Cancer Chemother Pharmacol (1994) 34: 209–215

Cancer Chemotherapy and Pharmacology

Methotrexate and 6-mercaptopurine maintenance therapy for childhood acute lymphoblastic leukemia: dose adjustments by white cell counts or by pharmacokinetic parameters?

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Received: 29 June 1993 / Accepted: 11 January 1994

Abstract. In a consecutive study of 14 boys and 17 girls with non-B-cell ALL who were ≥1 year of age at diagnosis, the degree of myelosuppression during the last year of MTX/6MP maintenance therapy was analyzed in relation to the erythrocyte concentration of MTX polyglutamates and 6-thioguanine nucleotides (E-MTX and E-6TGN, the respective major cytotoxic metabolites of MTX and 6MP). For each patient, E-MTX and E-6TGN levels were measured 2–15 (median, 6) and 2–17 (median, 7) times, respectively. From these measurements, arithmetic means of E-MTX and E-6TGN were calculated (mE-MTX and mE-6TGN, respectively). Since MTX and 6MP probably work synergistically, the product of mE-MTX and mE-6TGN was calculated for each patient (mE-MTX × 6TGN). The degree of myelosuppression was registered as the mean WBC determined following cessation of the therapy minus the mean WBC measured during the therapy ($mWBC_{shift}$). The mean WBCs measured on therapy ($mWBC_{on}$) and off therapy were highly correlated ($r = 0.48$, $P = 0.009$). The median $mWBC_{shift}$ was 2.7×10^9/l (range, $1.4–4.8 \times 10^9$/l). In a multivariate regression analysis, the best-fit model to predict the $mWBC_{shift}$ included mE-MTX × 6TGN, age at drug withdrawal, and mWBC in the order given [$mWBC_{shift} = 4.3 + 0.00089 \times$ (mE-MTX × 6TGN) $- 0.097 \times$ age $- 0.41 \times mWBC_{on}$; global $r_s = 0.66$, $P = 0.0002$]. Thus, the patients with higher mE-MTX × 6TGN values, the younger patients, and the patients with the lowest WBC during therapy had the most pronounced degree of myelosuppression as measured by $mWBC_{shift}$. These results indicate that E-MTX and E-6TGN may give a better reflection of the treatment intensity than do the WBCs alone.

This paper presents data from a larger study that has received financial support from the Carl and Ellen Hertz Foundation, the Danish Cancer Society (grants 88-8502, 90-051, 91-048), the Foersom Foundation, the Gerda and Aage Haensch Foundation, Inner Wheel Denmark, and the Ville Heise Foundation.

Abbreviations: ALL, Acute lymphoblastic leukemia; CV, coefficient of variation; dose-MTX/dose-6MP, mean dose of MTX and 6MP, respectively, during the last year of therapy; dose MTX × 6MP, the product of dose-MTX and dose-6MP; E-MTX, nmol MTX/mmol Hb in erythrocytes; E-6TGN, nmol 6TGN/mmol Hb in erythrocytes; mE-MTX, mean E-MTX; mE-6TGN, mean E-6TGN; mE-MTX × 6TGN, the product of mE-MTX and mE-6TGN; 6MP, 6-mercaptopurine; MTX, methotrexate, $mPLATE_{off}$, mean platelet count following cessation of therapy; $mPLATE_{on}$, mean platelet count during the last year of therapy; $mPLATE_{shift}$, change in mean platelet count following cessation of therapy; $mWBC_{off}$, mean WBC following cessation of therapy; $mWBC_{on}$, mean WBC during the last year of therapy; $mWBC_{shift}$, change in mean WBC following cessation of therapy; WBC, white blood cell count.

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Introduction

Oral MTX and 6MP constitute the core of most remission maintenance-therapy regimens to control childhood ALL. The interindividual variations in the pharmacokinetics of these antimetabolites are considerable and may influence the risk of therapy failure as well as of side effects [1, 5, 8, 10–14, 16, 17, 21, 22, 26]. Both MTX and 6MP should be regarded as prodrugs, their major cytotoxic metabolites being the MTX polyglutamates and 6-thioguanine nucleotides (6TGN), respectively [4, 24]. These metabolites have synergistic action in vitro [2]. During maintenance therapy, MTX-polyglutamates and TGN accumulate intracellularly, also in erythrocytes (E-MTX and E-6TGN), and it has been indicated that levels of E-MTX and E-6TGN reflect the treatment intensity [1, 12, 13, 17, 21, 22]. At the University Hospital, Rigshospitalet, Copenhagen, E-MTX and E-6TGN have been measured routinely since 1987 for children with ALL.

