Risk of Relapse in Childhood Acute Lymphoblastic Leukemia Is Related to RBC Methotrexate and Mercaptopurine Metabolites During Maintenance Chemotherapy

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Purpose: **During maintenance chemotherapy for childhood acute lymphoblastic leukemia (ALL), the cytotoxic metabolites of methotrexate (MTX polyglutamates) and mercaptopurine (6MP) (thioguanine nucleotides [6TGN]) accumulate intracellularly, including in erythrocytes (E-MTX and E-6TGN) with large interindividual variations. In the present Nordic Society for Pediatric Hematology and Oncology (NOPHO) study, the relation of E-MTX and E-6TGN to relapse risk was explored.**

Patients and Methods: **Two hundred ninety-seven patients with non-B-cell ALL, aged 1 to 14 years, on oral MTX and 6MP had E-MTX and E-6TGN levels measured three to 35 (median, eight) and three to 75 (median, nine) times, respectively. For each patient, a mean of all E-MTX (mE-MTX) and E-6TGN (mE-6TGN) measurements was calculated, as well as the product of mE-MTX and mE-6TGN (mE-MTX•6TGN), since MTX and 6MP may have synergistic action.**

Results: **For patients in remission, the median mE-MTX and mE-6TGN values were 4.7 nmol/mmol hemoglobin (Hgb) (range, 0.4 to 10.3) and 173 nmol/mmol Hgb (range, 58 to 846). With a median follow-up duration of 66 months for patients in remission, 64 patients relapsed. Cox regression analysis identified mE-MTX•6TGN and sex to be the most significant parameters to predict relapse (global $P = .001$). Factors that predicted a better prognosis were high mE-MTX 6TGN and female sex. Patients who had a mE-MTX•6TGN less than the product of the median mE-MTX and median mE-6TGN (813 [nmol/mmol Hgb]^2) had a significantly poorer event-free survival (EFS) than did patients with higher values (5-year probability of EFS [$pEFS_{5y}$], 0.70 *v* 0.86; $P = .001$).**

Conclusion: **The pharmacokinetics of MTX and 6MP may have significant influence on the risk of relapse. The value of dose adjustments by E-MTX and E-6TGN remains to be determined.**

J Clin Oncol 13:345-351. © 1995 by American Society of Clinical Oncology.

WITHIN THE LAST two decades, major changes in the treatment of childhood acute lymphoblastic leukemia (ALL) have increased the event-free survival (EFS) rate to greater than 70%.[1,2] Multidrug protocols, early and late treatment intensification, CNS-directed therapy, and improved standards for supportive care have all been important parts of this development. In contrast, the core of maintenance chemotherapy, ie, weekly oral methotrexate (MTX) and daily oral mercaptopurine (6MP), have remained almost unchanged. Although MTX and 6MP were among the first drugs proven effective toward childhood ALL,[3,4] the optimal way to apply MTX/6MP maintenance therapy remains to be determined. The interindividual variations in the bioavailability of MTX and 6MP are considerable.[5-8] Most protocols attempt to compensate for these variations by tailoring the dose of MTX and 6MP to the WBC counts.[9] Yet it remains uncertain whether this approach is sufficient or even relevant.[10,11]

The major cytotoxic metabolites of MTX and 6MP are the MTX polyglutamates and thioguanine nucleotides (6TGN), respectively.[12,13] MTX follows the polyglutamation pathways of the natural folates, having glutamate residues built on the maternal drug, which enhances the affinity for the MTX-sensitive enzymes and restricts the efflux of MTX from the cells.[13] Catalyzed by the hypoxanthine guanine phosphoribosyl transferase, 6MP is converted to 6TGN, which are retained in the cell due to their charged phosphate moieties, and which mediate the cytotoxic effects of 6MP through incorporation into DNA and RNA.[12,14,15] In cells without a nucleus, such as the erythrocytes, the 6TGN are the end products. During MTX/6MP therapy, these MTX and 6MP metabolites accumulate in erythrocytes (E-MTX and E-6TGN), and E-MTX and E-6TGN probably reflect treatment intensity.[15-19]

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Submitted May 31, 1994; accepted September 30, 1994.

