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Concomitant 5-aminosalicylic acid treatment does not affect 6-thioguanine nucleotide levels in patients with inflammatory bowel disease on thiopurines

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Abstract

Background There are conflicting data as to whether co-treatment with 5-aminosalicylic acid (5-ASA) in patients with inflammatory bowel disease (IBD) under azathioprine (AZA) or 6-mercaptopurine (6-MP) therapy may influence 6-thioguanine nucleotide (6-TGN) concentrations, and whether this combination puts patients at risk of side-effects. The aim of the study was to determine 6-TGN levels in patients treated with AZA/6-MP, either alone or in combination with 5-ASA.

Methods Available blood samples from patients treated with AZA or 6-MP were retrieved from the Swiss IBD Cohort Study (SIBDCS). The eligible individuals were divided into 2 groups: those with vs. without 5-ASA co-medication. Levels of 6-TGN and 6-methylmercaptopurine ribonucleotides (6-MMPR) were determined and compared. Potential confounders were compared between the groups, and also evaluated as potential predictors for a multivariate regression model.

Results Of the 110 patients enrolled in this analysis, 40 received concomitant 5-ASA at the time of blood sampling. The median 6-TGN levels in patients with vs. those without 5-ASA co-treatment were 261 and 257 pmol/8×10^8 erythrocytes, respectively (P=0.97). Likewise, there were no significant differences in 6-MMPR levels (P=0.79). Through multivariate analysis, 6-TGN levels were found to be significantly higher in non-smokers, patients without prior surgery, and those without signs of stress-hyperarousal.

Conclusions Blood concentrations of 6-TGN and 6-MMPR did not differ between patients with vs. those without 5-ASA co-treatment. Our data warrant neither more frequent lab monitoring nor dose adaptation of AZA in patients receiving concomitant 5-ASA treatment.

Keywords 6-thioguanine nucleotide level, 5-aminosalicylic acid, thiopurine, inflammatory bowel disease, azathioprine

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Introduction

For decades, immunomodulating drugs like thiopurines, e.g., azathioprine (AZA) or 6-mercaptopurine (6-MP), have been a mainstay in the long-term treatment of glucocorticoid-dependent or glucocorticoid-refractory inflammatory bowel disease (IBD). Thiopurines were introduced into clinical practice more than 60 years ago. In the 1980s, thiopurines were shown to be effective in Crohn's disease (CD), and later also in ulcerative colitis (UC) [1,2]. AZA and 6-MP are prodrugs that undergo a complex metabolic transformation, resulting in the formation of the pharmacologically active, immunosuppressive 6-thioguanine nucleotides (6-TGN) [3].

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The main metabolites of 6-TGN are active phosphorylated 6-thioguanosine triphosphate (TGTP) and its inactive precursor 6-thioguanosine diphosphate [2,4]. By acting as a purine antagonist, 6-TGN disrupts nucleic acid metabolism and also purine synthesis, which leads to cytotoxicity and immunosuppression by inhibiting DNA, RNA and protein synthesis [5-7]. Tiede *et al* demonstrated in 2003 that AZA and its metabolites induced apoptosis of T cells in both patients with CD and a control group, by co-stimulation of CD28. This was mediated by binding of AZA-generated TGTP to Rac1 instead of guanosine triphosphate, leading to a specific blockage of Rac1 activation [8]. Later, in 2006, the same investigators reported a suppression of T cell-APC conjugation through inhibition of Vav guanosine exchange activity on Rac proteins [9]. According to the literature, the therapeutic range of 6-TGN concentrations is 235-450 pmol/8×10^8 erythrocytes (ECs) [1,10].

The conversion of thiopurine drugs is a multistep enzymatic process initiated by hypoxanthine phosphoribosyltransferase [11,12]. Thiopurine methyltransferase (TPMT) is a cytosolic enzyme controlling one of the most important steps in the thiopurine metabolism [12]. It catalyzes the S-methylation of AZA and 6-MP to its ultimate conversion 6-methylmercaptopurine (6-MMP) and 6-methylmercaptopurine ribonucleotide (6-MMPR) [6]. Although 6-MMP is an inactive metabolite, a correlation has been revealed between 6-MMP levels and thiopurine-associated hepatotoxicity, pancreatitis and marrow suppression [13,14].

