Changes in Red Blood Cell Methotrexate Pharmacology and Their Impact on Outcome When Cytarabine Is Infused with Methotrexate in the Treatment of Acute Lymphocytic Leukemia in Children: A Pediatric Oncology Group Study


ABSTRACT

Since it is unclear whether methotrexate and cytarabine are synergistic or antagonistic in the treatment of acute lymphoblastic leukemia, the Pediatric Oncology Group studied the prognostic significance of a potential interaction between these agents.

RBC methotrexate concentrations were compared from 140 patients at lower risk of relapse randomized to two treatment groups: one receiving six methotrexate infusions with overlapping cytarabine; the other, six methotrexate infusions alone. Samples from 248 patients from all risk groups were studied to determine whether patients with extremely low RBC methotrexate concentrations had inferior outcomes.

Among low-risk patients studied 3 weeks after the sixth infusion, median RBC methotrexate concentrations were 0.13 nmol/ml RBCs (n = 71) for the methotrexate-only group and 0.02 nmol/ml RBCs (n = 69) for the methotrexate/cytarabine-treated low-risk patients, P = 0.001 by the two-sided Wilcoxon test. For low- and high-risk patients receiving methotrexate/cytarabine infusions, event-free survival at 1 and 3 years after RBC sampling was 97 ± 2% and 90 ± 3% for patients with concentrations greater than the median, and 88 ± 3% and 78 ± 4% for those with concentrations at or below the median. Log rank comparisons of event-free survival in the first year and overall yielded P = 0.005 and P = 0.04, respectively.

Cytarabine altered methotrexate pharmacology when the drugs were infused together. Patients whose levels were extremely low had an adverse prognosis. Although this study could not assess efficacy of the methotrexate/cytarabine combination, it appears that concurrent administration is not optimal.

INTRODUCTION

Chemotherapy regimens for childhood ALL usually use combinations of drugs active as single agents against the disease but which may not have established activity in the pairings and schedules utilized. MTX and cytarabine are commonly used in consolidation chemotherapy for childhood ALL, but the benefit and optimal timing of their combined administration have yet to be determined.

Laboratory studies of the combined use of MTX and cytarabine have usually shown dose- and schedule-related synergism (defined as greater than additive leukemia cell kill; Refs. 1–9). However, several reports of antagonism have also appeared (8–12). Jackson and Harkrader (9), working in four different cell lines, noted that synergism was observed when intracellular dCTP pools were decreased and dATP pools increased by MTX pretreatment, whereas in cell lines with pool alterations in the opposite direction, less than additive or antagonistic effects were seen.

In two clinical trials of the combined use of MTX and cytarabine, there was little evidence for efficacy in ALL (13, 14). However, a POG pilot study (15) in previously untreated patients tested higher doses of the drugs during continuation chemotherapy and reported 53% 4-year survival for poor-risk patients, supporting the possibility that the combination might be active when used in certain schedules. Newman et al. (16), in an analysis of cytarabine pharmacology in some of those POG patients, showed that the conditions for synergism suggested by laboratory studies were not met in the clinical trial.

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3 Deceased.

The abbreviations used are: ALL, acute lymphoblastic leukemia; MTX, methotrexate; POG, Pediatric Oncology Group.
Jackson and Harkrader (9) had been met in that (a) cytotoxic concentrations of the individual drugs were achieved; (b) there was no MTX antipurine effect (as measured by ATP, GTP, and dATP levels after administration of cytarabine alone versus cytarabine/MTX); and (c) that dCTP levels dropped considerably after MTX administration.

The effect of cytarabine administration on MTX pharmacology in patients has not been studied, although investigators have attempted to correlate MTX serum pharmacology with outcome. Craft et al. (17) showed that more rapid clearance of p.o. MTX during maintenance therapy correlated with shorter remission durations. A subsequent study by Pearson et al. (18) could not confirm this. Evans et al. (19, 20) showed increased early relapse rates (but not increased overall relapse rates) in patients who had faster clearance of MTX. Borsi and Moe (21) noted an inferior outcome in patients who had a more rapid clearance of MTX. Camitta et al. (22) noted improved survival in a group of poor prognosis patients treated with sequential MTX/6-mercaptopurine infusions whose median end-of-infusion MTX levels were higher.

