The Circadian Schedule for Childhood Acute Lymphoblastic Leukemia Maintenance Therapy does not Influence Event-Free Survival in the NOPHO ALL92 Protocol

Kim K.B. Clemmensen, Registe H. Christensen, Diana N. Shabaneh, Arja Harila-Saari, MD, PhD, Mats Heyman, MD, PhD, Olafur G. Jonsson, MD, Finn Wesenberg, MD, Susanne Rosthøj, PhD, Kjeld Schmiegelow, MD, DrMedSci, and On behalf of the Nordic Society of Pediatric Hematology, Oncology (NOPHO)

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood and with contemporary treatment the cure rate has surpassed 80% [1]. The treatment of ALL consists of induction, consolidation, reinduction, CNS-directed treatment, and maintenance therapy, of which the maintenance therapy phase is the longest. The backbone of most maintenance therapy programs is oral daily 6-Mercaptopurine (6MP) and weekly methotrexate (MTX) with dosages adjusted to keep the WBC below 3.0–3.5 × 10^9/L [2].

Although maintenance therapy is a crucial part of the treatment of childhood leukemia [3], the optimal strategy for dose adjustments, length and schedule of therapy is not established, primarily because its mode of action remains to be determined [2–5].

Circadian (from Latin circu diem, meaning "about a day") rhythm has a major influence on many aspects of human physiology, including the bone marrow activity and the endocrine system [6,7]. Studies in rodents have shown that circadian timing influences the extent of toxicity of 40 anticancer drugs, and affects antitumor efficacy of 28 of these, but with no consistent pattern of the most effective circadian schedule [8].

Two previous studies have reported significantly higher probability of event-free survival (pEFS) when oral MTX/6MP maintenance therapy was administered in the morning compared to in the evening [9,10]. In the latest of these studies, including 294 Nordic children with ALL pEFS of patients on morning schedule was only 0.57 ± 0.08, that is, as poor as if all therapy had been truncated at 12 months from diagnosis [3], and this inferiority could not be explained by co-administration of food or by poor treatment adherence when determined by the erythrocyte levels of the cytotoxic metabolites of 6MP and MTX; that is, 6-thioguanine nucleotides (E-6TGN) and methotrexate polyglutamates (E-MTX), respectively [10].

Since these earlier studies of circadian schedule were done during an era when cure rates were lower than those currently obtained, and since both of the previous studies [9,10] classified the individual patients circadian schedule by a single registration, the Nordic Society for Paediatric Haematology and Oncology (NOPHO) performed the present observational study with prospective registration of medication schedule to obtain detailed data on the circadian schedule of MTX/6MP administration throughout the NOPHO ALL92 oral 6MP/MTX maintenance therapy study [11].

Key words: acute lymphoblastic leukemia; child; Circadian rhythm; 6-mercaptopurine; methotrexate

Background. The event-free survival of childhood acute lymphoblastic leukemia (ALL) has been reported to be superior when oral methotrexate (MTX) and 6-mercaptopurine (6MP) maintenance therapy (MT) is administered in the evening compared to the morning. Procedure. In the ALL92 MT study we prospectively registered the intake of MTX/6MP. The registration was done when blood samples for erythrocyte MTX/6MP metabolite measurements were collected, and referred to the time of intake in the period since last registration. Nine thousand one hundred ninety-five registrations in total. The administration of MTX/6MP was scored as morning, midday, or evening. Results. Of 532 patients, 296 took their medication consistently in the evening, 129 in the evening 50.0–99.9% of the time, and 101 in the evening <50% of the time, six did not have any registrations. The circadian schedule did not differ significantly by age, sex, MTX/6MP doses, and average absolute neutrophil counts. The circadian schedule groups did differ on risk groups (P = 0.003) with fewer HR patients in the 50–99.9% group, and there was a negative correlation between percentage of time on evening schedule and average WBC (Spearman’s rho = −0.15; P = 0.0004). Average WBC was not associated with relapse on ALL92. In a Cox multivariate model the circadian schedule of MTX/6MP was not of prognostic significance for the risk of relapse, and the 10-year cumulative relapse risk was below 20% in all groups. Conclusion. An evening schedule may still be recommended based on the previous publications, but in this study morning administration of MTX and 6MP does not seem to impact EFS. Pediatr Blood Cancer 2014;61:653–658. © 2013 Wiley Periodicals, Inc.