TPMT is capable of influencing 6-TGN concentrations indirectly by shunting thiopurine drug metabolism away from 6-TGN [15]. The genetic polymorphism of the alleles encoding for TPMT results in various activities of this enzyme, leading to a significant interpatient variability of 6-TGN levels [16,17]. Most individuals are characterized by a high level of TPMT activity, though 11% have intermediate, and approximately 0.3% extremely low or even absent TPMT activity [18]. Patients who carry an intermediate or low TPMT activity phenotype are at elevated risk of myelosuppression under the standard therapeutic dose of thiopurines, due to a potential excess of 6-TGN [19-21]. However, mutation in the *TPMT* alleles is only responsible for myelosuppression in 27% of cases. Low leukocyte cell counts were more often caused by other factors [22].

In addition to thiopurine, 5-aminosalicylic acid (5-ASA) is frequently used as a co-treatment in IBD. There are conflicting data on whether 5-ASA may influence 6-TGN concentrations, and whether this co-treatment puts patients at greater risk of side-effects from AZA and 6-MP. Small, uncontrolled trials indicated that 5-ASA co-medication might rise 6-TGN levels. Hande *et al*, in 2006, reported in a retrospective study that 5-ASA therapy was associated with higher 6-TGN levels. However, a main limitation of the study was the inclusion of patients in clinical remission, without considering patients who had to stop the therapy early [5]. De Boer *et al* described a dose-dependent effect of 5-ASA on 6-TGN levels 1 year later. Co-medication with 2 g 5-ASA daily was associated with a statistically significant increase of 6-TGN levels by 40% (absolute 84 pmol/8x10^8 EC), whereas 4 g of 5-ASA elevated 6-TGN levels by 70% (absolute 154 pmol/8x10^8 EC) [23]. Lowry *et al* suggested that 5-ASA may reversely inhibit TPMT activity *in vitro*, supporting the experimental work of Szumlanski *et al* [24,25]. Later, De Graaf *et al* endorsed this concept by measuring smaller 6-MMPR levels after co-administration of 5-ASA in IBD patients [4]. However, the low absorption rate of 5-ASA raises the question whether this interaction is relevant *in vivo* [15]. Daperno *et al* drew the conclusion that 5-ASA seems to have no clinical influence on TPMT activity [26].

Therefore, the aim of the present study was to determine 6-TGN levels in ECs of IBD patients treated with AZA/6-MP, either as monotherapy or in co-treatment with 5-ASA, and whether elevated 6-TGN levels are associated with higher rates of side-effects/worse tolerability or higher therapeutic success.

Materials and methods

Study design

An electronic query of the Swiss IBD Cohort Study (SIBDCS) database was conducted for all available EDTA-blood samples from patients treated with AZA or 6-MP. The recommended dose was calculated according to the European Crohn's and Colitis Organization (ECCO) Consensus Guidelines [27,28], in order to achieve 2-2.5 mg/Kg/d for AZA and 1-1.5 mg/Kg/d for 6MP, once daily and *per os*. The individuals were divided into 2 groups: 5-ASA co-medication and no 5-ASA co-medication. In each group, 6-TGN and 6-MMPR levels in the archived blood samples were measured. During the first phase, these levels were compared between the 2 groups. A second comparison of the 2

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groups focused on the CD activity index (CDAI) for CD patients and the modified Truelove and Witts activity index (MTWAI) for patients with UC or indeterminate colitis (IC); disease duration, location and complications; therapy discontinuation; treatment side-effects (pancreatitis, hepatitis, leukopenia and flu-like symptoms); as well as quality of life and personality (e.g., Negative Affectivity Score, Social Inhibition Score, Type D-Personality), in order to identify potential confounders. The last part of the analyses consisted of multivariate models with 6-TGN and 6-MMPR levels as dependent variables and a selected subset of the abovementioned factors as predictors. Clinical data were available for the cohort based on enrolment and annual follow-up questionnaires. The study complied with the last revision of the Declaration of Helsinki principles and with the Guidelines of Good Clinical Practice [29,30].

