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REDUCED THIOPURINE METHYLTRANSFERASE ACTIVITY AND DEVELOPMENT OF SIDE EFFECTS OF AZATHIOPRINE TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Objective. **To investigate thiopurine enzyme activities for their possible value in predicting the development of azathioprine (AZA)–related toxicity in patients with rheumatoid arthritis (RA).**

Methods. **Patients with longstanding RA (n = 33) were enrolled in a study of treatment with AZA. Before the initiation of AZA treatment and at months 1 and 6 of treatment, we measured activities of the purine key enzymes hypoxanthine guanine phosphoribosyltransferase, 5′-nucleotidase, purine nucleoside phosphorylase, and thiopurine methyltransferase (TPMT). Controls included patients with early RA (n = 24) and healthy volunteers (n = 42).**

Results. **Fourteen of the 33 patients rapidly developed severe side effects, most frequently gastrointestinal (GI) intolerance. Compared with the other groups, the group with adverse effects had significantly lower TPMT activities ($P = 0.004$). Seven of 8 patients with reduced ("intermediate") baseline TPMT levels developed toxicity, resulting in a significant relationship ($P = 0.005$) between toxicity and "intermediate" TPMT activity. Compared with "high" activity, baseline intermediate TPMT activity gave a relative risk of 3.1 (95% confidence interval 1.6–6.2) for the development of severe toxicity with AZA treatment.**

Conclusion. **In RA patients, inherited intermediate TPMT activity seems predictive for the development of severe side effects of AZA. Clinicians should consider measuring TPMT prior to treatment initiation to improve the safety of AZA use. We hypothesize that GI intolerance may also be related to a thiopurine metabolic imbalance.**

Azathioprine (AZA) is a purine (hypoxanthine) analog with cytotoxic and immunosuppressive properties, used in the treatment of autoimmune rheumatic disorders, chronic inflammatory gastrointestinal (GI) diseases, hepatitis, inflammatory dermatologic diseases, and in the prevention of graft rejection in transplant recipients. After conversion to 6-mercaptopurine (6-MP), AZA metabolism largely follows the endogenous purine pathways (1) (Figure 1). It is generally believed that 6-thioguanine nucleotides (6-TGN) play a major role in the development of cytotoxicity via incorporation into DNA and RNA (2,3).

Rheumatoid arthritis (RA) patients treated with AZA show variable responses ranging from successful reduction of disease activity to severe toxicity. Prospective long-term studies in RA patients have demonstrated that in 10–30%, AZA had to be withdrawn due to side effects (4–6). These effects included severe bone marrow toxicity, manifested most of the time as leukopenia (15%), and less frequently as thrombocytopenia, anemia, pure red cell aplasia, or pancytopenia (7–10). Other side effects are GI intolerance (7,11), hepatotoxicity (11), and hypersensitivity manifestations (6,11,12).

It is known that substantial disturbances in the activities of hypoxanthine guanine phosphoribosyltransferase (HGPRT), thiopurine methyltransferase (TPMT), 5′-nucleotidase (5′NT), or xanthine oxidase (XO) influence the effect of AZA (13–16). In a previous study of patients with recent-onset RA who were not taking disease-modifying antirheumatic drugs (DMARDs), we