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METHODS

Patients

Patients were eligible for the present study if they were included in the randomized NOPHO ALL92 maintenance therapy study [11], which was approved by the ethical committee of Copenhagen (no. V.200.2080/91) as well as by local ethical committees. Participants gave informed consent according to the Helsinki Declaration. Patients included were diagnosed with non-B-cell childhood ALL in Denmark, Finland, Iceland, Norway and Sweden between January 1992 and December 1996, were between 1.0 and 14.9 years of age at diagnosis and in first remission after induction and consolidation therapy. Of the 538 patients that entered the ALL92 maintenance therapy study, six patients were excluded from the present analyses, because they had Down syndrome, leaving 532 patients for analysis. Of these, six patients did not have any data registered on circadian schedule. The patients were followed until first event or January 11, 2010, whichever came first.

Risk Grouping

The NOPHO ALL92 protocol has previously been described in detail [1,11,12]. The risk group assignment was based on age and white blood cell count (WBC) at diagnosis (standard risk (SR): age 2.0–9.9 years and WBC < 10.0 × 10^9/L; intermediate risk (IR): age 1.0–1.9 or ≥10.0 years and/or WBC 10–49.9 × 10^9/L); and presence of one or more high risk features, HR: WBC ≥ 50.0 × 10^9/L, T-lineage ALL, the presence of CNS or testicular leukemia, translocations t(9;22)(q34;q11) or t(4;11)(q21;q23), lymphomatous leukemia or mediastinal lymphoma, and a poor leukemia response (>25% leukemic blasts in the bone-marrow at day 15 or ≥5% day 29) [12].

Therapy

Induction and consolidation therapy have previously been described in detail [1,11]. The MTX/6MP maintenance therapy was initiated at treatment weeks 13 (SR), 32 (IR), or 63 (HR) and continued until 2 (IR and HR) or 2½ years (SR) after diagnosis. The starting dose of oral 6MP was 7.5 mg/m^2/day and starting dose of oral MTX was 20 mg/m^2/week. During the first year of maintenance therapy, patients with SR– or IR–ALL received alternate pulses at 4-week intervals of (1) vincristine (VCR) (2.0 mg/m^2 once) and prednisolone (60 mg/m^2/day for 1 week), and (2) high dose MTX (HD-MTX) 5 mg/m^2/24 hours with i.t. MTX and leucovorin rescue until five courses of HD-MTX had been given. Every 8 weeks throughout maintenance therapy, HR patients received reinductions of VCR (1.5 mg/m^2 once) and prednisolone (40 mg/m^2/day for 5 days) with i.t. MTX [12].

Maintenance Therapy Randomization in NOPHO ALL92

The ALL92 maintenance therapy study explored the prognostic impact of pharmacologically based monitoring and dose adjustments of oral MTX/6MP maintenance therapy by erythrocyte levels of MTX polyglutamates and 6TGN. The patients were randomized to two different dose-adjustment strategies (control and pharmacology group). This included more than 97% of all eligible Nordic patients during the study period. The dose-adjustment strategies have previously been published in detail [11]. In short, the control and pharmacology group had their dosing of oral 6MP and MTX targeted to a WBC of 1.5–3.5 × 10^9/L. Unless the WBC was < 1.5 × 10^9/L, the pharmacology group patients had in addition their doses of 6MP and/or MTX increased in steps of 20%, if E-6TGN/MTX was < 1.350 (nmol/mmol Hb)^2, and such dose increments were regarded as tolerable by the treating physician.

Circadian Time Schedule of MTX/6MP During Maintenance Therapy

An evening schedule of MTX/6MP was recommended in the NOPHO ALL92 protocol. As part of the study, data on all blood counts, E-MTX and E-6TGN levels, as well as all 6MP and MTX dose changes/modifications were collected for all patients on study. In total 9,195 blood samples were collected for E-MTX/E-6-TGN analyses. Together with each blood sampling, the current time for intake of MTX and 6MP (morning, midday, or evening) was registered. 879 (9.6%) of these blood samplings had no registered time schedule for MTX/6MP administration, 151 (1.6%) had missing data on MTX circadian schedule, and 1,163 (12.6%) had missing data on 6MP circadian schedule.