Patient selection

Initially, the electronic database of SIBDCS was searched for female and male patients with IBD who were treated with AZA or 6-MP without interacting co-medications, such as allopurinol, at the time of blood sampling. Patients suffering from CD, UC or IC were all included. In addition, availability of an EDTA-blood sample in the biobank (to measure the 6-TGN/6-MMPR levels) was a prerequisite and it had to be obtained ±4 weeks around the questionnaire. The patient's EC cell count was measured on the day of the EDTA-blood sample collection (±3 days) for the final estimation of the 6-TGN/6-MMPR levels.

6-TGN and 6-MMPR level analysis

Analysis of 6-TGN and 6-MMPR was conducted using a method based on that of Wusk *et al* [31]. In brief, 0.5 mL of stabilized whole blood was protein-precipitated using perchloric acid. After centrifugation, supernatants were hydrolyzed (6-MMPR to 6-MMP) for 45 min at 100°C. After neutralization, samples were analyzed using liquid chromatography coupled to mass spectrometry. Analytes were analyzed in positive heated electrospray ionization mode on a Q exactive mass spectrometer (Thermo Fisher Scientific, Reinach, Switzerland) and detection was performed in full-scan mode with a resolution of 70,000 full width at half maximum (calculated for m/z 200). Imprecision was <2% for 6-MMP and <4% for 6-TGN.

Statistical analysis

For comparisons between the 2 groups (5-ASA co-medication vs. no 5-ASA co-medication) in case of discrete outcomes (e.g., sex, disease location or complication [y/n]), we generally used chi-square (χ^2) tests. Fisher's exact tests were implemented when the sample size in a given category for a given group was less than 5. In case of continuous outcomes (for instance 6-TGN or 6-MMPR levels and age), Wilcoxon rank-sum tests were considered. A P-value <0.05 was considered statistically significant (2-tailed). Since 6-TGN and 6-MMPR levels are asymmetrically distributed, their natural logarithm was considered as dependent variable for the multivariate regression model. In order to design each of these 2 models, we first performed univariate regressions with each factor listed in Tables 1-5. Then we fit together all variables so that the corresponding P-value in univariate regressions was <0.2. In the presence of certain variables, others may cease to be significant. The multivariate model was then built by removing nonsignificant covariates one after the other, based on likelihood ratio tests. We then reconsidered each factor and tried to include them in the model. Finally, we checked that no factor in the model could be removed or added, based on likelihood ratio tests. For all analyses we calculated adjusted odds ratios (OR) with 95% confidence intervals (CI). As in the descriptive part above, a P-value <0.05 was considered statistically significant. For all of these analyses, Stata software for PC was used (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

A total of 144 IBD patients treated with AZA or 6-MP who met the inclusion criteria were identified in the SIBDCS database. Of this population, 34 patients were later excluded because of incomplete/missing information. Of the 110 patients finally enrolled in this study, 40 were receiving a co-medication with 5-ASA at the time of blood sampling.

6-TGN and 6-MMPR levels in patients with and without 5-ASA co-medication

The median 6-TGN level in the 5-ASA co-medicated group was 261 pmol/8×10^8 EC, while in the AZA monotherapy group, we found a median 6-TGN level of 257 pmol/8×10^8 EC. The difference was not statistically significant, according to a Wilcoxon rank-sum test (P=0.97). In line with these findings were the results of the 6-MMPR levels: the median 6-MMPR level was 745.5 pmol/8×10^8 EC under combination therapy and 722 pmol/8×10^8 EC under AZA monotherapy (P=0.79) (Table 3).