Supported by The Carl and Ellen Hertz Foundation; The Danish Cancer Society grants no. 88-8502, 90-051, and 91-048; Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands, and Greenland; The Gerda and Aage Haensch Foundation; The Gaardon Foundation; The Hede Nielsen Family Foundation; Inner Wheel; The Queen Louise Hospital's Research Foundation; and The Ville Heise Foundation, Copenhagen, Denmark; and the Children's Cancer Foundation, Stockholm, Sweden.

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0732-183X/95/1302-0007$3.00/0

The interindividual build up of E-MTX and E-6TGN varies considerably, which, at least in part, may be genetically determined.[15-20] In contrast, the intraindividual variations of E-MTX and E-6TGN are approximately 10% to 15% at an unchanged drug dosage.[17,18]

The present study by the Nordic Society for Pediatric Hematology and Oncology (NOPHO ALL-88) explores whether E-MTX and E-6TGN analyzed together with known prognostic factors are related to the risk of relapse, and thus whether these two parameters could be used to monitor and adjust oral MTX/6MP therapy. The study was approved by the local ethical committees.

PATIENTS AND METHODS

Patients

A new set of protocols for the treatment of childhood ALL was introduced in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) by July 1, 1986. The NOPHO ALL-88 study was opened for patient accrual by the end of 1988. Patients were eligible for the study and for statistical analyses, if they met the following criteria: (1) were diagnosed with non–B-cell ALL between July 1, 1986 and December 31, 1990 (end of accrual); (2) were ≥ 1 year and less than 15 years of age at diagnosis; (3) were receiving oral MTX and 6MP as part of maintenance therapy; (4) were in first remission when entering the study; and (5) had at least three measurements of both E-MTX and E-6TGN. Two hundred ninety-seven patients fullfilled these criteria. This encompassed 47% of 629 accruable patients 1 to 14 years of age diagnosed in the Nordic countries with non–B-cell ALL between July 1, 1986 and December 31, 1990, and who, when the study was opened, were in first remission and on MTX/6MP therapy (Table 1). Participation in the study was optional, and reasons for not entering eligible patients have not been registered. Seventy-two patients, who did enter the study and who otherwise were eligible, were excluded from statistical analyses due to fewer than three measurements of E-MTX and/or E-6TGN before relapse, end of maintenance therapy, or end of the follow-up period. The subjects included 131 girls and 166 boys, with 123 cases of standard-risk (SR), 122 cases of intermediate-risk (IR), and 52 cases of high-risk (HR) ALL. Risk classification was determined by age (SR, 2 to 10 years; IR, < 2 years or ≥ 10 years), WBC counts (SR, < 10; IR, 10 to 49; HR, ≥ 50 × 10^9/L), and presence of CNS or testicular leukemia, mediastinal mass, T-cell disease, and/or certain cytogenetic translocations (all HR criteria). At the time of diagnosis, the median age of the patients was 4.2 years. No patients in first remission died or were lost for follow-up evaluation, and no patients had received a bone marrow transplant while in first remission. Duration of remission was calculated as the number of months between achieved remission and end of the follow-up period (December 31, 1993) or relapse. For patients who stayed in remission, the median follow-up time from achieved remission was 66 months (range, 35 to 89). Cases referred to as extramedullary relapses are all isolated relapses and were counted as censoring events in respect to hematologic remission.

Table 1. Study and Nonstudy Patients

Characteristic	Study Patients	Nonstudy Patients
No. of patients	297	332
Male/female	165/192	171/161
Age at diagnosis (years)		
< 2	30	28
2-5	144	147
6-9	83	86
> 10	40	71
WBC count at diagnosis (× 10^9/L)		
<10	162	154
10-49	109	97
50-100	16	38
> 100	10	43
SR/IR/HR	121/123/15	107/111/114
T-/non–T-cell leukemia/ unknown	14/268/15	32/228/12

