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Maintenance chemotherapy for childhood acute lymphoblastic leukemia: relation of bone-marrow and hepatotoxicity to the concentration of methotrexate in erythrocytes*

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Summary. To explore the clinical significance of the concentration of methotrexate (MTX) in erythrocytes (E-MTX), 42 boys and 31 girls were studied during maintenance chemotherapy for childhood acute lymphoblastic leukemia for periods of 3–22 months (median, 8 months) at an unchanged dose of MTX. For each study period, a weighted mean of white cell counts (mWBC), absolute neutrophil counts (mANC), and serum aminotransferases (mAT) were calculated, using as weights the intervals from sampling until the next WBC, ANC, or AT determinations were done. In 17 patients who underwent at least six measurements of E-MTX during a period in which the MTX dose remained unchanged for up to 22-months, the median intraindividual coefficient of variation for E-MTX was 10% (range, 5%–22%). For each patient, a mean of all E-MTX values (mE-MTX) during a study period (range, 1–15 measurements; median, 3) was used as an index of the RBC accumulation of MTX at the prescribed dose of MTX. Among 42 patients receiving full-dose MTX (>17.5 mg/m^2), the mE-MTX ranged between 3.4 and 9.6 nmol hemoglobin (Hb) (interindividual coefficient of variation, 33%). The mE-MTX was significantly related to the MTX dose ($r = 0.45$, $P = 0.00003$). The mWBC and mANC were both significantly related to the mE-MTX (mWBC: $r = -0.31$, $P = 0.004$; mANC: $r = -0.35$, $P = 0.02$), but not to the dose of MTX (mWBC: $r = -0.08$, $P = 0.25$; mANC: $r = -0.22$, $P = 0.08$). Each of four patients with a persistent rise in AT above the upper-normal limit (40 IU/l) and an mAT of >80 IU/l had an mE-MTX of >6.5 nmol/mmol Hb. Due to its low intraindividual variation, E-MTX may be useful for detecting persistent or intermittent failure of patient compliance. Its prognostic significance and its clinical value in MTX dose adjustment should be explored in prospective studies.

Introduction

Maintenance chemotherapy (MT) for childhood acute lymphoblastic leukemia (ALL) in most cases includes oral methotrexate (MTX) (20–30 mg/m^2 per week) and oral 6-mercaptopurine (6MP) (50–90 mg/m^2 per day) with or without high-dose MTX and/or reinforcement pulses of vincristine (VCR) and prednisone [10]. Due to considerable interindividual variations in the pharmacokinetics of oral MTX and 6MP [7, 8, 12, 20], patients given an equal dose of these drugs may be treated with different intensity, and this could influence the clinical outcome as well as the development of toxicity during MT [3, 5, 11].

It has recently been demonstrated that the degree of bone-marrow depression as measured by the mean white-cell count during MT (MT-mWBC) as well as the presence of hepatotoxicity as measured by a rise in serum aminotransferases (AT) are significantly related to the risk of relapse, patients with the higher MT-mWBC or a lack of hepatotoxicity having the poorer outcome [Schmiegelow and Pulczynska, submitted for publication]. To investigate whether the differences in MT-mWBC and AT could reflect interindividual differences in the pharmacokinetics of MTX and its cytotoxic metabolites, we studied the relationship of bone-marrow depression and hepatotoxicity to the concentration of MTX in erythrocytes (E-MTX) during MT.

MTX is incorporated in the red blood cell (RBC) during erythropoiesis and retained as MTX polyglutamates in the circulating RBC pool, with a slow turnover [15, 16]. After 6–8 weeks of an unchanged weekly dose of MTX, a steady-state E-MTX level is reached [18]. Since E-MTX may reflect bone-marrow MTX exposure (i.e. treatment intensity), the former could turn out to be a useful clinical parameter for monitoring and adjusting the dose of MTX as well as for revealing non-compliance [18].

Patients and methods

Patients. The patient population included 42 boys and 31 girls with non-B-cell ALL, all of whom were >1 and ≤ 15 years of age at diagnosis. At the time of the study, the median age was 7 years and 4 months (range, 2 years and 8 months to 16 years and 2 months). In all, 36 children were classified as standard-risk (SR) patients at diagnosis, 22, as intermediate-risk (IR) cases, and 16, as high-risk (HR) patients according to the criteria given in Table 1.