Most ALL protocols recommend that the dose of MTX and 6MP be adjusted to keep WBCs low ($< 3.5–4.0 \times 10^9$/l), and retrospective studies have demonstrated a statistically significant correlation between

Table 1. Characteristics of E-MTX and E-6TGN

	E-MTX median (range)	E-6TGN median (range)
Method of analysis	Radioligand-binding assay [9]	HPLC [3] (duplicate assay)
Number of measurements during last year of therapy	6 (2–15)	7 (2–17)
Intraindividual CV	16% (1%–38%)	16% (1%–32%)
Interval between first and last measurements	39 (8–52) weeks	35 (4–52) weeks
Relative dose of MTX and 6MP during the period of E measurements[a]	101% (80%–133%)	101% (85%–134%)
mE-MTX and m-6TGN (nmol/mmol Hb)	4.8 (2.4–14.8)	144 (73–384)

[a] Dose of MTX or 6MP, respectively, given during the period of E-MTX or E-6TGN measurements in relation to the dose delivered during the entire last year of maintenance therapy

leuko-/neutropenia and cure rate [6, 8, 18, 20]. However, as the WBC levels measured during maintenance therapy reflect both the patients' normal WBC levels (i.e., the WBC levels determined off therapy) and the myelosuppressive influence of the treatment [19], the observation of similar WBC levels among patients on MTX/6MP therapy may reflect different impacts of the therapy.

The present study was done to explore the correlation of E-MTX and E-6TGN with treatment intensity. The dose of MTX and 6MP as well as E-MTX and E-6TGN were analyzed in relation to the mean WBC and the mean thrombocyte level determined during maintenance therapy and to the change in WBC and thrombocyte levels observed following cessation of the therapy.

Patients and methods

Patients. The criteria for entering the study were (a) a diagnosis of non-B-cell ALL and an age of ≥1 year at diagnosis; (b) treatment at the University Hospital, Rigshospitalet, Copenhagen; (c) ≥1 year of MTX/6MP therapy remaining when routine analyses of E-MTX and E-6TGN were introduced by July 1987; and (d) in first remission and ≥1 year from cessation of the therapy at the end of the follow-up period (March 31, 1993). A total of 34 patients fulfilled these criteria. Of these, 1 patient was excluded from the study due to follow-up elsewhere and only one measurement of E-MTX, and 2 patients were excluded due to long-term recurrent drug withdrawals during the last year of maintenance therapy because of febrile illnesses. The remaining 31 patients included 14 boys and 17 girls diagnosed between July 1985 and June 1989. In all, 7 patients had standard-risk (SR) ALL; 16 patients, intermediate-risk (IR) ALL; and 8 patients, high-risk (HR) ALL. Risk classification was determined by age (SR, 2–10 years; IR, <2 years or ≥10 years), WBC (SR, $<10 \times 10^9$/l; IR, $10–49 \times 10^9$/l; HR, $\geq 50 \times 10^9$/l), and the presence of central nervous system (CNS) disease, mediastinal mass, T-cell disease, and/or certain chromosomal translocations (all HR criteria). At the time of cessation of the therapy, the median age was 7 years and 4 months (range, from 3 years and 6 months to 17 years)