It was not possible to construct pure morning and midday groups, because there were too few patients that took their medication consistently at these time points. Instead the patients were divided into three circadian schedule groups, (1) patients that were on an evening schedule <50% of the time, (2) patients that were on an evening schedule 50.0–99.9% of the time, and (3) patients that were on evening schedule 100% of the time.

Statistics

To describe the course of the circadian schedule for each patient, the percentage of the duration of the therapy for which the patient had received the medication in the evening was determined at each time point during maintenance therapy. For a particular time point, the percentage was calculated as a weighted average based on all previous registrations, each registration counting until a new registration of circadian schedule or a maximum of 8 weeks had passed. Average blood counts, drug concentrations and doses of MTX and 6MP during maintenance therapy were calculated in the same manner.

The Kruskal–Wallis test was applied to test for differences between subgroups for continuous variables and the Pearson chi-squared test was applied for categorical variables. Spearman’s correlation coefficient was used to assess correlations. For the comparisons involving the time dependent variables (percentage evening medication, blood counts, drug concentrations, and doses) the averages obtained by the end of the maintenance therapy was used. In these analyses we included only the patients that were in remission at the end of maintenance therapy.

To investigate the development of the circadian schedule during the maintenance therapy, locally weighted polynomial regression (lowest regression) [13] of evening medication (1=evening medication of both drugs, 0=otherwise) was performed for each risk group. For each risk group, a test for trend over time was performed using an unadjusted logistic regression model with the number of months since diagnosis as explanatory variable. To account for the repeated measurements, an approach based on robust estimation of the standard errors was used (Generalized Estimating Equations) [14].

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### TABLE I. Patient Characteristics

<table>
<thead>
<tr>
<th>Circadian schedule</th>
<th>&lt;50% of the time on evening schedule</th>
<th>50–99.9% of the time on evening schedule</th>
<th>100 % of the time on evening schedule</th>
<th>Total</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td><strong>Baseline characteristics</strong> (n = 526)</td>
<td></td>
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<tr>
<td>Number of patients</td>
<td>101</td>
<td>129</td>
<td>296</td>
<td>526</td>
<td>—</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>4.0</td>
<td>4.5</td>
<td>3.8</td>
<td>4.0</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>49/52</td>
<td>73/56</td>
<td>159/137</td>
<td>281/245</td>
<td>0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>t(12; 21)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>High-hyperdiploidy</td>
<td>36</td>
<td>25</td>
<td>61</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>t(9; 22)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypodiploidy</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>t(1; 19)</td>
<td>15</td>
<td>24</td>
<td>36</td>
<td>75</td>
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<tr>
<td>Normal or missing</td>
<td>49</td>
<td>71</td>
<td>176</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>Risk groups (SR/IR/HR)</td>
<td>47/40/14</td>
<td>68/59/2</td>
<td>125/130/41</td>
<td>240/229/57</td>
<td>0.0026&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% (SR/IR/HR)</td>
<td>47/40/14</td>
<td>53/46/2</td>
<td>42/44/14</td>
<td>46/44/11</td>
<td></td>
</tr>
<tr>
<td>Maintenance therapy and follow-up for patients with no event during maintenance therapy (n = 512)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>14</td>
<td>18</td>
<td>51</td>
<td>83</td>
<td>—</td>
</tr>
<tr>
<td>DCR/SMN</td>
<td>1/0</td>
<td>0/3</td>
<td>2/5</td>
<td>3/8</td>
<td>—</td>
</tr>
<tr>
<td>Mean WBC under maintenance therapy (median; minimum, maximum)</td>
<td>$3.5 \times 10^7$/L (1.8, 5.7)</td>
<td>$3.4 \times 10^7$/L (1.7, 7.3)</td>
<td>$3.2 \times 10^7$/L (1.5, 5.7)</td>
<td>$3.3 \times 10^7$/L (1.5, 7.3)</td>
<td>0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean neutrophil count during maintenance therapy (median; minimum, maximum)</td>
<td>$2.0 \times 10^9$/L (1.0, 4.4)</td>
<td>$2.0 \times 10^9$/L (0.7, 4.1)</td>
<td>$1.9 \times 10^9$/L (0.7, 4.1)</td>
<td>$2.0 \times 10^9$/L (0.7, 4.4)</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MTX dosage during maintenance therapy (median; minimum, maximum)</td>
<td>14.8 mg/m²/week (3.5, 25.7)</td>
<td>15.8 mg/m²/week (2.5, 24.8)</td>
<td>15.6 mg/m²/week (3.8, 28.0)</td>
<td>15.5 mg/m²/week (2.5, 28.1)</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6MP dosage during maintenance therapy (median; minimum, maximum)</td>
<td>56.0 mg/m²/day (24.9, 101.6)</td>
<td>61.3 mg/m²/day (33.3, 106.0)</td>
<td>60.4 mg/m²/day (9.8, 109.4)</td>
<td>59.7 mg/m²/day (3.3, 109.4)</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Erythrocyte MTX-polyglutamates (median; minimum, maximum)</td>
<td>5.5 nmol/mmolHb (1.8, 16.8)</td>
<td>5.5 nmol/mmolHb (2.0, 11.6)</td>
<td>5.6 nmol/mmolHb (2.0, 14.1)</td>
<td>5.5 nmol/mmolHb (1.8, 16.8)</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Erythrocyte 6TGN (median)</td>
<td>147.6 mmol/mmolHb (75.7, 570.6)</td>
<td>175.9 mmol/mmolHb (69.7, 956.9)</td>
<td>173.9 mmol/mmolHb (30.3, 609.7)</td>
<td>171.1 mmol/mmolHb (30.3, 956.9)</td>
<td>0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9-Year pEFS [95% CI]</td>
<td>0.76 [0.42–0.92]</td>
<td>0.84 [0.75–0.89]</td>
<td>0.78 [0.49–0.83]</td>
<td>0.80 [0.74–0.85]</td>
<td>0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9-Year cumulative incidence of relapse [95% CI]</td>
<td>0.10 [0.06; 0.16]</td>
<td>0.14 [0.09; 0.22]</td>
<td>0.19 [0.14; 0.24]</td>
<td>0.15 [0.12; 0.19]</td>
<td>—</td>
</tr>
</tbody>
</table>