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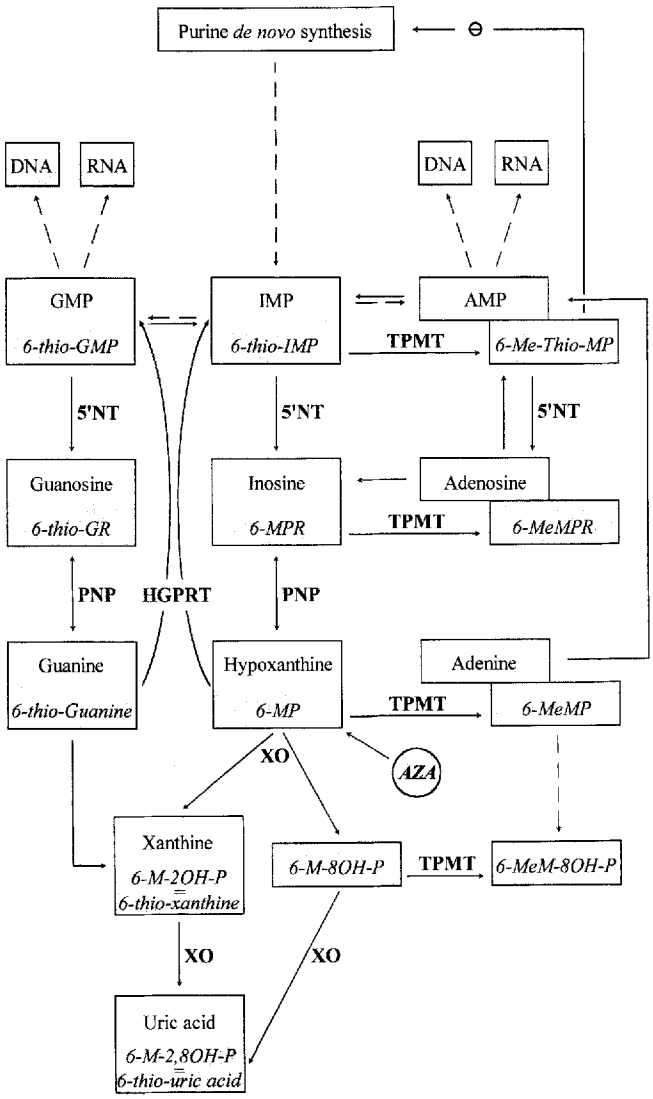

Figure 1. Simplified schema of purine and azathioprine (AZA)/6-mercaptopurine (6-MP) metabolism. TPMT = thiopurine methyltransferase; 6-Me-Thio-MP = 6-methyl-thio-MP; 5′NT = 5′-nucleotidase; 6-thio-GR = 6-thio-guanosine ribonucleoside; 6-MPR = 6-mercaptopurine ribonucleoside; 6-MeMPR = 6-methyl-mercaptopurine ribonucleoside; PNP = purine nucleoside phosphorylase; HGPRT = hypoxanthine guanine phosphoribosyltransferase; 6-MeMP = 6-methyl-MP; XO = xanthine oxidase (xanthine dehydrogenase); M = mercapto; P = purine.

demonstrated the absence of preexisting (RA-associated) abnormalities in activities of HGPRT, TPMT, 5′NT, or purine nucleoside phosphorylase (PNP) (17). Data from an uncontrolled cross-sectional pilot study of patients with longstanding RA, however, suggested an association between purine enzyme activities and the outcome of AZA treatment (18).

To determine whether the occurrence of toxicity may be predicted by changes in thiopurine enzyme activities, induced either by AZA itself or by alterations already present before the start of this treatment, we conducted a prospective longitudinal study of patients with established RA in whom AZA treatment was being initiated.

PATIENTS AND METHODS

Study design. Thirty-three RA patients were enrolled in the longitudinal study during a period of a year and a half. Other DMARDs had been discontinued at least 2 weeks before the initiation of AZA treatment. Prednisone could be continued at a maximum and stable daily dosage of ≤10 mg, as could nonsteroidal antiinflammatory drugs or acetaminophen in fixed doses. Laboratory and clinical assessments were performed just before AZA treatment was started (month 0) and at 1 month and 6 months thereafter. Between these times, blood samples were obtained every 2 weeks for monitoring of hematologic indices, liver enzyme levels, and kidney function. Patients made 2 clinical visits to their own rheumatologists between month 1 and month 6. During the first month, AZA was given in an increasing dosage, i.e., 1 50-mg tablet each day the first week, 50 mg twice daily the second week, and so on until a target value of ~2 mg/kg/day was attained. Tablets containing 50 mg or 25 mg of AZA were used. Patients were instructed to contact their doctor or the investigator in case of abnormalities or side effects that could possibly be related to the use of AZA. In case of a possible and persistent side effect, the dosage was reduced or the treatment stopped until the side effect disappeared. If, after rechallenge, the side effect recurred, AZA was withdrawn permanently.

To avoid the possible influence of circadian rhythms (19), clinical assessments and blood sampling were always performed between 9:00 am and 11:00 am. The clinical portion of the study was performed by one investigator (JNS).

At the time of analysis, the AZA-treated patients were divided into 2 subgroups. Group I consisted of patients who, throughout a complete 6-month followup, had no serious side effects. Group II comprised patients who had incomplete followup because of severe side effects necessitating withdrawal of AZA treatment. Results were compared with those of cross-sectionally studied groups of 24 patients with early RA who had not begun any DMARD treatment (group III) and 42 healthy controls (group IV). Some of the group III and group IV patients had been part of an earlier study (17). All participants were white.