Therapy. Induction and consolidation therapy depended on the risk group involved. The starting maintenance-therapy dose of MTX and 6MP were 20 mg/m^2 per week and 50–75 mg/m^2 per day, respectively, with recommended dose adjustments to a target WBC of 1.5–3.5 (or 4.0) $\times 10^9$/l. The total duration of the therapy following achieved remission was 2 (19 patients) or 3 years (12 patients). The median duration of maintenance therapy was 1 year and 9 months (range, from 1 year and 6 months to 2 years and 10 months). Data were analyzed for the last year of maintenance therapy and for a period after cessation of the therapy delimited by the date after which the interval between blood sampling exceeded 10 weeks (median period, 18 months; range, 12–26 months). The individual average dose of MTX and 6MP given during the last year of maintenance therapy was calculated per square meter of body surface area as the total cumulative prescribed dose divided by 52 weeks or 365 days, respectively (dose-MTX and dose-6MP). The median dose-MTX and dose-6MP were 18.8 (range, 7.5–24.1) and 63.0 (range, 34.4–98.8) mg/m^2, respectively. Due to the in vitro synergism between MTX and 6MP [2], the product of dose-MTX and dose-6MP was calculated for each patient (dose-MTX × 6MP). The median dose-MTX × 6MP was 1156 (mg/m^2)^2 [range, 338–1838 (mg/m^2)^2]. Neither other cytostatics nor *Pneumocystis carinii* prophylaxis was given during the last year of therapy.

E-MTX/6TGN. The concentrations of E-MTX and E-6TGN were expressed in nanomoles per millimole of hemoglobin (Hb). The method of analyses have been reported elsewhere [3, 9]. Sampling of red blood cells for E-MTX analyses were in all cases done at least 48 h after the latest dose of MTX. The dose of MTX and 6MP changed by <10% for at least 6 and 4 weeks, respectively, prior to the first measurement and during the whole period of E-MTX and E-6TGN measurements, respectively. Data on MTX/6MP dosage and E-MTX/6TGN are given in Table 1.

The median average dose of MTX and 6MP given during the period of E-MTX and E-TGN measurements in relation to the average dose given during the last year of maintenance therapy was 101% (range, 80%–133%) and 101% (range, 85%–134%), respectively. Prior to statistical analysis, the mean of all measurements of E-MTX for each patient was calculated and adjusted by these differences in MTX dose as follows: mE-MTX = the arithmetic mean E-MTX times the average dose of MTX given during the last year of maintenance therapy/the average dose of MTX given during the period of E-MTX measurements. Similarly, mE-6TGN values were calculated for all patients. The dose-adjusted calculations of mE-MTX or mE-6TGN were done because previous studies have indicated a proportional correlation between MTX/6MP dose and E-MTX/6TGN [17, 21]. Based on the possible synergism of MTX and 6MP [2], the product of mE-MTX and mE-6TGN (mE–MTX × 6TGN) was calculated for each patient. The product of the mean E-MTX and the mean E-6TGN during the period of E-MTX/6TGN measurements was also calculated, i.e., without adjustments by the average dose of MTX and 6MP given during the last year of maintenance therapy (mE-MTX × $6TGN_{unadjusted}$). mE-MTX × $6TGN_{unadjusted}$ and mE-MTX × 6TGN were highly correlated ($r_s = 0.94$, $P < 0.001$). The median mE-MTX × 6TGN was 709 (nmol/mmol Hb)^2 [range, 271–2032 (nmol/mmol Hb)^2]. mE-MTX and mE-6TGN were not correlated ($r_s = 0.10$, $P = 0.3$).

Blood counts. During maintenance therapy and for the first year off therapy, determinations of Hb, WBC, and platelet counts were done at least monthly. The median number of measurements obtained during the last year of maintenance therapy amounted to 19 (range, 12–37).