RBCs accumulate MTX only during the erythroblast stage (23) and retain MTX largely as polyglutamates throughout the RBC life-span (24). RBC MTX appears to rise with increasing exposure to the drug (25, 26), although the relationship is complex and schedule dependent (27–29). Following a specific treatment schedule of MTX, a new steady-state RBC MTX level is reached within 12 weeks (23, 30, 31). Schroder (32) determined the MTX content of RBCs in children receiving p.o. MTX maintenance therapy and could not find a correlation with outcome. The POG studied RBC MTX in patients receiving an intensive MTX consolidation and maintenance therapy and noted a trend to prolonged survival in patients with higher RBC MTX levels following consolidation, but no correlation of outcome with RBC MTX during maintenance therapy (31).

The findings described below result from a POG study of RBC MTX pharmacology in patients undergoing chemotherapy for ALL. We examined two specific questions: (a) Are RBC MTX levels altered in patients who receive cytarabine with MTX as opposed to MTX alone? (b) If there are alterations in RBC MTX levels, do patients with lower levels have a higher risk of relapse? Our report represents the first opportunity for noted a trend to prolonged survival in patients with higher RBC MTX levels following consolidation, but no correlation with out-

### TABLE 1
<table>
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<tbody>
<tr>
<td>WBC level (&lt;×10^9/liter)</td>
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<td></td>
<td></td>
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<tr>
<td>&lt;10</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>10–99</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>&gt;100</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Liver or spleen below umbilicus or extramedullary leukemia</td>
<td>B</td>
<td>B</td>
<td>B</td>
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</table>

*B, high risk; A, low risk.

**MATERIALS AND METHODS**

**Treatments.** Eligibility requirements for randomization on POG study 8602 included: age, 1–21.99 years, previously untreated non-B-, non-T-cell ALL, no central nervous system disease, achievement of complete remission with induction therapy, and signed informed consent. Patients were stratified for high- or low-risk features as defined in Table 1. After a common 6-week induction (prednisone, vincristine, asparaginase, triple intrathecal medications, and 6-mercaptopurine) schedule, patients were treated with one of the following four consolidation treatments: (a) regimen A: 200 mg/m² MTX by i.v. push, then 800 mg/m² i.v. over 24 h, followed by leucovorin rescue. This treatment was repeated every 3 weeks (weeks 7, 10, 13, 16, 19, and 22); (b) regimen B: MTX as in regimen A with 25,000 units/m² l-asparaginase i.m. weekly from weeks 7 to 30; (c) regimen C: MTX as in regimen A with 1 g/m² cytarabine over 24 h, beginning 12 h after the start of MTX; and (d) regimen D: MTX and cytarabine administered as in regimen C but given at weeks 7, 19, 31, 43, 55, and 67.

Since our report deals with the effects of cytarabine on RBC MTX and the correlation of RBC MTX changes with outcome, we have restricted our discussion to results for patients on regimens A and C. The two regimens are identical except for the addition of cytarabine to the MTX infusions in regimen C.

Patients with early pre-B ALL (i.e., those whose leukemia cells lacked cytoplasmic immunoglobulin) at lower risk of relapse were randomized to treatments A, B, C, and D. Patients with higher risk early pre-B ALL were randomized among regimens B, C, and D, while patients with pre-B ALL were randomized to treatments B or C. Thus, only low-risk patients were treated with regimen A, while both lower and higher risk patients were treated with regimen C.

Starting at week 25, patients received daily p.o. 6-mercaptopurine (75 mg/m²), weekly i.m. MTX, and pulse treatments with vincristine (1.5 mg/m²; maximum 2.0 mg i.v. on days 1 and 8), and p.o. prednisone (40 mg/m² for 7 days) every 16 weeks. i.m. MTX was replaced by triple intrathecal therapy during the first week of each pulse and 8 weeks later during each 16-week cycle.

**Patients.** Patients were accrued for the study between February 3, 1986 and November 23, 1989. The cutoff for this analysis was October 29, 1993.

For the comparison of RBC MTX levels at week 25, there were 114 patients randomized to regimen A and 117 randomized to regimen C. Of these, 111 and 112 on regimen A and
regimen C, respectively, were still on study at week 25 (the time of the RBC sample), and, of these, 71 and 69, respectively, had week 25 MTX samples. Since RBC transfusion might lower RBC MTX values by dilution, we verified the number of patients transfused during the consolidation therapy. Only three MTX patients and six MTX/cytarabine patients received transfusions between weeks 22 and 25.

For comparing the prognostic importance of RBC MTX levels in the MTX/cytarabine regimen (regimen C), 397 patients were assigned to this regimen. Of these, 380 were on study at week 25, of whom 248 had week 25 RBC MTX levels.