This protocol was approved by each of the participating hospital ethics committees, and each patient gave informed consent prior to enrollment in the study.

Selection of patients. The inclusion criteria were as follows: 1) RA fulfilling the 1987 American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria (20); 2) active disease with at least 3 of the following features: ≥3 swollen joints, ≥6 joints painful on motion or pressure, morning stiffness ≥45 minutes, and erythrocyte sedimentation rate (ESR) ≥28 mm/hour (21); 3) no prior treatment with AZA; and 4) age between 18 and 75 years.

Table 1. Baseline characteristics by group*

	AZA-treated RA		Group III: early, non–DMARD-treated RA (n = 24)	Group IV: healthy controls (n = 42)
	Group I: no side effects (n = 19)	Group II: side effects (n = 14)		
Age, mean ± SD years (range)	56.6 ± 11.5 (33–74)	55.6 ± 12.5 (36–72)	54.3 ± 13.8 (23–73)	43.6 ± 9.1 (26–63)
No. male/no. female	6/13	2/12	14/10	16/26
Months since diagnosis, mean ± SD	102.8 ± 71.0	80.7 ± 76.4	4.8 ± 3.8	NA
RF, no. positive/no. negative	15/4	12/2	21/3	NA
Prednisone, no. receiving/no. not receiving	5/14	2/12	0/24	NA
Washout period, mean ± SD weeks (median)	3.9 ± 3.3 (2.5)	6.0 ± 4.3 (4.0)	NA	NA
AZA†				
Mean ± SD mg/day (range)	140.8 ± 38.4 (75–200)	110.8 ± 52.5 (50–200)‡	NA	NA
Mean ± SD mg/kg/day (range)	2.0 ± 0.5 (1.3–2.9)	1.6 ± 0.7 (0.6–2.7)§	NA	NA

* AZA = azathioprine; RA = rheumatoid arthritis; NA = not applicable; RF = rheumatoid factor.
† Month-1 values in group I; values at the time of side effects in group II.
‡ $P = 0.07$ versus group I, by t-test.
§ $P = 0.03$ versus group I, by t-test.

Exclusion criteria consisted of 1) blood transfusion within the previous 4 months; 2) hematologic disease or abnormal hematologic parameters (hemoglobin <5.5 mmoles/liter, leukocyte count $<3.5 \times 10^9$/liter, platelet count $<150 \times 10^9$/liter); 3) kidney disease with creatinine clearance rates <50 ml/minute; 4) liver disease or transaminase levels more than twice the normal value; 5) malignancy; 6) acute or chronic infection; 7) insulin-dependent diabetes mellitus; 8) pregnancy; 9) alcohol abuse; 10) ACR functional class IV (22); 11) use of medication that may interact with AZA or affect purine metabolism; or 12) history of poor compliance with medication regimens.

Assessments of disease activity. Parameters of disease activity included patient assessments of general health and pain on 10-cm visual analog scales (0 = best possible; 10 = worst possible), the Ritchie Articular Index (23), the number of swollen joints, the number of tender joints, the duration of morning stiffness, and the disease activity score (DAS_4) (24).

Laboratory assessments. Venous blood samples for determination of purine enzyme activities, IgM rheumatoid factor (by enzyme-linked immunosorbent assay; positive if >10 IU/ml), HLA type, and routine blood testing (patients only) were collected immediately after the clinical assessments. Routine blood analyses included a complete blood cell count with differential count, determination of the ESR (mm/hour; Westergren method) and C-reactive protein level (mg/ml), and a blood biochemical profile including kidney and liver function and serum uric acid level.

The activities of 5′NT, PNP, and HGPRT were determined by nonradiochemical high performance liquid chromatography procedures, as described previously (25). TPMT activity was determined in red cell lysates as described by Weinshilboum et al (26) and was expressed in pmoles/10^6 erythrocytes/hour of incubation. Other enzyme activities were expressed in nmoles/10^6 viable mononuclear cells/hour of incubation. The enzyme assays had good reproducibility, with mean ± SEM coefficients of variation of 7.6 ± 0.7, 5.3 ± 0.9, 6.6 ± 0.8, and 4.0 ± 0.5 for HGPRT, 5′NT, PNP, and TPMT, respectively. All enzyme assays were carried out in quadruplicate.