Comparison of variables

Descriptive statistics from the 2 groups compared here are summarized in Table 1. There was no statistically significant difference between the 2 groups with respect to sex, age, weight, last smoking status or ethnicity. More specifically, 47.5% of the 40 patients co-medicated with 5-ASA and 54.3% of the 70 patients without co-medication were male. The median age and the median weight (last measure) were 41.8 years and 65 kg in the co-medicated group compared to 31.9 years and 70 kg

Table 1 Comparison of variables between patients taking and those not taking 5-ASA medication

Variables	No 5-ASA	5-ASA	P-value (χ^2 or Wilcoxon)
Sex			
Male	38 (54.3%)	19 (47.5%)	0.49
Female	32 (45.7%)	21 (52.5%)	
Age (years)			
median, q25-q75, min-max	31.9, 24.9-52, 12.2-77	41.8, 30.8-50.5, 14.8-76.1	0.13
Weight (kg)			
median, q25-q75, min-max	70, 59-78, 36-120	65, 57-78, 40-119	0.80
Last smoking status*			
No	47 (68.1%)	34 (85.0%)	0.052
Yes	22 (31.9%)	6 (15.0%)	
Unknown	1	0	
Ethnicity			
African	0	0	
Asian	3 (8.8%)	0	0.28**
Caucasian	29 (85.3%)	19 (90.5%)	0.70**
Hispanic	1 (2.9%)	0	>0.99**
Jewish	0	1 (4.8%)	0.38**
Unknown	36	19	

*According to the questionnaire for physicians; **Fisher's exact test

5-ASA; 5-aminosalicylic acid

in the control group, respectively. According to the physician's questionnaire, only 15.0% of all patients treated with 5-ASA were smokers at blood collection, compared to a smoking rate of 31.9% in the monotherapy group.

Comparison of disease characteristics

Disease characteristics are presented in Table 2. No differences were identified between the 2 groups regarding disease duration, CDAI (last measure), MTWAI (last measure), and disease location in CD/UC. In contrast, the distribution of the diagnosis of CD or UC in the cohort was different, reflecting the current treatment guidelines. The majority (67.5%) of patients co-treated with 5-ASA had the diagnosis of UC, whereas 94.3% of patients medicated with AZA or 6-MP monotherapy suffered from CD (P<0.001).

Comparison of disease course

Events during the disease course, such as complications, fistulas, stenosis, surgery, therapy with additional medication and therapy failure, are illustrated in Table 4. There was a statistically significant difference between the compared groups in 2 of the 12 investigated parameters. Fistulas, abscesses or anal fissures occurred in only 10.0% of patients with 5-ASA treatment at blood collection, whereas a manifestation rate of 41.4% was observed in the non-co-medicated group (P=0.001). Additionally, surgery for fistula was required more often in the monotherapy cohort (P=0.04). However, this result corresponds to the CD diagnosis and is not an independent variable.

With reference to treatment side-effects in our sample of 110 patients, we found no record of hepatitis, leukopenia, pancreatitis, or flu-like symptoms in the database.

Comparison of personality and quality of life

Table 5 shows the last measured scores for quality of life and personality in each group. The Hospital Anxiety and Depression Scale illustrating Anxiety (HADS-Anxiety) and the Negative Affectivity Score illustrating Personality were significantly different between the compared groups (P=0.03, P=0.01, respectively). Patients in dual therapy were more anxious and suffered from negative affectivity more often.

Factors associated with higher 6-TGN/6-MMPR levels by multivariate linear regression

The distribution of 6-TGN and 6-MMPR levels was asymmetrical. Consequently, we used the logarithm function for the multivariate linear regression model to test the relationship between 6-TGN levels and several clinical parameters (Table 6). The model selection for 6-TGN level returned a model with 3 significant parameters. We identified 6-TGN levels to be significantly higher in non-smokers, patients without prior surgery (intestinal or for fistula) and those without signs of stress-hyperarousal (P=0.03, P=0.01, P<0.001, respectively).