Chemotherapy

The patients received risk-adapted induction and consolidation therapy, which in all cases included four (IR), six (HR) or eight 24-hour infusions of MTX 500 (IR/HR) or 1,000 (SR) mg/m^2 with intrathecal MTX and leucovorin rescue. For IR and HR ALL patients, these MTX infusions were combined with oral 6MP (25 mg/m^2/d). In addition, during consolidation therapy, patients with IR or HR ALL received 4 weeks of daily oral 6MP (60 mg/m^2) and 2 weeks of daily oral 6TGN (60 mg/m^2) in combination with a 4-day series of cytarabine. Oral MTX/6MP therapy was started by week 17 (SR ALL), 31 (IR ALL), or 35 (HR ALL). The starting maintenance therapy dose of oral MTX was 20 mg/m^2/wk. The starting dose of oral 6MP was 50 to 75 mg/m^2/d. For all patients, the doses of oral MTX and 6MP were tailored to the WBC count (target WBC count, 1.5 to 3.5 × 10^9/L). During maintenance therapy, blood counts were performed at least monthly. Total length of therapy was 2 years (IR and HR patients) or 3 years (SR patients).

Methods

The treatment centers involved in the study were recommended to send blood for E-MTX and E-6TGN analyses when routine blood counts were performed and to avoid sampling within 4 weeks of the latest blood transfusion. Sampling for E-MTX was performed at least 48 hours after the latest dose of MTX. For patients who stayed in remission, the median numbers of E-MTX and E-6TGN measurements were eight (range, three to 35) and nine (range, three to 75), respectively. For patients who relapsed, the median numbers of E-MTX and E-6TGN measurements were seven (range, three to 28) and nine (range, three to 41), respectively. Eighty-nine percent of all patients had sampling performed at least every other month from the time of study entrance until relapse or end of therapy.

All E-MTX/6TGN analyses were centralized. E-MTX was analyzed with a radioligand enzyme-binding assay as described by Kamen et al.[21] E-6TGN analyses were performed with a high-performance liquid chromatography method as previously described, and all samples were assayed in duplicate.[22] Both E-MTX and E-6TGN were expressed in nanomolars per micromolars hemoglobin (Hgb). For each patient, an arithmetic mean of all measurements was calculated (mE-MTX and mE-6TGN). Since both in vitro and in vivo studies have indicated that MTX and 6MP have synergistic action, the product of mE-MTX and mE-6TGN was calculated for each patient (mE-MTX · 6TGN).[19,23] If mE-MTX · 6TGN was calculated as the mean of the product of E-MTX and E-6TGN for each of a

patient's samples, instead of the product of the mean E-MTX and mean E-6TGN, an almost identical value for mE-MTX · 6TGN was obtained. Thus, the coefficient of correlation of the mE-MTX · 6TGN values calculated by these two alternatives was 0.99.

Statistics

Statistical analyses were performed using the SPSS statistical software.[24] The Mann-Whitney *U* test was applied to compare parameters between subgroups.[25] Correlations between parameters were tested with Spearman's rank-order correlation analysis (r_s = correlation coefficient).[25] For the detection of possible prognostic factors, Cox multivariate proportional hazards regression analyses were performed.[26] Significance limits for including and excluding parameters from the models were 0.05 and 0.10, respectively. The Kaplan-Meier method was applied to estimate remission duration and generate survival curves.[27] Remission durations for subgroups were compared with the log-rank test.[28] Two-sided *P* values less than .05 were considered significant.

RESULTS

mE-MTX/mE-6TGN

The median mE-MTX and mE-6TGN values during maintenance for patients who stayed in remission were 4.7 nmol/mmol Hgb (range, 0.4 to 10.3) and 173 nmol/mmol Hgb (range, 58 to 846), respectively. mE-MTX and mE-6TGN were not correlated with age, risk group, year of diagnosis, number of E-MTX or E-6TGN samples, sampling frequency, or duration of maintenance therapy. Boys in remission had significantly higher mE-MTX values than did girls, although with considerable overlap, whereas their mE-6TGN values did not differ significantly (median mE-MTX values [75% range], males—5.0 [3.5 to 7.1] nmol/mmol Hgb; females—4.3 [3.0 to 6.3] nmol/mmol Hgb; $P = .001$). The intraindividual coefficients of variation of E-MTX and of E-6TGN were not correlated to RBC levels of the metabolites.

mE-MTX · 6TGN

The mE-MTX · 6TGN value for patients who stayed in remission ranged from 23 to 4,061 (nmol/mmol Hgb)^2. It was not related to age, risk group, year of diagnosis, number of E-MTX/6TGN-samples, sampling frequency, or duration of maintenance therapy. Boys had a higher mE-MTX · 6TGN value than did girls (median values, 903 *v* 720; $P = .003$). Figure 1 is a scattergram of mE-MTX by mE-6TGN for patients who stayed in remission. mE6TGN and mE-MTX were not correlated ($r_s = .01$). The curved line (mE-MTX = 813/mE-6TGN) is generated by the product of the median values of mE-MTX and mE-6TGN (ie, 4.7 · 173 = 813 [nmol/mmol Hgb]^2). Thus, all patients above this line had a mE-MTX · 6TGN value greater than 818 (nmol/mmol Hgb)^2.