Statistical analysis. The results were analyzed predominantly by parametric procedures, but in cases of small deviations from normal distribution (TPMT activity), nonparametric tests were used also. P values less than or equal to 0.05 were considered significant. The statistics were processed with SPSS computer software (version 6.1).

Correlations between enzyme activities and parameters of disease activity, disease duration, or functional class were examined at month 0 by Pearson or Spearman correlation analyses. The influence of age was studied by linear regression analysis using the baseline enzyme activities of all groups. Overall influences of group and sex on enzyme activities were analyzed by two-way analysis of variance (ANOVA). Since enzyme activities in the early RA group did not correlate with indices of disease activity or with disease duration and because they were not different from the values in healthy controls (17), the enzyme values for month 0 in groups III and IV were also used for comparisons at months 1 and 6. To prevent Type I errors according to the Bonferroni principle in multiple testing, P values less than or equal to 0.017 were then considered significant. Differences in enzyme activities between the groups were analyzed by one-way ANOVA followed by Tukey's highest significant difference test for post hoc pairwise multiple comparisons. In cases of violation of the assumptions for parametric testing, the Kruskal-Wallis test was used in combination with Mann-Whitney U tests with $\alpha = 0.017$. Within-group time-related differences in enzyme levels were analyzed by repeated-measures ANOVA or by Friedman's two-way ANOVA for group I (3 measurement points) and by paired t-tests or paired Wilcoxon tests for group II (2 measurement points). The effect of AZA treatment relative to TPMT activity was analyzed by Fisher's exact test.

RESULTS

Characteristics of the patients and healthy controls are shown in Table 1. One patient in group I left the study in the third month for personal reasons (not

Table 2. Purine enzyme activities by group*

	AZA-treated RA						
	Group I: no side effects			Group II: side effects		Group III: early RA†	Group IV: healthy controls†
	Month 0	Month 1	Month 6	Month 0	Month 1		
HGPRT	9.1 ± 3.5 (19)	9.6 ± 4.0 (19)	7.9 ± 2.2 (18)	8.6 ± 3.4 (14)	7.3 ± 3.1 (13)	11.0 ± 3.5 (23)	9.9 ± 3.8 (30)
5′NT							
Men	18.8 ± 11.1 (6)	16.2 ± 11.3 (6)	15.9 ± 5.5 (6)	31.0 ± 4.1 (2)	27.3 ± 5.5 (2)	14.2 ± 6.0 (14)	13.7 ± 4.6 (16)
Women	19.1 ± 8.3 (13)	20.9 ± 8.0 (13)	17.3 ± 7.3 (12)	19.8 ± 6.6 (12)	20.5 ± 8.4 (11)	19.1 ± 6.4 (10)	21.6 ± 8.3 (26)
PNP	195 ± 46 (19)	202 ± 76 (19)	169 ± 61 (18)	180 ± 50 (13)	191 ± 89 (12)	209 ± 55 (24)	198 ± 58 (42)
TPMT	22.4 ± 3.8 (19)	23.0 ± 3.9 (19)	24.8 ± 4.3 (18)	17.3 ± 5.9 (14)	17.1 ± 6.4 (13)	24.3 ± 4.8 (24)	22.1 ± 4.3 (39)

* Enzyme activities are expressed in nmoles/10^6 viable mononuclear cells/hour of incubation, except for thiopurine methyltransferase (TPMT) activity, which is expressed in pmoles/10^6 erythrocytes/hour of incubation. All enzyme activities are adjusted to the mean overall age of 50.4 years. Values are the mean ± SD (n). AZA = azathioprine; RA = rheumatoid arthritis; HGPRT = hypoxanthine guanine phosphoribosyltransferase; 5′NT = 5′-nucleotidase; PNP = purine nucleoside phosphorylase.
† Studied cross-sectionally.

because of side effects). At entry into the study all patients had normal kidney and liver function and normal serum urate levels. Some minor hematologic abnormalities (low hemoglobin concentration, elevated platelet count) (data not shown) were considered to be attributable to activity of the disease.

No significant associations were found between enzyme activities and indices of disease activity, or between enzyme activities and functional class. Enzyme activities were not different between the group of patients who were positive and the group who were negative for the RA-associated HLA types DR1, DR2, DR3, and DR4, or between those who were and those who were not treated with prednisone. TPMT showed a significant but weak relationship with disease duration (Spearman's correlation coefficient −0.29, $P = 0.03$). Disease duration did not differ significantly between groups I and II.