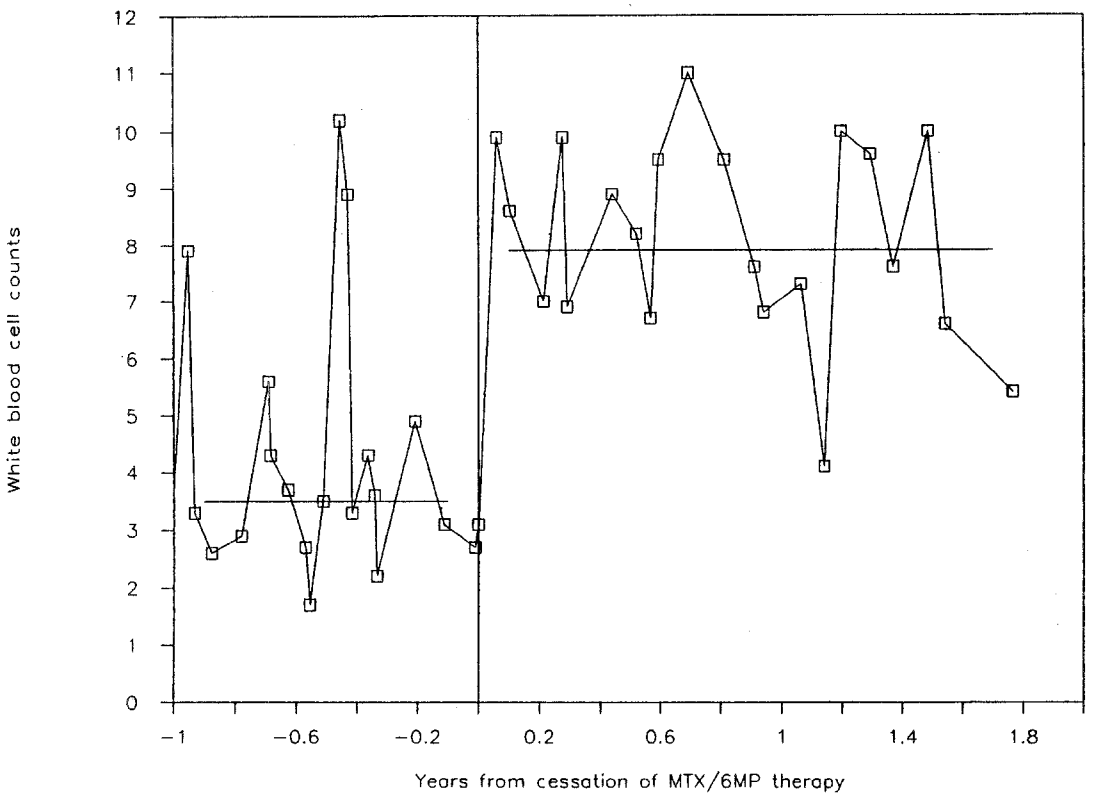

Fig. 1. Fluctuations in WBC recorded for an ALL patient during MTX/6MP maintenance therapy and following cessation of the therapy. *Horizontal lines* give the weighted mean WBCs measured during these periods

Mean WBCs were calculated for the last year of maintenance therapy ($mWBC_{on}$) and for the period following cessation of the therapy included in the analyses ($mWBC_{off}$). They were calculated as weighted means of all WBC measurements obtained within these periods using as weights the intervals between sampling:

$$mWBC = \left[\Sigma_F^L WBC_n \times (D_{n+1} - D_n)\right]/(D_L - D_F),$$

where WBC_n is the WBC at date n and D_F and D_L are the first and the last day, respectively, of the period included in the analyses. The change in WBC levels following cessation of the therapy ($mWBC_{shift}$) was calculated as $mWBC_{off} - mWBC_{on}$. Similarly, the mean thrombocyte counts on and off therapy as well as the change in thrombocyte counts were calculated for each patient ($mPLATE_{on}$, $mPLATE_{off}$, and $mPLATE_{shift}$, respectively).

Statistical analyses. Statistical analyses were done with SPSS statistical software [15]. Correlations between variables were tested with Spearman's correlation analyses (r_s, correlation coefficient). Distributions of parametric values among subgroups were compared with the Mann-Whitney *U*-test or the Kruskal-Wallis test [23]. Multivariate linear regression analyses to test best-fit models were performed with the forward stepwise method. The *P*-value limits for the inclusion or exclusion of variables from the best-fit models were 0.05 and 0.10, respectively. In all analyses, two-sided *P*-values of <0.05 were regarded as statistically significant.

Results

White cell counts

The median $mWBC_{on}$ was 3.5×10^9/l (range, $2.2-4.9 \times 10^9$/l). The median intraindividual coefficient of variation (CV) in WBC determined during the last year of therapy was 34% (range, 17%–68%). The intraindividual CV was related to neither gender, age, risk group, $mWBC_{on}$, duration of maintenance therapy, dose-MTX, dose-6MP, dose-MTX × 6MP, mE-MTX, mE-6TGN, nor mE-MTX × 6TGN. Figure 1 illustrates the fluctuations in WBC recorded for a patient during and after maintenance therapy. $mWBC_{on}$ was related to neither gender, age, risk group, duration of maintenance therapy, $mPLATE_{on}$, dose-6MP, dose-MTX × 6MP, mE-MTX, nor mE-MTX × 6TGN. However, a positive correlation between $mWBC_{on}$ and dose-MTX was demonstrated ($r_s = 0.37$, $P = 0.04$). Thus, patients having the highest WBCs were given the largest dose of MTX. In addition, a negative correlation between $mWBC_{on}$ and mE-6TGN was found ($r_s = -0.37$, $P = 0.04$).

