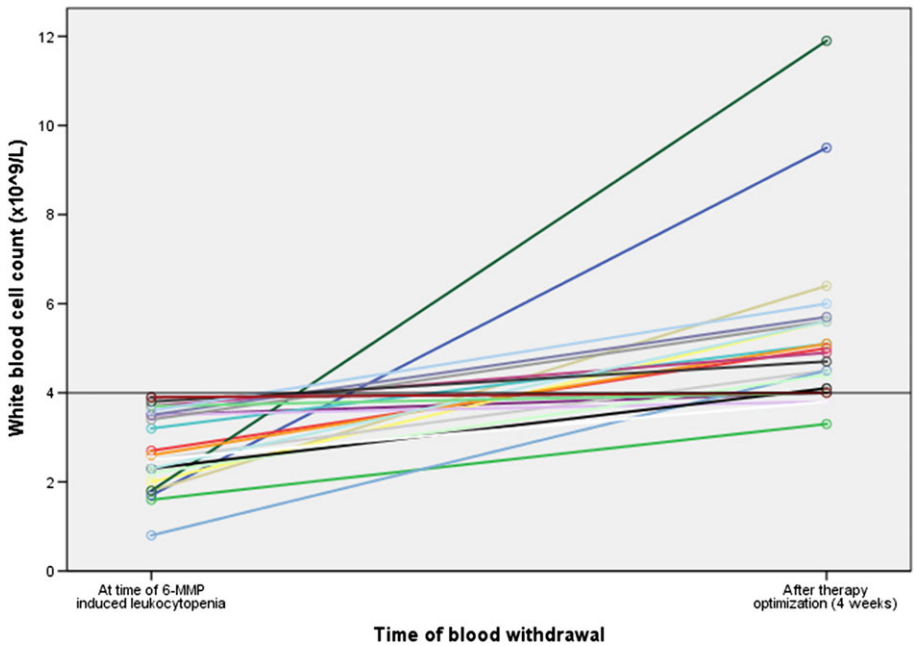


**Figure 1** Change in white blood cell count four weeks after optimizing thiopurine treatment. 6-MMP, 6-methylmercaptopurine. [Color figure can be viewed at wileyonlinelibrary.com]

**Table 4** Laboratory parameters of patients developing myelotoxicity on thiopurine therapy due to high 6-methylmercaptopurine concentrations after changing treatment strategy

| CASE | After changing treatment strategy | | | | | |
|---|---|---|---|---|---|---|
| | WBC ($\times 10^9$/L) | Hb ($\times 10^9$/L) | PC ($\times 10^9$/L) | 6-MMP | 6-TGN | Strategy |
| 1 | 4.4 | 8.7 | 264 | 0 | 695 | Thioguanine |
| 2 | 4.9 | 7.2 | 254 | 0 | 140 | Thioguanine |
| 3 | 5.1 | 9.4 | 207 | 2800 | 215 | Dose reduction |
| 4 | 5.0 | 8.4 | 177 | 6800 | 173 | Dose reduction |
| 5 | 6.0 | 5.8 | 226 | - | - | Discontinuation |
| 6 | 9.5 | 9.1 | 146 | 760 | 0 | Discontinuation |
| 7 | 4.1 | 7.1 | 164 | 4500† | 68 | Thioguanine |
| 8 | 5.6 | 9.0 | 318 | 310 | 273 | Allopurinol‡ |
| 9 | 4.5 | 7.6 | 153 | 200 | 207 | Allopurinol‡ |
| 10 | 4.0 | 8.7 | 264 | - | - | Dose reduction |
| 11 | 4.0 | 7.3 | 65 | - | - | Discontinuation |
| 12 | 5.1 | 7.6 | 247 | 280 | 188 | Allopurinol‡ |
| 13 | 3.8 | 7.9 | 221 | 190 | 219 | Allopurinol‡ |
| 14 | 4.0 | 8.5 | 228 | 254 | 204 | Allopurinol‡ |
| 15 | 6.4 | 7.3 | 120 | - | - | Allopurinol‡ |
| 16 | 4.7 | 8.0 | 240 | < 100 | 538 | Allopurinol‡ |
| 17 | 5.6 | 7.9 | 235 | 0 | 235 | Thioguanine |
| 18 | 11.9 | 8.2 | 395 | 240 | 327 | Allopurinol‡ |
| 19 | 5.6 | - | - | 1300† | 27 | Thioguanine |
| 20 | 3.3 | 8.2 | 316 | < 100 | 162 | Allopurinol‡ |
| 21 | 4.5 | 9.6 | 229 | < 100 | 62 | Allopurinol‡ |
| 22 | 3.2 | 7.4 | 81 | - | - | Discontinuation |
| 23 | 5.7 | 8.5 | 345 | 880 | 46 | Dose reduction |
| 24 | 3.8 | 8.9 | 183 | 724 | 127 | Allopurinol‡ |

†Detectable because mercaptopurine was only terminated recently.

‡Reduction of thiopurine dose (25–33%) combined with allopurinol 100 mg/day.

The symbol (-) means not available.

Hb, Hemoglobin concentration; PC, platelet count; WBC, white blood cell count.

6-MMP: 6-methylmercaptopurine; 6-TGN: 6-thioguaninenucleotides (Lennard). Metabolite concentrations are displayed as pmol/8 $\times 10^8$ red blood cells.

knowledge, there are no data available describing the relative incidence of neutropenia or lymphopenia in thiopurine users.

In this cohort, we did not determine TPMT genotyping systematically. However, we expect all patients to have normal/high TPMT activity (wild-type genotype), because 6-MMP formation is mainly driven by TPMT activity. Whereas it has been described that preferential 6-MMP production could occur in patients with TPMT mutations, we believe this will not be of added value to this paper, because other risk factors, besides 6-MMP, for developing leukocytopenia are not assessed in this retrospective study.[10] Furthermore, even though we ruled out common causes of leukocytopenia (e.g. viral infection or sepsis), it is not ruled out that other factors (e.g. hematologic or autoimmune disorders and deficiencies of dietary vitamins) might have contributed to the development of leukocytopenia in these patients, especially in those patients with only marginal elevated 6-MMP concentrations.[40]

With this detailed case series, we underline that myelotoxicity may also be caused by grossly elevated levels of 6-MMP. This is added to what has previously been demonstrated, namely that myelotoxicity is mainly caused by elevated cytotoxic levels of 6-TGN, the use of certain co-medications or intercurrent (viral) infections. Our findings might also be an explanation for unexplained leukocytopenia during thiopurine therapy without genetic variations (e.g. TPMT or NUDT15 mutation).[25,41,42]

## Conclusion

We demonstrated that leukocytopenia develops in patients with (extremely) elevated concentrations of 6-MMP. Almost all patients were successfully treated with allopurinol alongside thiopurines or from a switch to thioguanine. Adapted thiopurine therapy was successful in the majority of patients who developed leukopenia resulting from a skewed metabolism. As myelotoxicity mainly seems to occur shortly after introduction of thiopurine therapy, we stress the importance of therapeutic drug monitoring in case of myelotoxicity, especially in the first weeks after initiation.

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