Age and sex had a significant influence on 5′NT activity, similar to the findings in our previous study (17) (mean ± SEM annual change in activity with age −0.18 ± 0.06 nmoles/10^6 viable mononuclear cells/hour of incubation [$P = 0.005$]; sex influence $P = 0.01$, with lower activity in males). In the present study, PNP was also age dependent (mean ± SEM annual change in activity 1.02 ± 0.44 nmoles/10^6 viable mononuclear cells/hour of incubation [$P = 0.02$]). TPMT and HGPRT were not influenced by age or sex. Age-adjusted enzyme activities are shown in Table 2. One healthy control subject had undetectable TPMT activity and was excluded from further analysis.

ANOVA showed significant influences of the variable "group" on HGPRT activity at month 1 ($P = 0.02$) and month 6 ($P = 0.005$) and on TPMT activity at month 0 ($P = 0.004$) and month 1 ($P = 0.004$). These possible between-group differences were analyzed further as noted above. Mean levels of HGPRT in group II (AZA treatment with side effects) at month 1 and in group I (AZA treatment without side effects) at month 6 were significantly lower than in group III (early RA without DMARD treatment) ($P < 0.02$). TPMT activity in group II was significantly lower compared with all other groups, both at month 0 and at month 1 (all P values < 0.01) (Figure 2). Activities of 5′NT and PNP were not different between groups, except for a higher level of 5′NT among men in group II. Since there were only 2 male patients in group II, the latter finding was considered a coincidence.

In group II, we found no significant time-related differences in enzyme levels between the pretreatment

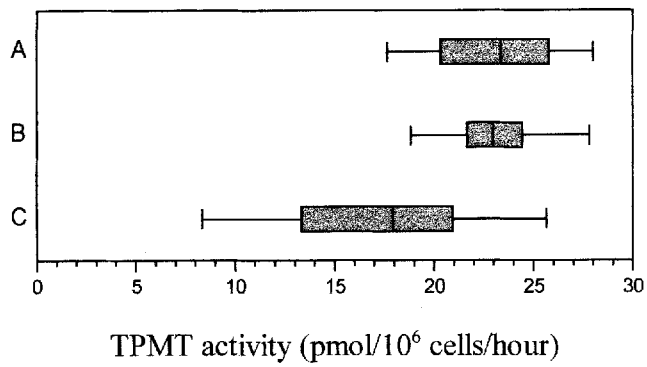

Figure 2. Baseline TPMT activities in patients with early rheumatoid arthritis (RA) and healthy controls (**A**), in AZA-treated RA patients without side effects (**B**), and in AZA-treated RA patients with side effects (**C**). Boxes represent the twenty-fifth–seventy-fifth percentile range, bars within the boxes the median value, and lines outside the boxes the tenth–ninetieth percentile range. See Figure 1 for other definitions.

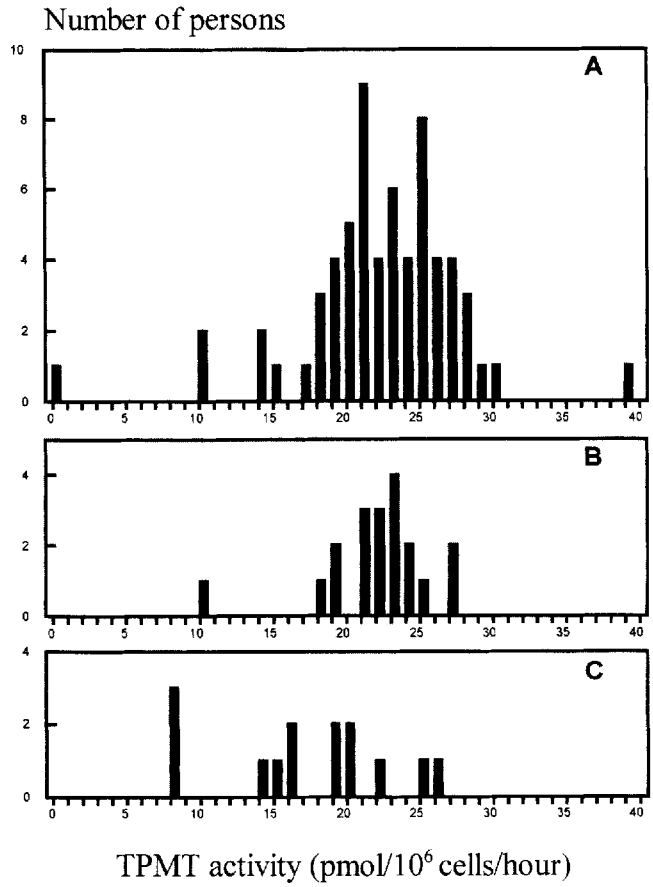

Figure 3. Frequency distribution of baseline TPMT activities in the reference group of patients with early rheumatoid arthritis (RA) and healthy controls (**A**), in AZA-treated RA patients without side effects (**B**), and in AZA-treated RA patients with side effects (**C**). See Figure 1 for other definitions.