The median $mWBC_{off}$ was 6.0×10^9/l (range, $4.7-8.6 \times 10^9$/l). The median interindividual CV in WBC determined off therapy was 0.22 (range, 0.14–0.38). Figure 2 demonstrates the relationship between $mWBC_{on}$ and $mWBC_{off}$. The patients who had the lowest WBCs during maintenance therapy also tended to do so after cessation of the therapy ($r_s = 0.48$, $P = 0.009$). All patients demonstrated a rise in their WBC following cessation of the therapy. The median $mWBC_{shift}$ was 2.7 (range, 1.4–4.8) $\times 10^9$/l. In univariate analyses, $mWBC_{shift}$ significantly correlated with mE-MTX × 6TGN ($r_s = 0.44$, $P = 0.02$) and borderline-correlated with mE-6TGN ($r_s = 0.35$, $P = 0.05$) but did not correlate with dose-MTX, dose-6MP, dose-MTX × 6MP, or mE-MTX ($P > 0.05$). In a multivariate regression analysis the best-fit model to predict the rise in WBC in-

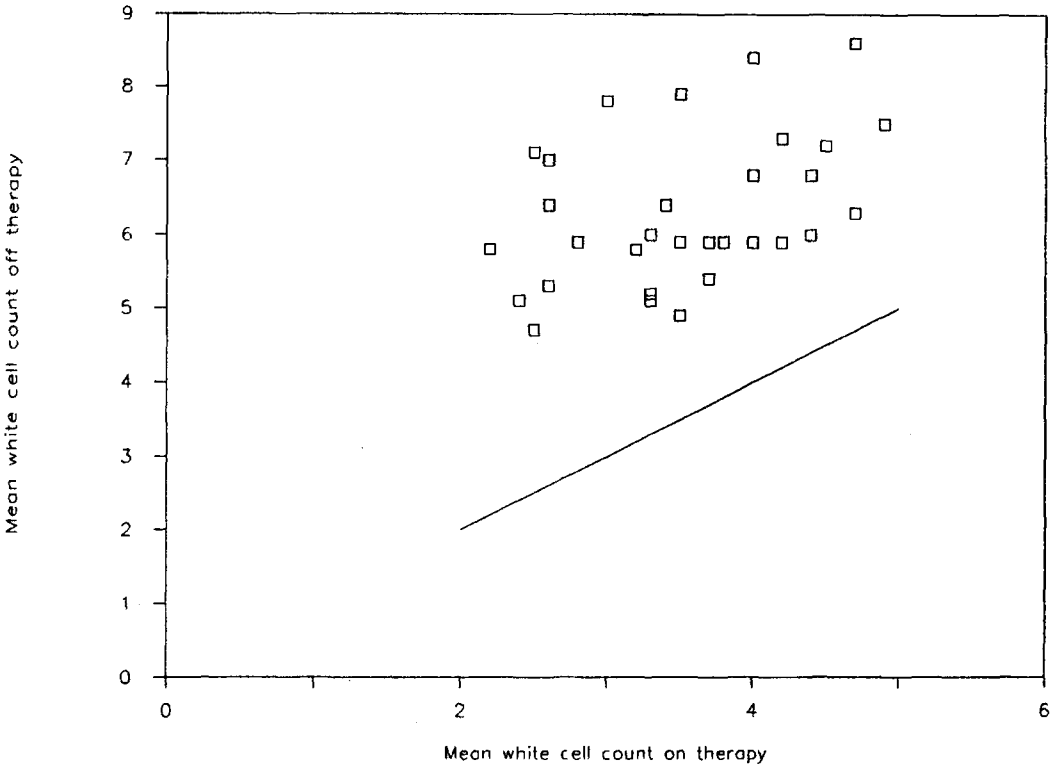

Fig. 2. Mean WBCs measured during MTX/6MP maintenance therapy in relation to the mean WBCs determined following cessation of the therapy. The vertical distance from the symbols to the line represents the rise in mean counts observed following discontinuation of the therapy ($r_s = 0.48$, $P = 0.009$)