DCRI, death in first clinical remission; SMN, second malignant neoplasm; SR, standard risk; IR, intermediate risk; HR, high risk; pEFS, probability of event-free survival from end of maintenance therapy. <sup>a</sup>Kruskal–Wallis test; <sup>b</sup>Pearson chi-square test; <sup>c</sup>Log rank test with delayed entry at end of maintenance therapy.
With a basic time scale defined by the date of diagnosis, survival probabilities for each circadian schedule group were based on the Kaplan–Meier estimator and cumulative incidences were based on the Aalen–Johansen estimator [15]. Calculation of confidence intervals was based on the cloglog transform. Since the circadian schedule group is not known until the end of the therapy, the patients entered these analyses at the time point of cessation of the maintenance therapy (delayed entry) [16].

A multiple Cox regression analysis [17] was performed to assess the relative risk of relapse according to circadian schedule group. The patients who died in remission or developed a second malignancy were censored at the time point of these events. The circadian schedule group was considered as a time-dependent categorical variable, for each time point during the maintenance therapy dividing the percentage with evening medication into groups of <50%, 50–99%, and 100%. The model was further adjusted for sex, randomization group [11], WBC at diagnosis, TPMT activity [18], mean absolute neutrophil count (time-dependent), and stratified on risk group as in the original analysis of these data [11]. The entry time of the patients in the analysis was defined as the first time point during the maintenance therapy for which the three time-dependent variables (neutrophil, MTX, and 6MP schedule) had been registered. The proportional hazards assumption was assessed by the score processes using graphical methods [19] and the Lin, Wei, and Ying test [20]. Calculations were performed using SPSS 18 [21], SAS version 9.2 [22], and R version 2.12.2 [23].

RESULTS

Of the total cohort of 532 patients, 56 males and 28 females experienced a relapse, of which 72 involved the bone-marrow and only 11 occurred during maintenance therapy. One of the six patients with no registration on circadian schedule had a relapse. Nine patients developed a second malignancy, and three patients died in first remission. The median follow up of the 436 patients that stayed in first remission was 14.9 years (range, 7.0–18.0 years). The overall 10-year pEFS was 0.83 (95% CI: 0.80–0.86).