period and the time that side effects occurred. In group I, overall time effects for HGPRT ($P = 0.06$) and TPMT ($P = 0.09$) could not be entirely excluded; in this group, paired t-tests revealed significant differences between month 1 and month 6 for HGPRT ($P = 0.04$) and between month 0 and month 6 for TPMT ($P = 0.04$), HGPRT being lower and TPMT being higher at month 6.

The distribution of TPMT activity in the patients and controls is shown in Figure 3. The results are consistent with the known trimodality of this activity (27). Since TPMT activity was not different between healthy controls and patients with early, non–DMARD-treated RA (17), their data were combined and used as reference (Figure 3A) for the AZA-treated patients. One control subject had undetectable TPMT activity. The "intermediate" activities ranged from 8.3 to 18.0 pmoles/10^6erythrocytes/hour (below or at percentile 11.1) and the "high" activities from 18.1 to 39.4 pmoles/10^6erythrocytes/hour (above percentile 11.1). Of the AZA-treated patients without signs of toxicity (n = 18), all but 1 had a baseline TPMT activity in the high range (Figure 3B). In the group with side effects (n = 14), the baseline TPMT level was shifted downward: only 50% of these patients had high TPMT activity, while the other half had activity in the intermediate range (Figure 3C). Fisher's exact test showed a highly significant association ($P = 0.005$) between the occurrence of side effects and intermediate TPMT activity. The relative risk of developing toxicity in patients with baseline intermediate TPMT activity, versus baseline high activity, was 3.1 (95% confidence interval 1.6–6.2). With a baseline high TPMT activity, the relative chance of not developing side effects with AZA treatment was 5.8 (95% confidence interval 0.91–36.6). When only healthy controls were used as a reference group, we obtained similar results.

Side effects and other characteristics of AZA-treated patients in AZA group II are shown in Table 3. Of the patients with bone marrow depression, 1 had GI symptoms first and gradually developed complete pancytopenia within the second month despite reduction of the AZA dosage from 150 mg/day to 50 mg/day; the other underwent the same dosage reduction but nonetheless had complete pancytopenia within the third month. In all patients, the side effects disappeared after discontinuation of AZA treatment. In the patients with fever, no signs of infection were found. Eight patients were rechallenged with AZA, and all of them again developed side effects. Two patients were not rechallenged because of suspected hypersensitivity and 1 because of pancytopenia. Three patients refused rechallenge. We found no significant correlation between AZA dosage and TPMT activity.

Previous use of DMARDs was not essentially different in the AZA-treated patients with versus those without side effects. The AZA-treated patients showed significantly lower serum uric acid levels after 1 month of treatment as compared with baseline levels (mean ± SD 0.28 ± 0.07 mmoles/liter at baseline versus 0.26 ± 0.05 at month 1 in group I [$P = 0.016$ by paired t-test]; 0.30 ± 0.08 at baseline versus 0.26 ± 0.08 at month 1 in group II [$P = 0.004$ by paired t-test]). Percentile decreases were 7.1 in group I and 13.3 in group II, respectively. In group I, disease activity according to the DAS_4 decreased significantly in 9 of the 18 patients after 6 months of AZA treatment (good responders, i.e.,