Relapse Risk

Fourty-five patients relapsed in the bone marrow and 19 patients had an extramedullary relapse (nine in CNS, seven in testes, and three elsewhere). The 5-year probability of EFS ($pEFS_{5y}$) was 0.77 ± 0.03. This outcome was not significantly different from the outcome of the total accruable population of 629 patients diagnosed between July 1, 1986 and December 31, 1990 (N = 629; $pEFS_{5y}$ = 0.75 ± 0.02) or from the outcome of the 72 patients, who were otherwise eligible, but who had zero to two measurements of E-MTX and/or E-6TGN ($pEFS_{5y}$ = 0.82 ± 0.05). For the 297 study patients, none of the following factors were of significant prognostic value in univariate Cox analyses: sex, year of diagnosis, age or WBC count at diagnosis, risk group, mE-MTX, mE-6TGN, and number or frequency of E-MTX or E-6TGN measurements. No subgroups of patients defined by E-MTX or by E-6TGN only (ie, with very high or low metabolite levels of E-MTX or of E-6TGN) could be identified who had a clinical outcome that differed significantly from that of the remaining patients.

Figure 2 gives the distribution of mE-MTX by mE-6TGN for patients who relapsed. The curved line is the product line of the median mE-MTX and mE-6TGN for patients who stayed in remission as defined earlier. Patients with a mEMTX · 6TGN value less than 813 (nmol/mmol Hgb)^2 had an increased risk of both hematologic relapse and of any relapse compared with patients with mE-MTX · 6TGN values greater than 813 (nmol/mmol Hgb)^2 (probability of continuous hematologic remission, 0.79 ± 0.04 *v* 0.88 ± 0.03 [$P = .06$];

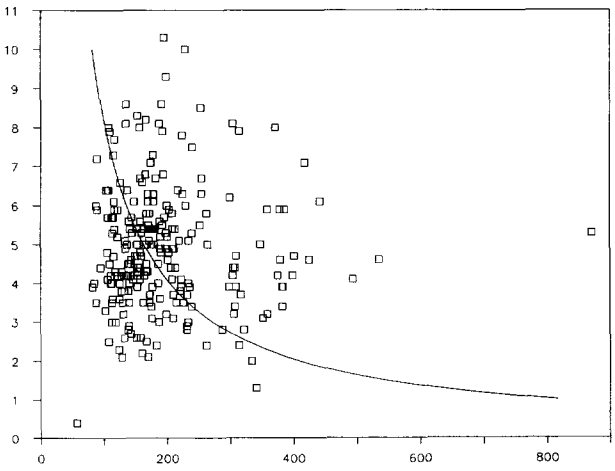

Fig 1. Scattergram of mE-MTX in relation to mE-6TGN for patients who stayed in remission. Curved line (mE-MTX · 6TGN = 813) reflects the product of the median mE-MTX multiplied by the median mE-6TGN.

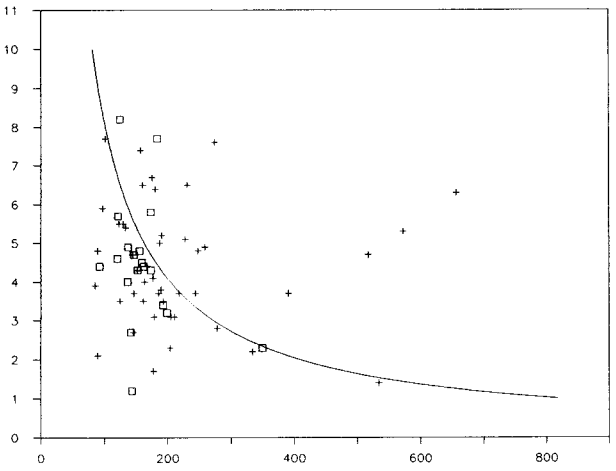

Fig 2. Scattergram of mE-MTX in relation to mE-6TGN for patients who relapsed. (□) Bone marrow relapses; (+) extramedullary relapses. Curved line (mE-MTX · 6TGN = 813) reflects the product of the median mE-MTX multiplied by the median mE-6TGN for patients who stayed in remission.