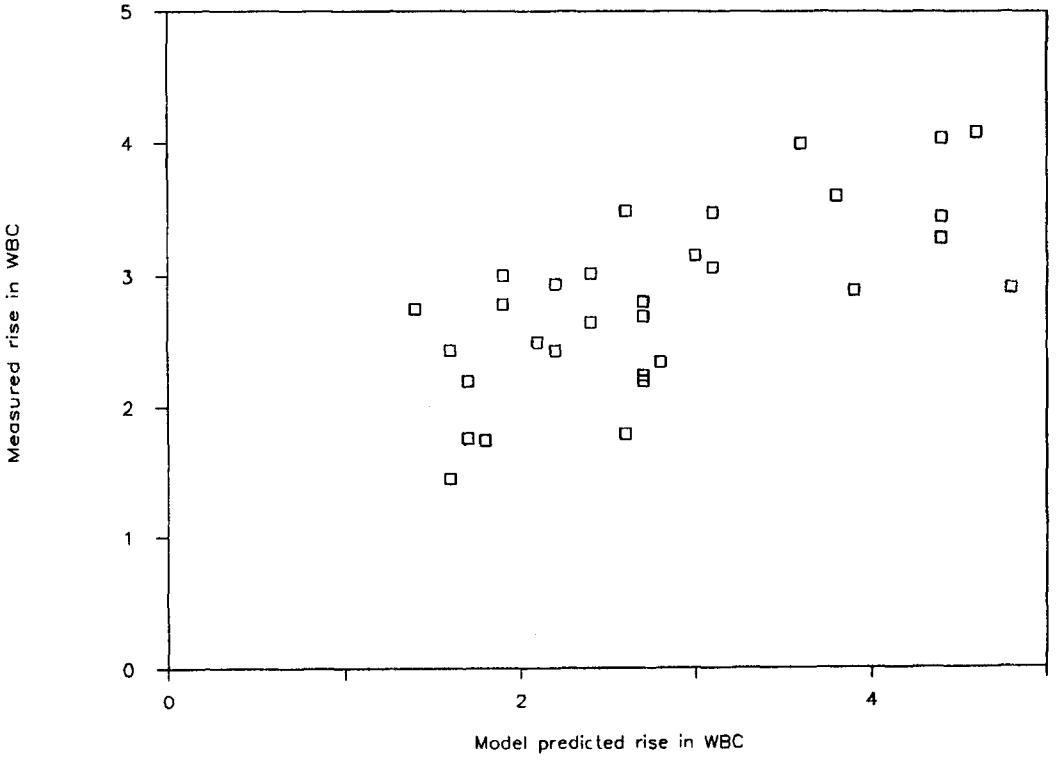

Fig. 3. The best-fit model-predicted rise in mean WBCs following cessation of the therapy in relation to the measured rise in mean WBCs: $mWBC_{shift} = 4.3 + 0.00089 \times (E\text{-}MTX \times 6TGN) - 0.097 \times age - 0.41 \times mWBC_{on}$ (global $r_s = 0.66$; $P = 0.0002$)

cluded in the following order: mE-MTX × 6TGN, age at drug withdrawal, and $mWBC_{on}$ [$mWBC_{shift}$ = 4.3 + 0.00089 × (mE-MTX × TGN) − 0.097 × age − 0.41 × $mWBC_{on}$; global $r = 0.66$, $P = 0.0002$]. Parameters not included in the model were gender, year of diagnosis, risk group, dose-MTX, dose-6MP, dose-MTX × 6MP, mE-MTX, mE-6TGN, duration of maintenance therapy, and duration of follow-up after cessation of the therapy. Figure 3 shows the relation of this best-fit model-predicted $mWBC_{shift}$ to the measured $mWBC_{shift}$. A similar model was generated if mE-MTX × $6TGN_{unadjusted}$ was included in the analysis instead of mE-MTX × 6TGN [$mWBC_{shift}$ = 4.3 + 0.00086 × (E-MTX × $6TGN_{unadjusted}$) − 0.51 × $mWBC_{on}$ − 0.093 × age; global r = 0.68, $P = 0.0007$].