Table 3. Characteristics of the AZA-treated RA patients with side effects*

Patient	Side effect	Interval to first side effects (weeks after start)	Permanent withdrawal (weeks after first start)†	TPMT activity	HGPRT relative activity‡	Rechallenge	Side effect after rechallenge, type, and time interval	Considered as hypersensitivity manifestation
1	GI, HT	2	4	H	>50	No	–	No
2	GI, flu-like, rash	3	3½	H	<50	No	–	Yes
3	GI	3½	5½	H	<10	Yes	+, same, at 1½ weeks	No
4	GI, flu-like	3½	5½	I	<25	No	–	No
5	GI, HA	<1	3	H	<25	Yes	+, same, immediately	Yes
6	Recurrent UAI	2½	10	I	<50	Yes (2×)	+, same, at 3 weeks	No
7	GI, pancytopenia	3	7§	I	<10	Yes	+, same GI, at 2½ weeks	No
8	Vasculitis, fever, A	1	1	H	<50	No	–	Yes
9	GI	2½	4½	I	>50	Yes	+, same, at 1½ weeks	No
10	GI	4	5½	I	<25	Yes	+, same, at 1 week	No
11	Pancytopenia	8	10	H¶	<50	No	–	No
12	GI	1	2	H	>50	Yes	+, same, at <½ week	Yes
13	GI, HA, fever	2	3	I	<50	Yes	+, same, immediately	Yes
14	HT	4	4	I	>50	No	–	No

* GI = gastrointestinal; HT = hepatotoxicity; H = high activity; I = intermediate activity; HA = headache; UAI = upper airway infection; A = arthralgia. See Table 2 for other definitions.
† Including rechallenge.
‡ Percentile enzyme activity of the reference group.
§ Development of pancytopenia.
¶ Low end of the high range.

≥1.1-point improvement in the score). We found no differences in enzyme activities between good responders and the other patients in this group.

DISCUSSION

Despite the use of new drugs, AZA still has an important role in the treatment of autoimmune rheumatic disorders and other inflammatory diseases, and in transplantation medicine. Plasma concentrations of AZA or 6-MP have no prognostic value for the predicting effect of treatment (28), and as yet there is no consensus regarding the optimal way to predict the toxicity of this drug. For those reasons, it seemed promising to investigate AZA metabolism during treatment; especially since in various conditions, changes in thiopurine enzyme activities may be responsible for treatment outcome (13–16,18,29). We therefore conducted this investigation which, to our knowledge, is the first longitudinal observational study of AZA metabolism in RA patients beginning this treatment.

The most important finding of this study was the significantly reduced TPMT activity in the group of patients who experienced side effects. This reduced activity was already present before the start of AZA treatment and did not change. In the AZA-treated patients without signs of toxicity, baseline TPMT activity was higher and comparable with that in the control groups. In addition, we noted a significant increase (~10%) in TPMT levels after 6 months of treatment. This increase is consistent with previous reports and may be regarded as an "induction effect" by mercaptopurine therapy, the underlying mechanism and clinical relevance of which are as yet unknown (15,30,31).

Previous investigations of TPMT have shown that its activity is controlled by genetic polymorphism resulting from an autosomal codominant inheritance. Very low TPMT activity is found in 0.3% of the population, intermediate activity in 11.1%, and high activity in ~88.6% (26,30). Our data also clearly showed the trimodal distribution of TPMT activity. Interestingly, 50% of our patients with AZA-related side effects had TPMT activities below the 11th percentile reference value, corresponding with intermediate activity (27). Conversely, 7 of the 8 patients with an intermediate baseline TPMT level developed side effects.

We also found lower baseline HGPRT activity in the patients with side effects compared with controls. Notably, the activity in these group II patients decreased further and became significantly lower at month 1, while AZA-treated patients without side effects showed an increase in their HGPRT activities at that time.

Another striking observation in this study was the high rate of side effects necessitating the withdrawal of AZA (42%). Compared with some other studies (23,32)

this percentage was not exceptional, but lower rates have also been reported (4,6,12,33). Most patients (71% of those with side effects) developed severe GI intolerance, often accompanied by other symptoms.

Previous studies have demonstrated that low activity, and especially, deficiency, of TPMT in patients treated with AZA or 6-MP in conventional doses is a serious risk factor for the development of severe bone marrow depression (15,34–36). Two of the patients in this study developed pancytopenia. Since the first signs were obvious after 1 month of treatment, it is not very likely that these cytotoxic events were due to a true TPMT deficiency (15,34–36). Although we did not find a true deficiency, 1 of the 2 patients had reduced TPMT activity, which likely explained the bone marrow depression. Other possible explanations, such as low 5′NT activity (16) or extremely high HGPRT activity, were not found. Since we observed a significant relationship between side effects and intermediate TPMT activity, it is likely that in most of the remaining patients with intermediate TPMT activity, nonhematologic side effects were also caused by a thiopurine enzymatic imbalance.