Therapy. All patients were treated at one of the three major departments of pediatric oncology in Denmark. Induction and consolidation therapy differed among the patients, depending on the risk classification. For all patients, MT comprised weekly oral MTX and daily oral 6MP (target

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Table 1. Criteria for risk classification

Standard risk	1. WBC $\leq 20 \times 10^9$/l 2. Age ≥ 2 and < 10 years 3. No CNS leukemia or mediastinal mass 4. Non-T-cell ALL
Intermediate risk	1. WBC ≥ 20 and $< 50 \times 10^9$/l 2. Age < 2 or ≥ 10 years and WBC $< 50 \times 10^9$/l 3. No CNS leukemia or mediastinal mass 4. Non-T-cell ALL
High risk	One or more of the following: 1. WBC $\geq 50 \times 10^9$/l 2. CNS leukemia 3. Mediastinal mass 4. T-cell ALL

Patients with B-cell ALL and those < 1 year of age at diagnosis are not included

dose: 20 and 75 mg/m^2, respectively). MTX and 6MP doses were reduced or completely withdrawn when the WBC reached $< 1.5 \times 10^9$ cells/l or when the patient developed thrombocytopenia measuring $< 160 \times 10^9$ cells/l or febrile illness. Recommendations as to dose regulation due to hepatotoxicity were not given in the protocols. In the absence of leukopenia (WBC of $> 4 \times 10^9$ cells/l), dose escalation was often not effected, and patients with an intermittent WBC of $> 4 \times 10^9$ cells/l were in most cases given no more than the target dose.

For the periods of MT included in the analyses, the prescribed drug doses ranged between 1.8–24 mg/m^2 MTX (mean ± 1 SD, 17.6 ± 4.2 mg/m^2) and 31–85 mg/m^2 6MP (mean ± 1 SD, 62 ± 15 mg/m^2). A total of 42 patients (approx. 60%) received the full dose of MTX, defined as > 17.5 mg/m^2. In addition to oral MTX and 6MP, 3 patients with IR-ALL and 11 with HR-ALL received pulse VCR-prednisone reinductions at intervals of 3–4 months as part of their MT. No other medication was given during the periods included in the analyses, and none of the patients had been treated with intermediate- or high-dose MTX in the 3 months prior to the study.

Hb measurements and WBC and platelet counts, together with physical examinations, were done at least once a month during MT. In addition, differential counts and AT measurements were carried out at intervals of no more than 1 month in 39 patients; only these patients were included in the analyses of the two parameters.

Analyses. For the purpose of calculations, all data on E-MTX, WBC and differential counts, serum AT, MTX and 6MP doses, body surface, and the dates relating to these data were entered into a computer-file data base designed for the study. The duration of the MT periods included in the analyses ranged from 3 to 22 months (median, 8 months). At entry into the study, all patients had been on MT for at least 3 months. For each study period, the MTX and 6MP doses remained unchanged. However, eight patients given a different dose of MTX were studied during two MT periods at least 2 months apart.

E-MTX was measured with a modified enzyme-inhibition assay, as previously described [17], and expressed as nmol MTX/mmol Hb. All E-MTX analyses were done at the Department of Clinical Chemistry, University Hospital, Arhus, by one of the authors (H. S.). In all cases, sampling of RBCs for E-MTX analyses was done at least 48 h after the latest dose of MTX. E-MTX measurements were done 1–15 times during the MT courses included in the analyses (median number of measurements, 3); only those measurements taken after a minimum of 8 weeks of unaltered MTX doses were included in the analyses. When several measurements were available, E-MTX was calculated as the mean (mE-MTX). In the following, mE-MTX also designates E-MTX in patients with only one measurement.

Even at unchanged MTX and 6MP doses, large oscillations in WBCs are registered during MT in most patients, but the level at which the WBCs fluctuate changes only little during therapy [14]. To compensate for these oscillations in WBCs, a mean WBC (mWBC) was calculated for each MT course as a weighted mean of all WBC measurements within the study period, using the intervals between sampling as weights. Thus, for the purpose of calculations, we assumed that the WBC remained unchanged until the next was determined. To reduce the influence of infectious leukocytosis on the mWBC, all WBCs of $> 10 \times 10^9$ cells/l were set to 10×10^9 cells/l prior to calculations. These methods for calculation give a better index of the WBCs than does a simple arithmetic mean, which tends to favor periods with multiple WBC measurements taken at short intervals due to severe leukopenia or febrile illness. Following the same principles, a weighted mean ANC (mANC) and a weighted mean serum AT (mAT) (reflecting the degree of hepatotoxicity) were calculated.