**Laboratory Techniques for RBC MTX.** Serum MTX concentrations were determined for each MTX infusion between 12 and 24 h after the start of each 24-h MTX infusion (referred to below as "12-h levels") and at 36 h after the start of each infusion. Blood samples were drawn prior to starting maintenance therapy at week 25, collected in tubes containing EDTA, and sent by overnight mail to a central laboratory at the Johns Hopkins Hospital for analysis. Previous work from our laboratory demonstrated that RBC MTX measurements did not change when blood samples were sent by carrier and kept in the laboratory in evacuated tubes for up to 10 days at ambient temperatures.

**Statistical Methods.** For the first major objective, the week 25 comparison of RBC MTX, levels were compared for the lower risk patients randomized to regimen A versus regimen C (Fig. 1) by a two-sided Wilcoxon test. Patients with missing values were excluded. In addition, a plot of the empirical distributions is given excluding patients who received transfusions (Fig. 2). This plots percentile against RBC MTX level. For the second major objective, the prognostic importance of RBC MTX levels in patients undergoing the MTX/cytarabine infusions, the event-free survival was compared for patients with RBC MTX levels at or below the median versus those with greater than the median levels in two ways. Since the presumed major biological effect might be short term, we considered the first year post-week 25 as well as overall event-free survival. The two-sided log rank test, stratified for the Trueworthy risk

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Fig. 1 Chemotherapy schema. All patients received identical therapy for weeks 1 through 6 and weeks 25 through 154. Treatments diverged only during weeks 7–24, during which patients received either six infusions of MTX or six infusions of MTX with cytarabine. VCR, vincristine; HC, hydrocortisone; Ara-C, cytarabine; Triple IT, triple intrathecal medications.
Significance of Methotrexate Pharmacology

![Graph showing empirical distribution of week 25 RBC MTX levels divided by treatment group.](image)

**Fig. 2** Empirical distribution of week 25 RBC MTX levels divided by treatment group [MTX (---), n = 68; MTX + cytarabine (——), n = 63]. Patients transfused between weeks 22 and 25 are excluded.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Week 25 RBC MTX levels in patients treated with regimens A and C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients enrolled</td>
<td>Regimen A (low-risk patients)</td>
</tr>
<tr>
<td>Patients on study at week 25</td>
<td>114</td>
</tr>
<tr>
<td>Number of week 25 RBC samples</td>
<td>111</td>
</tr>
<tr>
<td>Week 25 mean ± SD RBC MTX (nmol/ml RBC)</td>
<td>71</td>
</tr>
<tr>
<td>Week 25 median RBC MTX (nmol/ml RBC)</td>
<td>0.18 ± 0.13</td>
</tr>
</tbody>
</table>

*Patients enrolled refer to all patients entered on the noted treatment regimens. Patients on study at week 25 were those not removed from the treatment study due to death, unacceptable toxicity, withdrawal, or relapse.*

Groups (33), was utilized in both cases. Since unnatural weights of patients were assigned to regimen C, as POG 8602 was a two-, three-, or four-arm study depending on risk group and phenotype, Kaplan-Meier estimates (34) had to be stratified by their natural weights in Table 4 to remove bias. Stratum-specific estimates are also given, with SEs of Peto et al. (35) in Table 4. It should be noted that 40% of the eligible patients did not have week 25 RBC MTX samples. The resulting potential for selection bias is addressed further in the discussion.

Secondary comparisons were conducted by the two-sided Wilcoxon test for quantitative variables and by the Pearson χ² test for qualitative variables.

**RESULTS**

Comparison of RBC MTX in Patients Treated with MTX with or without Cytarabine (First Major Objective). The first two columns of Table 2 provide the randomized comparison. Within the group with good prognosis and early pre-B phenotype, the MTX/cytarabine regimen produced significantly lower RBC MTX levels (*P* < 0.001). Fig. 2 demonstrates the quantitative difference between the regimens after exclusion of the three regimen A and six regimen C patients who received RBC transfusions between weeks 22 and 25. There is a more than a 6-fold difference in the medians. The 10th percentile of the MTX curve corresponds to the 80th percentile of the MTX/cytarabine curve.