The multivariate linear regression model testing the correlation of 6-MMPR levels resulted in 2 statistically significant parameters (Table 7). We found 6-MMPR levels to be higher in patients without complications and stress-hyperarousal (P=0.02, P=0.001, respectively).

Discussion

The most substantial finding from this SIBDCS cohort was that co-medication with 5-ASA was not associated with differences in 6-TGN or 6-MMPR levels, as similar 6-TGN and 6-MMPR levels were measured in both groups. Previous small-scale trials described significantly higher levels of whole blood 6-TGN concentration in patients with IBD who received AZA or 6-MP with co-administration of 5-ASA [4,15,32]. However, these studies included only up to 34 patients. limiting their overall power. An expanded investigation was performed by Hande *et al* in 2006 [5]. They reported an association between 5-ASA and higher 6-TGN levels, but found no difference in 6-MMP levels. A critical point is that this study only included

Table 2 Comparison of disease characteristics between patients taking and those not taking a 5-ASA medication

Characteristics	No 5-ASA	5-ASA	P-value (χ^2 or Wilcoxon)
Diagnosis			
CD	66 (94.3%)	13 (32.5%)	<0.001
UC	4 (5.7%)	27 (67.5%)	
Disease duration (years)			
median, q25-q75,	5, 1.6-14.9,	9.0, 3.1-15.2,	0.30
min-max	0.1-55.5	0.1-31.2	
CDAI			
median, q25-q75,	20, 6-63,	35, 13-71,	0.20
min-max, n	0-177, n=66	6-133, n=13	
MTWAI			
median, q25-q75,	3, 1-7.5,	2, 1-3,	0.59
min-max, n	1-10, n=4	0-11, n=27	
Last CD location			
L1 (ileal)	20 (30.8%)	4 (33.3%)	0.61*
L2 (colic)	16 (24.6%)	5 (41.7%)	
L3 (ileo-colic)	27 (41.5%)	3 (25.0%)	
L4 only (upper GI)	2 (3.1%)	0 (0.0%)	
Unknown	1	1	
Maximal UC extent			
Proctitis	0 (0.0%)	1 (3.8%)	0.39*
Left-sided colitis	3 (75.0%)	10 (38.5%)	
Pancolitis	1 (25.0%)	15 (57.7%)	
Unknown	0	1	

*Fisher's exact test

5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; UC, ulcerative colitis; CDAI, CD activity index; MTWAI, modified Truelove and Witts activity index; GI, gastrointestinal

Table 3 Comparison of 6-TGN and 6-MMPR levels in patients on monotherapy and those receiving 5-ASA co-medication

Metabolites	No 5-ASA	5-ASA	P-value (Wilcoxon)
6-TGN level [pmol/8×10^8 EC]			
median, q25 – q75,	257, 141-463,	261, 126.5-499.5,	0.97
min – max	50-1232	50-1069	
6-MMPR level [pmol/8×10^8 EC]			
median, q25 – q75,	722, 306-2517,	745.5, 207-3147.5,	0.76
min – max	100-22360	100-13976	

6-TGN, 6-thioguanine nucleotide; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 5-ASA, 5-aminosalicylic acid; EC, erythrocytes

patients in clinical remission, and no patients who had to stop the therapy early.

In a large-scale trial in 2009 (n=183), Daperno *et al* observed that there was no significant difference in 6-TGN and 6-MMP blood concentrations associated with co-medication in patients on active thiopurine [26]. A potential influence of dual therapy on TPMT activity was not found. Although we did not measure TPMT activity in our cohort, we hypothesize that TPMT activity is not influenced by 5-ASA therapy *in vivo*. As this enzyme catalyzes the S-methylation of thiopurine to its ultimate conversion 6-MMP and 6-MMPR, an inhibition of TPMT activity would consequently lead to lower 6-MMP/6-MMPR blood concentrations. Remarkably, Hande *et al* also reported non-altered 6-MMP levels (see above) [5]. These different findings could be interpreted in the context of an adaption that leads to minor interference between thiopurines, 5-ASA and TPMT [25]. By including not only patients in clinical remission, but also in any stage of disease activity, we minimized the influence of a potential adaption.