Platelet counts

The median $mPLATE_{on}$ was 264 × 10^9/l (range, 147–401 × 10^9/l). $mPLATE_{on}$ was related to neither gender, risk group, duration of maintenance therapy, dose-MTX, dose-6MP, dose-MTX × 6MP, mE-MTX, mE-6TGN, nor mE-MTX × 6TGN. However, a negative correlation between $mPLATE_{on}$ and age was demonstrated (r_s = –0.57, P = 0.002). Thus, the younger patients had the highest platelet counts during maintenance therapy.

The median $mPLATE_{off}$ was 233 × 10^9/l (range, 157–348 × 10^9/l). $mPLATE_{off}$ correlated highly with $mPLATE_{on}$ ($r_s = 0.74$; $P < 0.0001$). Thus, the patients with lower thrombocyte counts during maintenance therapy also had lower thrombocyte counts following cessation of the therapy.

Most patients demonstrated a fall in their platelet count following cessation of the therapy, but the relative change in the thrombocyte level following treatment withdrawal was less than that observed for WBC. The median intraindividual $mPLATE_{shift}$ was −10 × 10^9/l (range, from −104 to +40). In a multivariate regression analysis the best-fit model to predict the change in platelet counts included $mPLATE_{on}$ and age at drug withdrawal ($PLATE_{shift}$ = 145 − 0.51 × $mPLATE_{on}$ − 3.7 × age; global $r_s = 0.66$, $P = 0.0003$). The patients with the lower $mPLATE_{on}$ and the younger patients thus had greater rises in their platelet counts following cessation of the therapy. Parameters not included in the model were gender, year of diagnosis, risk group, dose-MTX, dose-6MP, dose-MTX × 6MP, mE-MTX, mE-6TGN, mE-MTX × 6TGN, duration of maintenance therapy, and duration of follow-up following cessation of the therapy.

In univariate analysis, $mWBC_{shift}$ and $mPLATE_{shift}$ did not correlate ($r_s = 0.29$, $P = 0.11$). When $mPLATE_{shift}$ was included in a multivariate regression analysis, with $mWBC_{shift}$ serving as the dependent parameter, the significant parameters included in the best-fit model were in the following order: $mWBC_{shift}$ = 2.8 + 0.0011 × (mE-MTX × 6TGN) − 0.086 × age + 0.0084 × $mPLATE_{shift}$ (global $r_s = 0.69$; $P = 0.0001$). The patients with the higher mE-MTX × 6TGN, the lower age, and the greatest rise in platelet counts following cessation of the treatment thus had the largest rise in WBC following cessation of the therapy. Prior to the third step in the analysis, the statistical significance of $mWBC_{on}$ and $mPLATE_{shift}$ was almost equal, and when either parameter was included in the model, the other lost its significance.

Discussion

In recent years the importance of improving MTX/6MP maintenance therapy has been stressed in a number of studies. The interindividual variations in MTX/6MP pharmacokinetics are considerable, and the optimal way to monitor and adjust for these variations has not yet been established. Targeting the dose of MTX and 6MP to the WBC may be both impractical and insufficient: impractical, due to the large fluctuations in WBC counts even at an unchanged dose as demonstrated in this investigation and previous studies [18, 20]; insufficient, since the observation of similar WBC levels during maintenance therapy may reflect different treatment intensities. This is illustrated by the highly significant correlation shown between WBC_{on} and WBC_{off} in this investigation and a previous study [19]. The insufficiency of dose adjustments by WBC was demonstrated in a randomized study by van Eys and co-workers [7], who found an equal risk of relapse for patients targeted to a WBC of 1.5–3.0 × 10^9/l as compared with 3.0–4.5 × 10^9/l.

At present, it is not known whether the risk of relapse is most strongly correlated with the absolute WBC measured during MTX/6MP therapy or to the actual degree of myelosuppression (which corresponds to the WBC_{shift} in the present study). No consensus now exists as to how maintenance therapy should be monitored. The NOPHO (Nordic Society for Pediatric Hematology and Oncology) protocols recommend dose adjustments by WBC, thrombocyte counts, and hepatotoxicity parameters. The UKCCSG (United Kingdom Childrens Cancer Study Group) protocols adjust dose by neutro- and thrombocytopenia, whereas the BFM (Berlin-Frankfurt-Münster) protocols recommend dose adjustments by WBC and lymphocyte counts. In spite of these differences, the results obtained are by and large similar among these groups. Since patients with low WBCs during maintenance therapy also tend to have low WBCs off therapy, some endogenous factors besides therapy must have a major influence on $mWBC_{on}$. If patients are to receive equal treatment intensity, additional parameters for adjustment of MTX and 6MP doses must be identified.