Statistical analyses. The correlation between any two variables was calculated using linear regression analysis by the least-squares smethod (r = correlation coefficient). In addition, the non-parametric Spearman's rank-correlation analysis was applied for the analysis of the correlation between two variables not showing homogeneity of variance (r_s = Spearman's correlation coefficient). All analyses were carried out with SPSS statistical software [2].

Results

E-MTX

In 17 patients in whom E-MTX was measured at least six times during a study period lasting up to 22 months (i.e. at an unchanged dose of MTX), the median intraindividual coefficient of variation (CV) for E-MTX was 10% (range, 5%–22%). When all patients were included, the CV was not related to the magnitude of E-MTX, the MTX dose, or the length of the study period. Thus, there did not seem to be any major difference in short- and long-term intraindividual variability.

In each of the 73 patients, we used mE-MTX as an index of the RBC accumulation of MTX at the prescribed MTX dose and found that mE-MTX ranged from 2.6 to 10.0 nmol/mmol Hb (median, 6.1 nmol/mmol Hb). In patients receiving > 17.5 mg/m^2 MTX, the interindividual CV for mE-MTX was 33%. mE-MTX was not significantly related to gender, age at the time of analysis, previous medication (i.e. risk group), duration of MT, or the preceding total dose of oral MTX given during MT. As previously reported [18], mE-MTX was significantly related to the MTX dose ($r = 0.45$, $P = 0.00003$; $r_s = 0.43$, $P = 0.001$) (Fig. 1). However, there was an up to 3-fold variation in mE-MTX among patients receiving an equal dose of MTX.

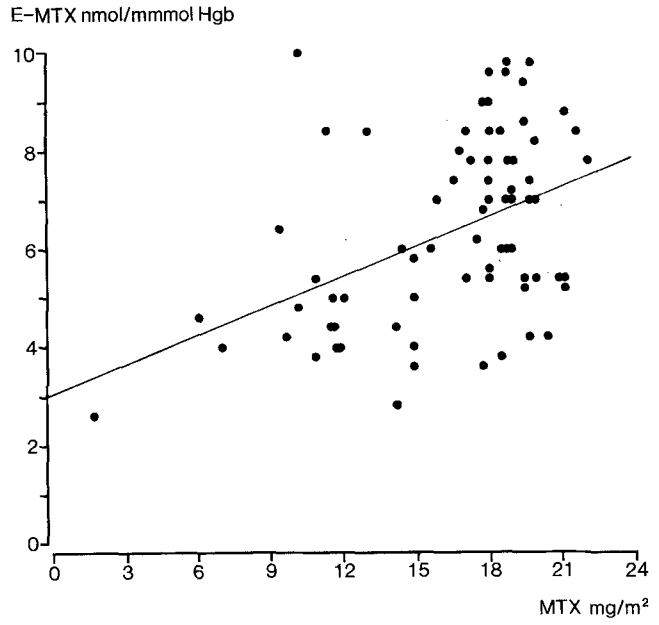

Fig. 1. Scattergram of the steady-state levels of E-MTX in relation to the weekly dose of MTX ($r = 0.45$, $P = 0.00003$; $r_s = 0.43$, $P = 0.001$)

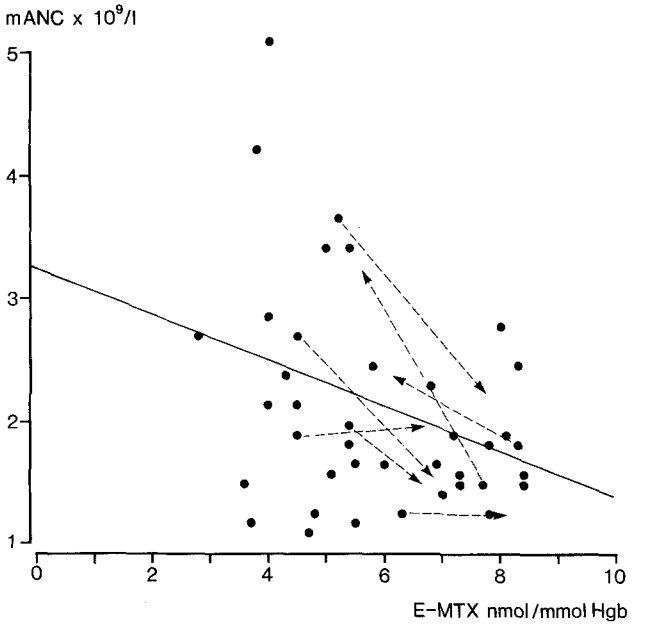

Fig. 3. Scattergram of the mean absolute neutrophil counts in relation to the steady-state levels of E-MTX ($r = -0.35$, $P = 0.02$)