In addition, a potentially reduced absorption rate in patients suffering from IBD should be acknowledged. This could lead to a false negative result, due to malabsorption of the administered medication. However, an altered absorption rate would mainly influence the results if the 2 compared groups had significantly different disease activity indexes (CDAI and MTWAI). However, in our study, no asymmetrical distribution of disease activity between the compared patients

Table 4 Comparison of disease courses between patients taking and those not taking a 5-ASA medication*

Disease course	No 5-ASA	5-ASA	P-value (χ^2)
Complication**			
No	40 (57.1%)	17 (42.5%)	0.14
Yes	30 (42.9%)	23 (57.5%)	
Fistula, abscess or anal fissure			
No	41 (58.6%)	36 (90.0%)	0.001
Yes	29 (41.4%)	4 (10.0%)	
Stenosis			
No	49 (70.0%)	32 (80.0%)	0.25
Yes	21 (30.0%)	8 (20.0%)	
Intestinal surgery			
No	49 (70.0%)	34 (85.0%)	0.08
Yes	21 (30.0%)	6 (15.0%)	
Surgery for fistula			
No	54 (77.1%)	37 (92.5%)	0.04
Yes	16 (22.9%)	3 (7.5%)	
Therapy with antibiotics			
No	42 (60.0%)	29 (72.5%)	0.19
Yes	28 (40.0%)	11 (27.5%)	
Therapy with biologics			
No	46 (65.7%)	31 (77.5%)	0.19
Yes	24 (34.3%)	9 (22.5%)	
Therapy with steroids			
No	9 (12.9%)	5 (12.5%)	0.96
Yes	61 (87.1%)	35 (87.5%)	
Failure of antibiotic therapy			
No	68 (97.1%)	40 (100%)	0.53***
Yes	2 (2.9%)	0 (0%)	
Failure of immunomodulator therapy			
No	66 (94.3%)	36 (90.0%)	0.46***
Yes	4 (5.7%)	4 (10.0%)	
Failure of biologic therapy			
No	62 (88.6%)	38 (95.0%)	0.32***
Yes	8 (11.4%)	2 (5.0%)	
Failure of steroid therapy			
No	61 (87.1%)	37 (92.5%)	0.53***
Yes	9 (12.9%)	3 (7.5%)	

*For each event (complication, fistula, stenosis, etc.), a yes means that it occurred at least once during follow up or before the enrolment in Swiss inflammatory bowel disease cohort; ** at least once during follow up; *** Fisher's exact test *

was found. The physician's decision to prescribe dual therapy or not may have had an impact on the results. The symmetrical distribution of different disease activities/courses in our cohort reduces the risk of such selection bias.

From a mechanistic perspective, combination treatment with 5-ASA and AZA offers a plethora of beneficial effects. In particular, it was revealed in a recent study, utilizing intestinal organoids derived from wild-type mice, that the abovementioned co-medication offered a junctional complex modulation and restoration of epithelial barrier function in a setting of intestinal inflammation. Moreover, 5-ASA—in contrast to AZA (which demonstrated antiproliferative effects)—promoted wound healing of colonic epithelial cells [33].

Within our study, it was not possible to verify adherence to 5-ASA medication. The overall long-term adherence rate in patients with UC taking mesalamine was found to be lower than 50% [34,35]. Therefore, given the absence of such relevant adherence information, the actual administered dosage could not be analyzed meaningfully, although it was between 2.4 and 4.8 g, in accordance with ECCO Guidelines [36]. This might lead to an underestimation of the effect of dual therapy on 6-TGN levels. For future studies, a verification of pill adherence is advisable to reduce the impact of adherence bias. Nevertheless, recent evidence endorses a high dosage of 5-ASA to induce remission in patients with IBD, without monitoring administered dosage or estimating the level of 5-ASA/metabolites [37], since 5-ASA ranks at the top among comparator treatments regarding safety and tolerability [38].