It is clear that in RA, as in other diseases (15,33,37), TPMT plays a critical role in AZA treatment. The rare deficiencies of TPMT are strongly related to severe cytotoxicity (15,34,36). Moreover, in our RA group, the distinction between intermediate and high transmethylation activity also appears to be important. A baseline inherited intermediate TPMT value results in a significant relative risk of 3.1 for the development of severe, predominantly nonhematologic, side effects. This finding emphasizes the importance of pretreatment measurement of TPMT activity, as stated previously by others (15,29,34). Since the number of laboratories performing TPMT enzyme assays is increasing, this measurement should be easily obtained. In addition, a polymerase chain reaction–based method has been recently described for molecular diagnosis of TPMT deficiency or heterozygosity (29,38).

However, as noted previously (35), the TPMT level is not the only factor responsible for AZA intolerance, since about half of our AZA-treated patients with side effects had TPMT activities in the lower region of the "high" range. Other purine enzymatic causes for AZA-related toxicity, such as low 5′NT activity (16), were found in 1 female patient (6.7 nmoles/10^6 cells/hour versus a mean activity of 19.8 in women). She had GI symptoms rather than the expected bone marrow depression. Abnormal XO activity was likely not present in our patients, since all of them had normal serum uric acid levels.

Another explanation for development of side effects may be the large interindividual variability in bioavailability of AZA (39). Although cytotoxicity is related to intracellular 6-TGN concentrations and not to plasma concentrations (40), it is possible that other side effects may be related to plasma concentrations, in which case bioavailability may be an important factor. The related factors of washout period and previously used DMARDs may also play a role. For ethical reasons, the minimum washout period in this study was set at 2 weeks, not at 1 month. It is conceivable that after this relatively short period, the previous DMARD or its metabolite(s) may still interfere with AZA and subsequently lead to development of side effects. Recently we examined the influence of sulfasalazine on the activities of the same thiopurine enzymes as were measured in this study, and found no significant changes (Stolk JN et al: unpublished observations). Methotrexate interferes with de novo synthesis of purine, but probably not with that part of the "salvage pathway" that is involved in AZA metabolism. For the other DMARDs taken by our patients (hydroxychloroquine, D-penicillamine, aurothioglucose, auranofin), there are no data addressing possible interference. However, the mean washout period in the AZA group with side effects was longer than in the group without side effects, and the previously used DMARDs were comparable in both AZA-treated groups, so these variables probably did not have an influence on the results of the study.

Finally, AZA may cause several side effects that are attributed to hypersensitivity reactions (11,12). Features of anaphylaxis are sometimes reported, but we found none. Based on the temporal relationship between the start of AZA treatment and the first signs of side effects and on the symptom-free interval after rechallenge, we believe hypersensitivity was probably causal in 5 of our patients. Four of them had high TPMT levels. From these observations, the possibility arises that the same kind of side effect, GI intolerance, may develop from 2 different pathophysiologic mechanisms: a hypersensitivity reaction on the one hand and an imbalance in thiopurine metabolism on the other. The higher percentile decrease of serum uric acid levels in patients with side effects compared with those without side effects may be evidence in support of an altered (thio-)purine catabolism.

GI intolerance may or may not be seen as a relative contraindication for the use of AZA. In our opinion, severe and persistent GI symptoms are not acceptable, interfere with patient compliance, and should be considered as a contraindication.

We realize that the number of AZA-treated patients in this study (n = 33) may seem rather small, but statistical analyses showed clear differences regarding the main points of interest in the investigation. Therefore, we conclude that, in AZA-treated RA patients, inherited intermediate TPMT activity has a predictive value for the development of severe side effects that necessitate the withdrawal of AZA. The implication of our findings for daily clinical practice is that one should consider measuring TPMT activity before initiation of AZA treatment, in order to reduce the chance of development of serious adverse effects. Additionally, the data presented support the observations of TPMT "inducibility" by AZA and, as expected, the studied white Dutch population showed a trimodal distribution of TPMT activity. As mentioned above, the GI side effects may be based on 2 different pathophysiologic mechanisms. To our knowledge, these findings have not been reported before. Because TPMT activity is largely genetically controlled, it would not be surprising if our findings also hold true for other AZA-treated nonmalignant conditions, all the more so since the dosages of AZA used for other conditions are largely comparable with those used in RA. Our results and opinions have to be confirmed by other studies in different groups of patients and with inclusion of TPMT genotyping. One outcome could be more appropriate use of AZA, with dosage decisions made based on TPMT characteristics.

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