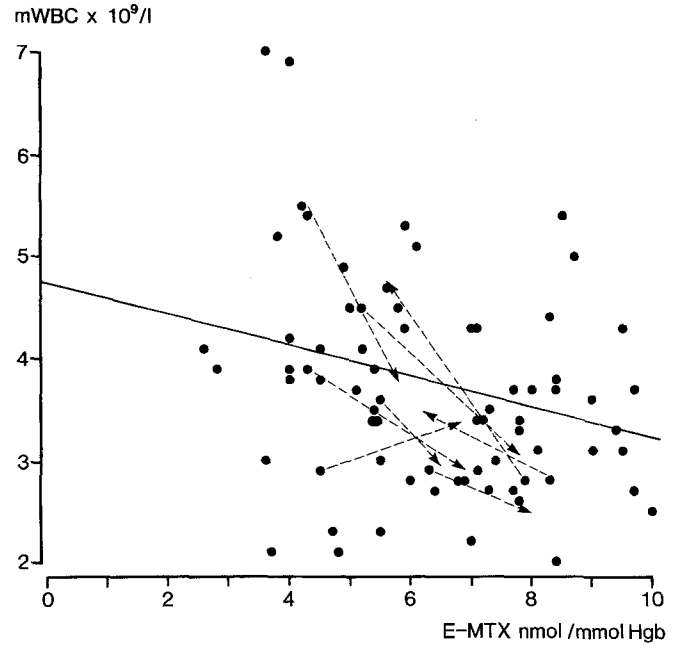

Fig. 2. Scattergram of the mean white-cell counts in relation to the steady-state levels of E-MTX ($r = -0.31$, $P = 0.004$)

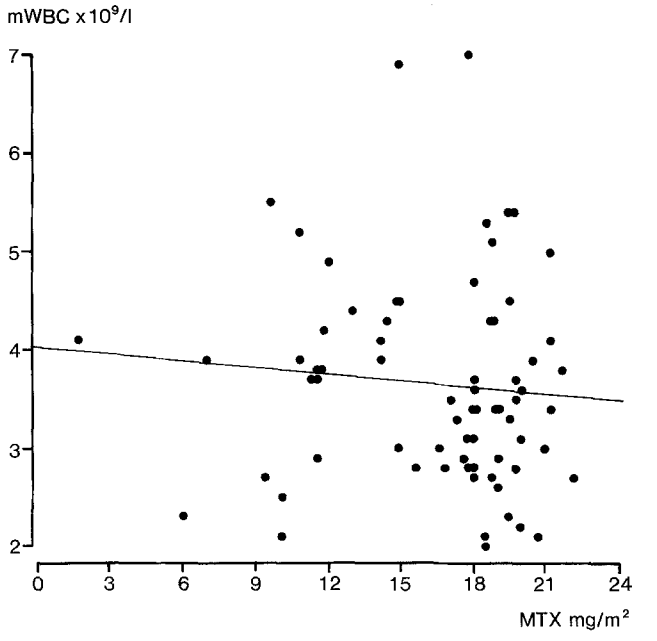

Fig. 4. Scattergram of the mean white-cell count in relation to the weekly dose of MTX ($r = -0.08$, $P = 0.25$)

mWBC and mANC

mWBC and mANC ranged between $2.2-7.0 \times 10^9$ cells/l (median, 3.4×10^9 cells/l) and $1.1-5.2 \times 10^9$ cells/l (median, $1.7 \times 10.^9$ cells/l), respectively. Neither gender, age at the time of analysis, nor duration of MT seemed to influence mWBC and mANC significantly. However, these WBC parameters were both significantly related to mE-MTX, as shown in Figs. 2 and 3 (mWBC: $r = -0.31$, $P = 0.004$; mANC: $r = -0.35$, $P = 0.02$). These figures also illustrate changes in mWBC and mANC together with the corresponding mE-MTX values for patients who were studied for two courses of MT. An increase in mE-MTX was accompanied by a decrease in mWBC in seven of eight patients and by a decrease in mANC in five of seven patients, but the relationship between the mE-MTX values and the WBC parameters varied among the patients.