$pEFS_{5y}$, 0.70 ± 0.04 *v* 0.86 ± 0.03 [*P* = .001]) (Fig 3). The poorer outcome for patients with a mE-MTX · 6TGN value less than 818 (nmol/mmol Hgb)^2 was demonstrated for all risk groups, for both sexes, and for the different age groups (Table 2). The difference was statistically significant in analyses of boys, of SR patients, of the age groups 1 to 5 years and 6 to 9 years, and for extramedullary relapses. When analyzing all patients, those with a mE-MTX · 6TGN value less than 813 versus ≥ 813 (nmol/mmol Hgb)^2 did not differ significantly in respect to age (median, 4.0 *v* 4.6), risk groups (SR/IR/HR, 60/64/31 *v* 63/58/21), or WBC count at diagnosis (median, 9.0 *v* 8.0 × 10^9/L), but more boys were included among the patients with the higher mE-MTX · 6TGN values (male/female, 77/78 *v* 89/53) (Tables 2 and 3).

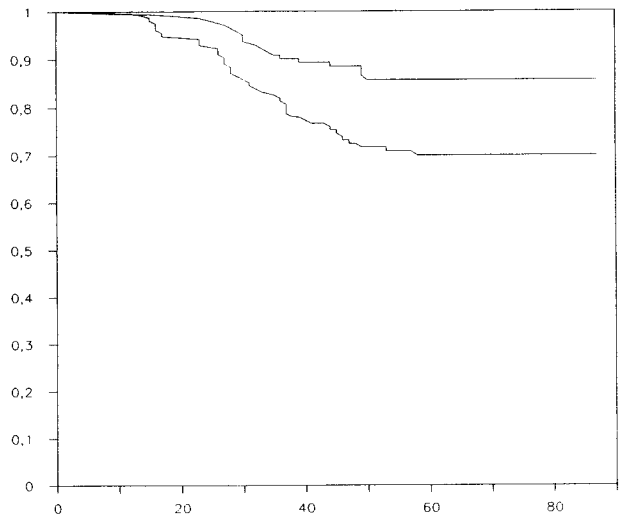

Fig 3. EFS curves for patients with a mE-MTX 6TGN ≥ 813 *v* < 813 (nmol/mmol Hgb)^2. Upper curve reflects patients with higher values ($pEFS_{5y}$ = 0.86 *v* 0.70; *P* = .001).

In multivariate Cox regression analyses of relapse risk, the following parameters were tested: year of diagnosis, sex, age and WBC count at diagnosis, risk group (SR = 1, IR = 2, HR = 3), mE-MTX (≥ 4.7 *v* < 4.7 nmol/mmol Hgb), mE-6TGN (≥ 173 *v* < 173 nmol/mmol Hgb), mE-MTX · 6TGN (≥ 818 *v* < 818 [nmol/mmol Hgb]^2), total number of E-MTX/6TGN measurements, and frequency of measurements. In these analyses, mE-MTX · 6TGN could not be entered into the models together with mE-TX and/or mE-6TGN. The best-fit model to predict any relapse included in the following order, mE-MTX · 6TGN and sex (best prognosis for high mE-MTX · 6TGN and girls) (global *P* = .001). The best-fit model to predict bone marrow relapse included in the following order, mE-MTX · 6TGN and age (best prognosis for high mE-MTX · 6TGN and younger age) (global *P* = .02). The best-fit model to predict extramedullary relapses included only mE-MTX · 6TGN (global *P* = .01).