Circadian Schedule

Of the 532 patients, 296 patients took their 6-MP and MTX consistently in the evening, 129 patients had evening schedule registered in 50.0–99.9% of the maintenance therapy, 101 patients had an evening schedule registered in <50% of the time (Table I). Among the latter group only 40 patients took their medication consistently in the morning. The pEFS of these 40 patients did not differ from that of the remaining 61 patients in the <50% evening schedule group and they are in the following not analyzed separately. Figure 1 shows the smoothed average circadian schedule over time for all patients in each of the three risk groups. Overall there were no differences in the trend for the three risk groups, \( P = 0.34 \). However, potentially reflecting patient numbers and duration of maintenance therapy, the SR- patients were increasingly on evening schedule over time (OR = 1.04 per 1 month duration of MT, 95% CI: 1.02–1.06, \( P < 0.0001 \)), whereas the IR- and HR-patients did not change significantly from initiation to cessation of maintenance therapy (OR = 1.02 per 1 month duration of MT, 95% CI: 0.99–1.06, \( P = 0.09 \) and OR = 1.04, 95% CI: 0.89–1.21, \( P = 0.29 \), respectively). Figure 2 shows for each patient the average percentage of treatment with MTX evening medication versus average percentage of treatment with 6MP evening medication. Both numbers are based on the entire maintenance therapy and only patients in first remission by the end of the maintenance therapy are included. Patients experiencing a relapse are marked with black dots. Patients with identical timeschedule (e.g., continuous evening dosage) will have overlapping dots. There were 48 relapses among the 286 patients consistently receiving both drugs in the evening (upper right corner).

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schedule for their entire maintenance therapy. Figure 3 shows the cumulative incidence of relapse with entry delayed to the end of maintenance therapy for the three circadian schedule groups.

The circadian schedule did not differ significantly by age or sex (Table I). The circadian schedule groups did differ on risk groups ($P = 0.03$) with fewer HR patients in the 50–99.9% group. Furthermore, the average doses of MTX and 6MP, the red blood cell levels of MTX and 6TGN, and the average absolute neutrophil counts did not differ between the circadian schedule groups, but there was a small but significant negative correlation between percentage of time on evening schedule and the average white blood cell count (Spearman’s rho $-0.15$; $P = 0.0004$). Average white blood cell count was not associated with relapse on ALL92 [11].

Circadian Schedule and Clinical Outcome

Overall the circadian schedules of MTX and 6MP dosages were not a significant risk factor of relapse in a Cox model that included sex, white blood cell count at diagnosis, the average absolute neutrophil count during maintenance therapy, and the thiopurine methyltransferase activity (Table II). The hazard ratio of relapse in the 100% evening group compared to the <50% group was 1.48 (95% CI: 0.80–2.77), while it for the 50–99.9% evening group was 1.23 (95% CI: 0.60–2.55) compared to the <50% evening group. The overall $P$-value of no effect of circadian schedule was 0.44.

DISCUSSION

Two previous publications have indicated that an evening schedule of oral MTX/6MP maintenance therapy reduces the relapse rate [9,10,24], but both studies were burdened by their retrospective nature and the single time point for registration of the patients in first remission by the end of the maintenance therapy (delayed entry). Only patients in first remission by the end of the maintenance therapy were included.

![Fig. 3. Cumulative relapse incidence curves for the three circadian schedule groups defined by the observed percentage of evening medication obtained by the end of the maintenance therapy (delayed entry).](image)