The relationship of mWBC and mANC to mE-MTX were almost unaltered, when patients who received pulses of VCR and prednisone as part of their MT were excluded from the analyses (mWBC: $r = -0.30$, $P = 0.01$; mANC: $r = -0.32$, $P = 0.03$). When only patients receiving >17.5 mg/m^2 MTX were included, the correlation of

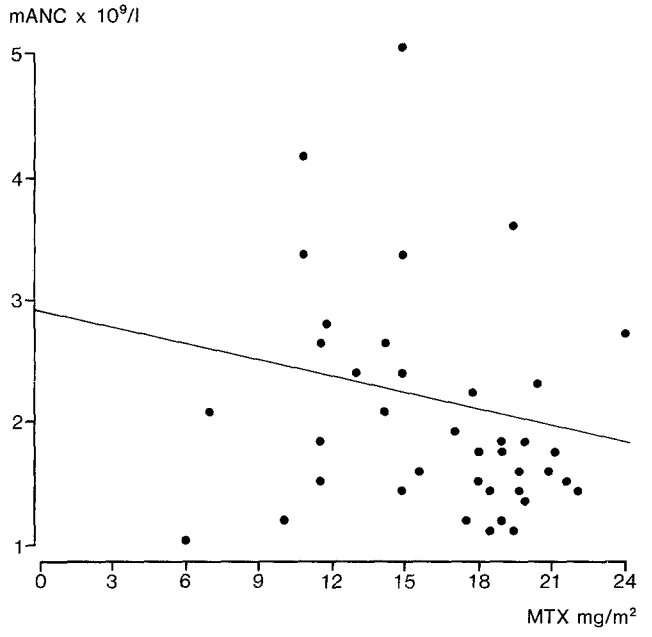

Fig. 5. Scattergram of the mean absolute neutrophil count in relation to the weekly dose of MTX ($r = -0.22$, $P = 0.08$)

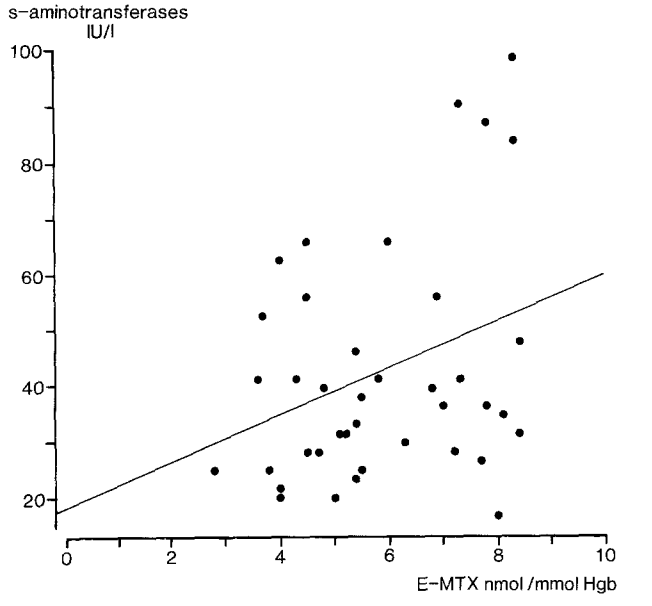

Fig. 6. Scattergram of mean serum aminotransferases in relation to the steady-state levels of E-MTX ($r = 0.32$, $P = 0.02$; $r_s = 0.16$, $P = 0.16$)

mWBC and mANC to mE-MTX lost its significance (mWBC: $r = -0.26$, $P = 0.05$, 43 patients; mANC: $r = -0.13$, $P = 0.30$, 21 patients), which to some extent could have been due to the smaller study population.

Patients with an mE-MTX of >6.1 nmol/mmol Hb (the median value of the study population) received a somewhat higher dose of 6MP than did those with an mE-MTX of <6.1 nmol/mmol Hb (mean dose, 65 ± 13 and 56 ± 19 mg/m^2, respectively), this difference in the means being significant ($P = 0.01$). When either mWBC or mANC was plotted against the prescribed dose of MTX, no significant relationship between these parameters could be demonstrated ($r = -0.08$, $P = 0.25$; mANC: $r = -0.22$, $P = 0.08$) (Figs. 4, 5).