The unbalanced distribution of the diagnosis of CD and UC in our selection of patients may have had a significant impact on the basic 6-TGN concentration of each group. The majority (67.5%) of patients co-treated with 5-ASA had the diagnosis of UC, while 94.3% of patients medicated with only AZA or 6-MP suffered from CD. It was suggested in a previous study that the diagnosis of CD is independently associated with elevated 6-TGN blood concentrations [5]. Further descriptive statistics of co-treated patients were performed to evaluate these concerns for each diagnosis. Using a Wilcoxon rank-sum test, we were unable to establish a significant difference in 6-TGN levels between patients with UC and those suffering from CD (P=0.39).

The shift in the distribution of diagnosis was most probably responsible for the statistically significant difference between the compared groups in 2 of the 12 investigated parameters of disease courses. Fistulas, abscesses or anal fissures occurred in only 10.0% of patients receiving dual therapy at blood collection, whereas a manifestation rate of 41.4% was observed in the non-co-medicated group. Additionally, surgery for fistula was required more often in the non-co-medicated, predominantly CD cohort.

Using a multivariate linear regression model, we tested the relationship between 6-TGN levels and different parameters. We found that the 6-TGN level of a non-smoker was on average 1.54 times greater than that of a smoker. In this respect, emerging scientific evidence supports that nicotine possesses anti-inflammatory properties for both UC and CD [39,40]. In contrast, it is postulated that 6-TGN levels correlate with therapeutic success/remission [13,41]. Further investigations into this interaction are warranted. The multivariate regression model revealed that the parameter of surgery was associated with lower TGN levels, providing further support for the abovementioned idea of Cuffari *et al* [42]. This theory implies that higher 6-TGN levels may be associated with a better disease course. As we did not observe altered 6-TGN concentrations or disease courses in either of the compared groups, we cannot

Table 5 Comparison of personality and quality of life between patients taking and those not taking a 5-ASA medication

Scales	No 5-ASA	5-ASA	P-value (χ^2 or Wilcoxon)
SF-36 – Physical Component Summary median, q25-q75, min-max, n	51.2, 42.0-57.1, 25.3-59.6, n=32	53.2, 47.0-55.6, 25.6-57.3, n=18	0.92
SF-36 – Mental Component Summary median, q25-q75, min-max, n	49.0, 43.5-55.2, 21.8-62.3, n=32	44.4, 41.5-51.9, 20.7-56.9, n=18	0.10
HADS – Anxiety median, q25-q75, min-max, n	4.5, 1-9, 0-14, n=32	7, 4.3-10.5, 2-14, n=20	0.034
HADS – Depression median, q25-q75, min-max, n	2, 1-6, 0-15, n=32	4, 2-7, 0-14, n=20	0.18
Stress – Re-experiencing median, q25-q75, min-max, n	0, 0-2, 0-12, n=32	1, 0-2.75, 0-7, n=20	0.33
Stress-Avoidance median, q25-q75, min-max, n	1, 0-4, 0-13, n=32	2.5, 1.5-4.5, 0-13, n=20	0.10
Stress – Hyperarousal median, q25-q75, min-max, n	2, 1-3, 0-11, n=32	3, 2-4.5, 0-10, n=20	0.20
Stress – TOTAL median, q25-q75, min-max, n	4, 2-8.5, 0-33, n=32	6.5, 4-11, 0-24, n=20	0.15
Personality–Negative Affectivity Score median, q25-q75, min-max, n	9, 3.5-11.5, 0-17, n=32	14, 8.5-15.6, 2-24, n=20	0.006
Personality–Social Inhibition Score median, q25-q75, min-max, n	7, 2-15, 0-24, n=32	8.5, 4-16.5, 1-25, n=20	0.42
Type-D Personality No Yes	24 (75.0%) 8 (25.0%)	12 (60.0%) 8 (40.0%)	0.25