Table 2. Outcome in Relation to mE-MTX·6TGN

Variable	mE-MTX·6TGN < 818 (nmol/mmol Hgb)^2 N	$pEFS_{5y}$ ± SEM	≥ 818 (nmol/mmol Hgb)^2 N	$pEFS_{5y}$ ± SEM	*P*
All patients	155	0.70 ± 0.04	142	0.86 ± 0.03	.001
Sex					
Male	77	0.64 ± 0.06	89	0.85 ± 0.04	.003
Female	78	0.78 ± 0.05	53	0.84 ± 0.06	.10
Risk group					
SR	59	0.73 ± 0.05	63	0.89 ± 0.05	.003
IR	64	0.75 ± 0.06	58	0.81 ± 0.06	.49
HR	31	0.61 ± 0.09	21	0.84 ± 0.08	.07
Age (years)					
1-5	109	0.77 ± 0.04	90	0.88 ± 0.04	.02
6-9	26	0.62 ± 0.10	31	0.83 ± 0.08	.03
10-14	19	0.55 ± 0.12	21	0.71 ± 0.13	.27
Probability of bone marrow RFS		0.79 ± 0.04		0.88 ± 0.03	.06
Probability of CNS RFS		0.95 ± 0.02		0.98 ± 0.01	.10
Probability of testicular RFS		0.96 ± 0.02		1.00	.009
Probability of extramedullary RFS		0.89 ± 0.03		0.97 ± 0.02	.003

Abbreviation: RFS, relapse-free survival.

Table 3. Characteristics of Patients Defined by mE-MTX/6TGN

Characteristic	mE-MTX · 6TGN > 818 (nmol/mmol Hgb)[2]	mE-MTX/6TGN < 818 (nmol/mmol Hgb)
No. of patients	155	142
Year of diagnosis (1986-1987 v 1988-1990)	58/97	44/98
Male/female	77/78	89/53
Median age at diagnosis (years)	4.0	4.6
Median WBC count at diagnosis (× 10⁹/L)	9.0	8.0
Risk groups (SR/IR/HR)	60/64/31	63/58/21
Median mE-MTX (nmol/mmol Hgb)	4.0[2]	5.4[2]
Median mE-6TGN (nmol/mmol Hgb)	143[2]	207[2]
Median no. of E-MTX/6TGN measurements	7/8	8/10
Bone marrow/CNS/testicular/other relapses	29/7/7/2	16/2/0/1

DISCUSSION

Although Pinkel et al[29] demonstrated as early as 1971 the importance of treatment intensity (as measured by dosage) in MTX/6MP maintenance chemotherapy of childhood ALL, it is only within recent years that this issue has received major clinical attention. The interindividual variations in the pharmacokinetics of oral MTX and 6MP have been recognized in a number of studies, and during the last two decades, cellular MTX and thiopurine metabolite accumulation have been recognized as potentially useful parameters reflecting MTX/6MP treatment intensity and possibly drug exposure.[14-19,30,31] The present study is the first major prospective study of relapse risk in relation to mE-MTX, mE-6TGN, and mE-MTX · 6TGN.

Lennard and Lilleyman[16] have previously found E-6TGN to be negatively related to the risk of relapse. In the present study, this could not be confirmed by univariate analysis. An explanation for this discrepancy could be that their ranking of patients was not based on repetitive measurements of E-6TGN during therapy, but on classification of patients according to E-6TGN level achieved following full-dose treatment with oral 6MP (75 mg/m²/d) during a 14-day period. Thus, what they detected was not the E-6TGN level during maintenance therapy, but biologic features that reflected interindividual variations in 6MP absorption and excretion, as well as in metabolic polymorphism.[7,8,20,32] Given the relatively small interpatient and intrapatient variations in mean cellular Hgb concentration (MCHC) and mean cell volume (MCV) compared with the interpatient variations in mE-6TGN, it is unlikely that the differences between the results reported by Lennard and Lilleyman and ourselves reflect only differences in the calculation of E-6TGN levels. We expressed E-MTX/6TGN levels in relation to micromolars of Hgb, whereas Lennard and Lilleyman expressed E-6TGN with $8 \cdot 10^8$ cells as the denominator, which approximates a 100-μL RBC concentrate. At a MCV of 85 fL and a MCHC of 20 mmol/L, an E-6TGN of 284 pmol/$8 \cdot 10^8$ cells (the median value reported by Lennard and Lilleyman) would equal 209 nmol/mmol Hgb.