The last 10 years have broadened our understanding of the pharmacokinetics and pharmacodynamics of MTX and 6MP with major clinical focus on E-MTX and E-6TGN, which have been explored for their relation to side effects and relapse risk [1, 12, 17, 21]. Previous studies have demonstrated both E-MTX and E-6TGN to be negatively related to the mean WBC and mean absolute neutrophil count measured during maintenance therapy [13, 17, 21]. The interindividual CV in $mWBC_{on}$ explained by E-MTX and E-6TGN in these studies was ≤20% [17, 21], which has raised doubt as to the usefulness of these metabolites as dose-targeting parameters. However, the significant correlation demonstrated between mE-MTX × 6TGN and WBC_{shift} in the present study and the previously reported low intraindividual variation in E-MTX and E-6TGN at an

unchanged dose as compared with the large interindividual variation in E-MTX and E-6TGN [17, 21] emphasize the clinical relevance of E-MTX and E-6TGN for monitoring of MTX and 6MP therapy. In addition, the study indicates that the doses of MTX and 6MP are probably of minor importance as compared with the variations in drug metabolism.

E-MTX and E-6TGN may not be the optimal metabolites by which MTX/6MP therapy should be monitored. The different MTX polyglutamates could be more informative than the total MTX pool [4]. Similarly, the total intracellular 6MP metabolite concentration (methylated and nonmethylated derivates) could be of greater clinical significance than just the TGN [1, 14, 25]. However, this possibility needs to be tested in prospective clinical studies.

MTX is an inhibitor of de novo purine synthesis and thus promotes the incorporation of 6TGN into DNA and RNA through the purine salvage pathway [2]. The clinical relevance of this pharmacodynamic interaction was demonstrated in the present study, in which the product of E-MTX and E-6TGN was of stronger significance than both E-MTX and E-6TGN. This finding also stresses the importance of doing combined analyses of MTX and 6MP metabolism and their clinical significance.

A few publications have demonstrated a significant correlation between the pharmacokinetics of MTX and 6MP, respectively, and the risk of relapse [1, 5, 8, 11, 12]. In a recent Nordic study (NOPHO ALL-88) of 296 ALL patients on maintenance chemotherapy, a statistically significant negative correlation was demonstrated between mE-MTX × 6TGN and the risk of relapse [Schmiegelow et al, submitted for publication]. To explore the univariate and combined clinical significance of WBC, neutrophil and lymphocyte counts, the presence of hepatotoxicity, the dose of MTX and 6MP, and E-MTX and E-6TGN during maintenance therapy, the NOPHO ALL-92 protocol randomizes patients with SR-, IR-, and HR-ALL to have their MTX/6MP maintenance therapy adjusted by either WBC or by a combination of WBC and E-MTX/6TGN. For the latter group, patients with an E-MTX × 6TGN below a given limit will have their dose of MTX and 6MP adjusted upward until an E-MTX × 6TGN at or above this limit has been achieved or the WBC falls below 1.5×10^9/l. For all patients, determinations of blood counts, hepatotoxicity parameters, drug dose, and E-MTX/6TGN will be done at least monthly and registered centrally. The study will accrue approximately 150 patients/year over the next 3- to 4-year period and give important information on how the MTX and 6MP dose should be targeted during maintenance therapy.

Large, prospective clinical studies such as NOPHO ALL-92 are needed to evaluate whether all patients need to achieve a specific degree of leuko-/neutropenia during maintenance therapy. Until data from such studies have become available, we will have to accept that major pharmacokinetic variations render the actual dose of MTX and 6MP given per square meter of body surface area to be of minor importance and that the basic concept in clinical oncology of treating to maximal tolerance (i.e., toxicity) seems to be as important during maintenance therapy as during the earlier phases of ALL treatment.

Acknowledgements. The skillfull technical laboratory assistance of Michael Timm, Anne Sørensen, and Inger-Marie Jensen is greatly acknowledged.

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