5-ASA, 5-aminosalicylic acid; HADS, Hospital Anxiety and Depression Scale; SF-36, Short Form (36) Health Survey

Table 6 Factors associated with elevated 6-TGN levels by multivariate linear regression model

Response: logarithm of 6-TGN level, n=54	Coefficient Beta (95%CI; P-value)
Last smoking status (y/n)	-0.433 (-0.819 to -0.047; 0.029)
Any surgery (y/n)	-0.466 (-0.829 to -0.103; 0.013)
Stress – hyperarousal	-0.183 (-0.279 to -0.087; <0.001)
Intercept	7.447 (6.141 to 8.754; <0.001)

A coefficient value of -0.433 (95% CI: -0.819 to -0.047; P=0.03) in last smoking status means that, all other things being equal, the logarithm of the 6-TGN level of a smoker will be on average 0.433 units lower compared to a non-smoker. Conversely, the 6-TGN level of a non-smoker was on average 1.54 times higher compared to smokers

6-TGN, 6-thioguanine nucleotide; CI, confidence interval

Table 7 Factors associated with elevated 6-MMPR levels by multivariate linear regression model

Response: logarithm of 6-MMPR level, n=54	Coefficient Beta (95%CI; P-value)
Complication (y/n)	-0.914 (-1.669 to -0.158; 0.019)
Stress – hyperarousal	-0.350 (-0.549 to -0.151; 0.001)
Intercept	10.740 (7.927 to 13.552; <0.001)

We found a coefficient value of -0.914 (-1.669 to -0.158; P=0.02) in relation to complications, meaning that the 6-MMPR level of patients without complications was 2.494 times higher than that of patients who had complications

6-MMPR, 6-methylmercaptopurine ribonucleotides; CI, confidence interval

offer a more specific statement regarding the advantages of elevated 6-TGN levels in relation to a better therapy outcome.

For the same reason, we cannot assert that higher 6-TGN levels are associated with more side-effects. However, in contrast to other studies claiming an elevated risk up to 47% for leukopenia when AZA is paired with 5-ASA [43], in our cohort no hepatitis, leukopenia, pancreatitis or flu-like symptoms were reported. It is worth noting that this finding referred to a monotherapy group with a maximum 6-TGN level of 1232 pmol/8×10^8 EC, compared to 1069 pmol/8×10^8 EC in the dual therapy group.

A further limitation of our study was the missing documentation regarding the different formulations of 5-ASA; in this respect, it was recently demonstrated [44] that 5-ASA and N-acetyl-5-ASA levels were significantly higher in IBD individuals receiving time-dependent mesalazine compared to those with pH-dependent mesalazine and multimatrix mesalazine, and that this was also accompanied by greater TPMT inhibition. Moreover, the rather small number of recruited patients has to be acknowledged, even though the present study included more patients than the ones of the past.

In conclusion, our data support no effect of concomitant 5-ASA treatment on 6-TGN and 6-MMPR levels. Treatment-associated side-effects or worse tolerability never occurred. Nevertheless, physicians should be careful in administrating 5-ASA co-medication, although our data warrant neither more frequent laboratory monitoring, nor dose adaptation of AZA in thiopurine patients receiving concomitant 5-ASA treatment.

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Summary Box

What is already known:

- Inflammatory bowel disease almost always requires long-term treatment
- Azathioprine (AZA) and 5-aminosalicylic acid (5-ASA) are among its fundamental pharmaceutical treatments
- AZA metabolite levels in blood are influenced by a plethora of parameters
- There are conflicting data as to whether co-medication of AZA with 5-ASA is associated with more side-effects or less efficient treatment

What the new findings are:

- AZA metabolite levels do not differ between patients under AZA monotherapy and those under co-medication with 5-ASA
- More frequent lab monitoring is probably not advised for such patients
- Adaptation of AZA dosage in patients with concomitant 5-ASA is not necessary

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