E-MTX levels have not previously been reported to be significantly related to treatment outcome. In a recent Pediatric Oncology Group study of 84 patients, children with the highest E-MTX levels had a slightly better outcome, although the difference was not statistically significant.[33] However, E-MTX was measured infrequently in the majority of these patients and no data on E-6TGN were given.

It is noteworthy that, except for patients 10 to 14 years of age, the $pEFS_{5y}$ was greater than 80% for all sex, age, and risk subgroups of patients with a mE-MTX/6TGN value greater than 818 nmol/mmol Hgb. If these data reflect the clinical impact of interindividual drug metabolism, adjustment of therapy according to relevant pharmacokinetic parameters could lead to significant improvement in the outcome of childhood ALL.

Since MTX inhibits purine de novo synthesis, combined MTX and 6MP therapy will promote the incorporation of 6TGN into DNA and RNA due to increased dependency on the purine salvage pathway.[23] The necessity of combined analyses of MTX and 6MP pharmacokinetics is supported by the present study and by previous in vivo and in vitro studies.[19,23]

Although the correlation of mE-MTX · 6TGN to clinical outcome does indicate that the pharmacokinetics of oral MTX/6MP maintenance chemotherapy have clinical significance, other explanations are possible. All of the patients studied received intrathecal or high-dose MTX during induction and/or consolidation therapy, and most received 6MP or the related thiopurine analog, 6TGN, during consolidation therapy. Thus, the relation of mE-MTX · 6TGN to relapse risk could reflect interindividual pharmacodynamic features of MTX and 6MP, the clinical importance of which was manifest during previous phases of treatment.[34] If this is the case, adjustments of MTX/6MP maintenance therapy dosage based on monitoring of E-MTX and E-6TGN levels could increase the risk of toxicity without reducing the risk of treatment failure. Since E-MTX and E-6TGN were measured during maintenance therapy only, it is not known whether patients with high E-MTX and E-6TGN levels during maintenance therapy also had high metabolite levels during the

earlier MTX and thiopurine consolidation treatment. Lack of compliance could explain low E-MTX/6TGN values and an increased risk of relapse, at least for some of the patients. However, since the intraindividual coefficients of variation of E-MTX and of E-6TGN were not correlated to the RBC level of the metabolites, intermittent lack of compliance does not seem a likely explanation for the relation of mE-MTX · 6TGN to remission duration.

This study does not establish E-MTX and E-6TGN as the optimal metabolites by which to monitor MTX/6MP treatment intensity. The different MTX polyglutamates rather than the total MTX pool, or the total intracellular 6MP metabolite concentration (methylated and nonmethylated derivates) rather than just 6TGN, could be of more clinical significance.[13,15,35]

This study does not clarify whether toxicity parameters (ie, myelosuppression or hepatoxicity), pharmacokinetic parameters, or a combination of both will be the most useful to monitor and adjust maintenance therapy, since blood counts and liver function parameters were not registered centrally.[36] In a recently published study, we found that mE-MTX · 6TGN correlated better to treatment intensity than did the mean WBC count during maintenance therapy.[19] However, the answer as to which parameters will be most useful to adjust MTX/6MP maintenance therapy can be given only by prospective, randomized studies.

To explore the feasibility and clinical value of dose adjustments of MTX and 6MP based on E-MTX and E-6TGN, the NOPHO initiated a study on January 1, 1992 (NOPHO ALL-92) in which patients with non–B-cell ALL are randomized to have maintenance therapy MTX/6MP dosages adjusted by WBC counts only or by a combination of E-MTX/6TGN measurements and WBC counts. In the latter group, patients with a mE-MTX · 6TGN value less than 1,350 (nmol/mmol Hgb)^2 will have the dose of MTX and/or 6MP increased until a mE-MTX · 6TGN value $\geq$ 1,350 (nmol/mmol Hgb)^2 is achieved or the WBC decreases to less than 1.5×10^9/L. Whole blood counts and E-MTX/6TGN measurements are performed monthly. The limit of 1,350 (nmol/mmol Hgb)^2 was chosen as the NOPHO ALL-88 data indicated that these patients had an even better (although not statistically significant) prognosis than did the remaining patients with a mE-MTX · 6TGN value of greater than 818 (nmol/mmol Hgb)^2. By August 1994, more than 250 patients had entered the study, which is more than 95% of all eligible patients in the Nordic countries. Preliminary data indicate that, through dosage targeting by E-MTX and E-6TGN, mE-MTX · 6TGN values of greater than 1,350 (nmol/mmol Hgb)^2 can be achieved without unacceptable toxicity. The NOPHO ALL-92 study will accrue approximately 500 patients within 3 to 4 years.