Prognostic Significance of RBC MTX Levels in Patients Treated on Regimen C (Second Major Objective). Tables 3 and 4 present the event-free survival comparison, subdivided at the median for RBC MTX, 0.02 nmol/ml RBCs. Differences were most pronounced in the higher risk groups (strata 2 and 3). A significant difference was not found for patients with lower risk early pre-B ALL treated with regimen C. The latter result should be viewed as inconclusive. It is important to note that the prognostic significance of RBC MTX was derived from strata 2 and 3 (higher risk), whereas stratum 1 (lower risk) from patients treated with regimens A and C were used to establish the pharmacological effect of MTX versus MTX/cytarabine.

Secondary Analyses. To determine whether the marked drop in RBC MTX seen with simultaneous cytarabine adminis-
Table 3  Event-free survival among patients treated on regimen C (ara-C containing) with low and high week 25 RBC MTX

<table>
<thead>
<tr>
<th>MTX ≤ 0.02 nmol/ml RBCs</th>
<th>MTX &gt; 0.02 nmol/ml RBCs</th>
</tr>
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<tbody>
<tr>
<td>n Fail Expected</td>
<td>n Fail Expected</td>
</tr>
<tr>
<td>EFS overall</td>
<td>142 38 30.3</td>
</tr>
<tr>
<td>EFS (to 25 wk + 12 mo)</td>
<td>142 18 11.6</td>
</tr>
<tr>
<td></td>
<td>106 18 25.7</td>
</tr>
<tr>
<td></td>
<td>106 3 9.4</td>
</tr>
<tr>
<td>P = 0.040</td>
<td>P = 0.005</td>
</tr>
</tbody>
</table>

*aEFS, event-free survival.

Table 4  Kaplan-Meier and stratified Kaplan-Meier estimates of post-week 25 event-free survival for patients treated on regimen C

<table>
<thead>
<tr>
<th>MTX ≤ 0.02</th>
<th>&gt;0.02</th>
<th>≤ 0.02</th>
<th>&gt;0.02</th>
<th>≤ 0.02</th>
<th>&gt;0.02</th>
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</thead>
<tbody>
<tr>
<td>1 yr</td>
<td>98% (3%)</td>
<td>97% (3%)</td>
<td>73% (8%)</td>
<td>96% (3%)</td>
<td>88% (4%)</td>
</tr>
<tr>
<td>2 yr</td>
<td>95% (3%)</td>
<td>93% (5%)</td>
<td>64% (8%)</td>
<td>93% (5%)</td>
<td>83% (5%)</td>
</tr>
<tr>
<td>3 yr</td>
<td>87% (5%)</td>
<td>90% (6%)</td>
<td>64% (9%)</td>
<td>89% (6%)</td>
<td>79% (5%)</td>
</tr>
<tr>
<td>4 yr</td>
<td>84% (6%)</td>
<td>79% (9%)</td>
<td>60% (9%)</td>
<td>82% (7%)</td>
<td>76% (6%)</td>
</tr>
<tr>
<td>5 yr</td>
<td>81% (9%)</td>
<td>79% (14%)</td>
<td>60% (10%)</td>
<td>77% (10%)</td>
<td>73% (7%)</td>
</tr>
</tbody>
</table>

Patients 40 29 33 28 69 49

*Stratum 1, low-risk early pre-B (natural distribution = 43%); stratum 2, high-risk early pre-B (natural distribution = 28%); and stratum 3, pre-B (natural distribution = 29%). RBC MTX is expressed as nmol/ml RBCs.

Table 5  Week 22 serum MTX levels in low-risk patients treated on regimens A and C

<table>
<thead>
<tr>
<th>Regimen A</th>
<th>Regimen C (low-risk patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with week 25 RBC MTX and 12-h serum MTX level</td>
<td>69</td>
</tr>
<tr>
<td>Mean 12-h MTX level ± SD</td>
<td>1.43 ± 1.43 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Median 12-h MTX level</td>
<td>1.1 × 10⁻⁵ M</td>
</tr>
<tr>
<td>No. with week 25 RBC MTX and 36-h serum MTX level</td>
<td>70</td>
</tr>
<tr>
<td>Mean 36-h MTX level ± SD</td>
<td>5.8 ± 4.0 × 10⁻⁷ M</td>
</tr>
<tr>
<td>Median 36-h MTX level</td>
<td>5.0 × 10⁻⁷ M</td>
</tr>
</tbody>
</table>

*All serum levels refer to those drawn during the week 22 (sixth and final) MTX infusion. "Twelve-h" MTX levels were drawn between 12 and 24 h after the start of MTX infusion.