TABLE II. Coefficients in the Cox Hazard Models: Relapses Only

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard ratio [95% CI]</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex*</td>
<td>8.09 [2.45; 26.67]</td>
<td>0.0006</td>
</tr>
<tr>
<td>WBC at diagnosis</td>
<td>1.01 [1.00; 1.01]</td>
<td>0.0185</td>
</tr>
<tr>
<td>Randomization group*b</td>
<td>6.99 [2.07; 23.64]</td>
<td>0.0018</td>
</tr>
<tr>
<td>Randomization group $\times$ sex*c</td>
<td>0.14 [0.04; 0.53]</td>
<td>0.0039</td>
</tr>
<tr>
<td>mANCd</td>
<td>1.99 [1.37; 2.89]</td>
<td>0.0003</td>
</tr>
<tr>
<td>TPMT activity</td>
<td>1.10 [1.00; 1.01]</td>
<td>0.0022</td>
</tr>
<tr>
<td>100% evening</td>
<td>1.56 [0.84; 2.89]</td>
<td>0.1603</td>
</tr>
<tr>
<td>50–99.9% evening</td>
<td>0.93 [0.43; 2.03]</td>
<td>0.8621</td>
</tr>
<tr>
<td>&lt;50% (reference group)</td>
<td>1 — — —</td>
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</tbody>
</table>

mANC, mean absolute neutrophil count; TPMT, thiopurinemethyltransferase. *Death in remission and second malignancies were counted as censoring events; $^b$0 for females, 1 for males; $^c$0 for control, 1 for pharmacology; $^d$The interaction between sex and randomization group, 1 for males in pharmacology group, 0 for all other; $^e$Mean absolute neutrophil count during maintenance therapy was analyzed as a time-dependent continuous variable.

time of medication. Thus, the failure to replicate these findings in the present far larger study with more detailed registration of the circadian schedule may have several explanations.

Firstly, the importance of the circadian schedule would have been diminished in contemporary protocols including NOPHO ALL92 compared to those applied in the eighties, if maintenance therapy overall had become less important because the burden of residual leukemic at the start of maintenance therapy had become significantly lower due to improved earlier treatment phases. However this can only be a minor part of the explanation, since the overall improved cure rate for childhood ALL also reflects more attention to maintenance therapy. Thus, the study by Toyoda and co-workers showed that with only 1 year of therapy approximately 40% of all patients would relapse irrespective of risk groups [3], and the intensity of maintenance therapy as measured by the neutrophil counts even in the present cohort was significantly related to the risk of relapse.

Secondly, since the NOPHO ALL92 protocol recommended an evening schedule and furthermore paid specific attention to this at each time point of blood sampling for MTX and 6MP metabolite measurements, those 42% who chose to take their MTX and 6MP at other time points for a shorter or longer part of their therapy may have been burdened by side effects when on evening schedule, making medication more tolerable at other hours of the day. A shift towards morning schedule could in part be a surrogate marker for a high adherence to therapy. An association of evening schedule with side effects is supported by the lower white cell counts for patients on evening schedule in spite of no significant differences in their MTX and 6MP doses. Such differences in treatment response could reflect MTX and 6MP bioavailability, although previous studies have been discordant in their findings [25–30].

Thirdly, the overall adherence to the prescribed dose of MTX and 6MP could have been significantly lower in those on morning schedule in the Canadian study that was the first to link circadian schedule with relapse rates in childhood ALL [9,24]. However, this is unlikely to have been the explanation in the subsequent Nordic study, where we found similar red blood cell levels of the cytotoxic MTX and 6MP metabolites in the morning and evening schedule.
groups [10]. Still, the median number of metabolite measurements in the previous NOPHO ALL88 and the current NOPHO ALL92 studies were 3 and 16, respectively. Thus, the MTX and 6MP metabolite levels could have underestimated the degree of non-adherence in the morning schedule group of our previous study [10].

In the current large patient cohort with more than 9,000 metabolite measurements, the adherence to medication seemed independent of their circadian schedule, and the red blood cell levels of MTX, which (in contrast to 6TG levels) are significantly related to the prescribed MTX dose, was approximately 25% higher in the current study compared the ALL88 study [10]. This seemingly better overall adherence to the oral medication could thus have eliminated the association between morning schedule and risk of relapse.

Finally, the recommendation to take MTX and 6MP in the evening in the NOPHO ALL92 protocol, which was not the case in the two earlier studies, resulted in a relatively small group being consistently on morning schedule, which has weakened the power to detect differences not least since the overall relapse rate was low.

Based on the current and the previous studies, an evening schedule for oral MTX/6MP maintenance therapy should still be recommended, but our findings indicate that an intermittent or consistent shift to a morning schedule in modern ALL regimens may not jeopardize the patient’s chance of cure.

ACKNOWLEDGMENTS

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