MTX and 6MP are not only the drugs with the longest history in ALL therapy, they are also among the drugs with the fewest long-term side effects. Optimal use of MTX and 6MP could result in the cure of more children with ALL at a lower cost.

ACKNOWLEDGMENT

The skillful technical assistance of Michael Timm, Anne Sørensen, and Inger-Marie Jensen is acknowledged.

APPENDIX

Participating Pediatric Departments (alphabetic order, country and city)

Denmark: Schmiegelow K., Rigshospitalet, Copenhagen; Peitersen B., University Hospital, Hvidovre; Jacobsen B. B., University Hospital, Odense; Østergård E., University Hospital, Ålborg; Schrøder H., University Hospital, Århus. *Finland:* Siimes M., University Hospital, Helsinki; Perkkiö M., University Hospital, Kuopio; Lanning M., University Hospital, Oulu; Mäkipernaa A., University Hospital, Tampere; Salmi T., University Hospital, Turku. *Iceland:* Kristinsson J., Landspitalinn, Rejkjavik. *Norway:* Danielsen O., Municipal Hospital, Arendal; Wesenberg F., University Hospital, Bergen; Nielsen B., Municipal Hospital, Bodö; Stensvold K., Municipal Hospital, Drammen; Lund J. H., Municipal Hospital Frederiksstad; Danielsen K., Municipal Hospital, Kristiansand; Lie S., Rikshospitalet, Oslo; Hellebostad M., Ullevål Sykehus, Oslo; Zanussi G., Municipal Hospital, Stavanger; Stokland T., University Hospital, Tromsø; Moe P. J., University Hospital, Trondheim; Halvorsen B., Municipal Hospital, Tønsberg; Spangen S., Municipal Hospital, Ålesund. *Sweden:* Carlsson G., Boden Hospital, Boden; Lindh A., Borås Hospital, Borås; Lundmark K. M., Eskilstuna Hospital, Eskilstuna; Fröstad B., Falun Hospital, Falun; Dimberg A., Gällivare Hospital, Gällivare; Adran B.-A., Gävle Hospital, Gävle; Mellander L., Eastern Hospital, Gotenburg; Aronson S., Halmstad Hospital, Halmstad; Jensen D., Helsingborg Hospital, Helsingborg; Winiarski J., Huddinge Hospital, Huddinge; Berglund K., Hudiksvall Hospital, Hudiksvall; Jonsson N.-O., Jönköping Hospital, Jönköping; Cervin T., Kalmar Hospital, Kalmar; Malmport S., Karlskrona Hospital, Karlskrona; Berg A., Central Hospital, Karlstad; Nilsson H., Kristianstad Hospital, Kristianstad; Ludvigsson J., Linköping Hospital, Linköping; Wiebe T., University Hospital, Lund; Ljung R., Malmö Hospital, Malmö; Tessin I., Mölndal Hospital, Mölndal; Ljungren C. G., Norrköping Hospital, Norrköping; Dohlwitz A., Nyköping Hospital, Nyköping; Christensen H. O., Skellefteå Hospital, Skellefteå; Appelby G., Sundsvall Hospital, Sundsvall; Eriksson M., Uddevalla Hospital, Uddevalla; Forestier E., University Hospital, Umeå; Kreuger A., University Hospital Uppsala; Michanek K., Visby Hospital, Visby; Samuelsson G., Trollhättan Hospital, Trollhättan; Eriksson B., Västervik Hospital, Västervik; Berg T., Västerås Hospital, Västerås; Hedling L., Växjø Hospital, Växjø; Forsberg T., Ängelholm Hospital, Ängelhjolm; Wranne L. and Lindquist B., Örebro Medical Center, Örebro; Kriström B., Örnsköldsvik Hospital, Örnsköldsvik; Gustafsson G., Östersund Hospital, Östersund.

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