Hepatotoxicity

A scattergram of mAT and mE-MTX is shown in Fig. 6. Although linear regression analysis indicated a significant correlation between these parameters ($r = 0.32$, $P = 0.02$), this was due to the fact that all four patients with persistently elevated AT values of >40 IU/l during the study period and an mAT of >80 IU/l had an mE-MTX of >6.5 nmol/mmol Hb. Using non-parametric correlation analysis, no significant relationship could be demonstrated ($r_s = 0.16$, $P = 0.16$).

Discussion

Approximately every third child with ALL who achieves clinical remission will later experience recurrent disease [6]. To what extent this is influenced by intraindividual variations in the pharmacokinetics of the cytostatics given is as yet unknown, although a few studies have indicated that these differences have significant clinical importance [1, 4, 8].

According to most protocols, the MTX and 6MP doses should be adjusted to keep the WBC between 1.5 and $3.5(-4.0) \times 10^9$ cells/l. The clinical value of these recommendations have been stressed by the correlation between bone-marrow depression (as measured by mWBC) and relapse risk, as approx. 90% of patients with an mWBC of $<3.5 \times 10^9$ cells/l achieve a 4-year relapse-free survival [14]. However, the recommendations can be difficult to apply due to considerable fluctuations in WBC counts [14] and may thus be insufficient for dose regulation in a number of patients.

Several previous studies have dealt with the pharmacokinetics of E-MTX, but little has been published concerning the clinical significance of this parameter and its possible role as a guideline for dose adjustments. Its relationship to the MTX dose [18] and its incorporation in RBS precursors [15] suggest that E-MTX might reflect bone-marrow drug exposure as well as the ability of the RBC line to form polyglutamates [16]. The correlation of bone-marrow and hepatotoxicity to E-MTX, as demonstrated in this study, lends support to the hypothesis that E-MTX reflects treatment intensity. In addition, the very small intra-individual variation of E-MTX at an unaltered MTX dose seems to exclude that the findings could be attributable to differences in drug compliance.

In a previous report, a correlation could not be demonstrated between E-MTX and the total WBC or neutrophil count measured at the time of blood sampling for E-MTX analysis [19]. This could have been due to fluctuations in WBC and ANC, which in the present study were adjusted through calculations of weighted means. In the aforementioned paper, folic acid supplementation during MT was suggested to be a significant determinator of hematological drug tolerance [19]. Since RBC-folic acid values were available for only a minority of the present patient population, this variable was not included in the analyses. However, investigations of the influence of RBC-folic acid on the relationship between mWBC and mE-MTX are in progress.

The lack of a statistically significant relationship of both leukopenia and relapse risk to the MTX dose, which were demonstrated in the present investigation and a previous study [14], emphasizes the need for parameters that reflect the interindividual variations in MTX pharmacokinetics and are applicable for monitoring MT. Although we demonstrated that mE-MTX was correlated to mWBC (which may be a prognostic factor), this does not necessarily imply a correlation of mE-MTX to relapse risk. A Nordic, population-based, prospective multicentre study has been initiated by the Nordic Society for Pediatric Hematology and Oncology (NOPHO) to determine whether such a correlation exists.

Although 70% of the patients in the present study who had an mWBC of $< 3.5 \times 10^9$ cells/l also had an mE-MTX of > 6.1 nmol/mmol Hb, a number of patients with such mE-MTX values did not manifest bone-marrow depression. Studies dealing with the clinical outcome of these patients would be very valuable for the assessment of the independent prognostic value of E-MTX and WBC.

Beyond the possible usefulness of E-MTX for dose adjustments, the stability of E-MTX at an unchanged MTX dose makes it a valuable parameter for revealing an intermittent or persistent lack of therapy compliance. When enzyme inhibition assays are used for the detection of E-MTX, blood samples should be obtained no less than 48 h after the latest dose of MTX, as the peaks in serum and tissue MTX following oral MTX are added to the RBC MTX-polyglutamate pool. In addition, intermediate- or high-dose MTX should not be given in the 3 months prior to E-MTX measurements [13].

Since the present MT includes both MTX and 6MP, there is an equal need for studies exploring the clinical importance of the variation in the pharmacokinetics of the latter. The RBC concentration of 6-thioguanine nucleotides (the major intracellular cytotoxic metabolite of 6MP) have been found to be significantly correlated to ANC and mWBC [9, Schmiegelow and Bruunshuus, submitted for publication]. In the aforementioned inter-Nordic study, the prognostic significance of this parameter will be investigated along with that of E-MTX.

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