Table 6  Comparison of leukemia prognostic features among arm C patients with low and high week 25 RBC MTX levels

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>MTX ≤ 0.02 nmol/ml RBCs (n = 142)</th>
<th>MTX &gt; 0.02 nmol/ml RBCs (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI</td>
<td>Unknown 20 96 (79%)&lt;sup&gt;b&lt;/sup&gt; 61 (76%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26 (21%) 19 (24%)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Truerworthy risk group</td>
<td>DI &gt; 1.16, any age or WBC 26 (18%) 19 (18%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25 (18%) 19 (18%)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>WBC × 10⁻⁹ /liter by quartile</td>
<td>4 4</td>
<td>4 4</td>
</tr>
<tr>
<td>Age (yr) by quartile</td>
<td>3.1 3.0</td>
<td>3.1 3.0</td>
</tr>
</tbody>
</table>

*DI, DNA index.
<sup>a</sup>Numbers in parentheses, number of patients in each MTX group with known DI levels.
<sup>b</sup>Pearson’s χ².
<sup>c</sup>Wilcoxon test.
tation was related to alterations in serum pharmacokinetics of MTX, we compared levels obtained during (12 h) and 36 h after the start of the last MTX given at week 22 to lower risk patients treated with regimens A and C (Table 5). A small number of patients were excluded from analysis due to missing serum levels. Based on the two-sided Wilcoxon test, there was a significant difference at 12 h, with patients who received MTX/ cytarabine having lower levels of RBC MTX (P = 0.049), but no statistically significant difference at 36 h (P = 0.16). The median 12 h levels were quite close, however, suggesting that clinical relevance of the reduction in serum MTX was likely to be minor.

Association of Other Leukemia Prognostic Factors and RBC MTX. As shown in Table 6, there were no major clinical correlations between previously established major prognostic features and RBC MTX levels. The Trueworthy risk groups (33) were derived after studying all documented prognostic factors. After adjustment for age at diagnosis, WBC count at diagnosis, and blast DNA index, no other factors were significantly prognostic.

DISCUSSION

This study establishes that administration of MTX and cytarabine in the schedule used results in a markedly lower RBC MTX levels when compared to levels of patients treated with MTX alone. Since the effect size is so dramatic (more than a 6-fold difference in medians) and since compliance rates for obtaining samples were similar (71/111 versus 69/112), it is unlikely that the difference could be attributed entirely to selection bias. Again, from the minor nature of the differences in serum levels at 12 and 36 h compared to the large effect size, it seems unlikely that the entire difference in RBC MTX could be due to lower serum MTX levels.

There are several possible explanations for the interaction between MTX and cytarabine. The markedly lower RBC MTX levels might be due to a cytotoxic effect of cytarabine against developing RBCs, inhibiting or preventing erythroid differentiation and resulting in fewer RBCs precursors to take up MTX during the time that cytarabine circulates. A comparable effect in leukemic cells, with cytarabine altering but not destroying such cells and rendering them less susceptible to MTX, would be necessary to support this hypothesis. A second possible explanation is that cytarabine interferes with an aspect of MTX metabolism, such as cellular uptake or polyglutamation, resulting in lower RBC MTX levels. Finally, patients receiving MTX/cytarabine tended to have more nausea and vomiting associated with administration and greater marrow suppression shortly thereafter. This might lead to more prolonged i.v. fluid and/or leukovorin administration, either of which might result in a lower net MTX exposure beyond the 36 h at which the last serum MTX level was drawn.

It also appears that the patients treated with MTX and cytarabine are at increased risk of relapse (particularly early relapse) if their RBC MTX levels are low. Although no direct cause-effect relationship can be inferred from the association, the clinical features of the low and high RBC MTX groups are similar. It would appear that RBCs may act as pharmacological surrogates for leukemia cells with regard to MTX biochemistry and interactions. These data do suggest that the use of cytarabine as an overlapping infusion with MTX may not be optimal. Future studies might consider using nonsimultaneous infusions of the drugs to avoid possible drug antagonism. Alternatively, if MTX and cytarabine are infused together, consideration should be given to the use of subsequent intensification treatment for patients with low RBC MTX levels at the end of consolidation therapy, since such patients appear to be at increased risk of relapse.

ACKNOWLEDGMENTS

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REFERENCES


Changes in red blood cell methotrexate pharmacology and their impact on outcome when cytarabine is infused with methotrexate in the treatment of acute lymphocytic leukemia in children: a pediatric oncology group study.
