Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with Down syndrome and acute lymphoblastic leukemia

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ABSTRACT

Background
Children with Down syndrome have an increased risk of developing acute lymphoblastic leukemia and a poor tolerance of methotrexate. This latter problem is assumed to be caused by a higher cellular sensitivity of tissues in children with Down syndrome. However, whether differences in pharmacokinetics play a role is unknown.

Design and Methods
We compared methotrexate-induced toxicity and pharmacokinetics in a retrospective case-control study between patients with acute lymphoblastic leukemia who did or did not have Down syndrome. Population pharmacokinetic models were fitted to data from all individuals simultaneously, using non-linear mixed effect modeling.

Results
Overall, 468 courses of methotrexate (1-5 g/m^2) were given to 44 acute lymphoblastic leukemia patients with Down syndrome and to 87 acute lymphoblastic leukemia patients without Down syndrome. Grade 3-4 gastrointestinal toxicity was significantly more frequent in the children with Down syndrome than in those without (25.5% *versus* 3.9%; P=0.001). The occurrence of grade 3-4 gastrointestinal toxicity was not related to plasma methotrexate area under the curve. Methotrexate clearance was 5% lower in the acute lymphoblastic leukemia patients with Down syndrome (P=0.001); however, this small difference is probably clinically not relevant, because no significant differences in methotrexate plasma levels were detected at 24 and 48 hours.

Conclusions
We did not find evidence of differences in the pharmacokinetics of methotrexate between patients with and without Down syndrome which could explain the higher frequency of gastrointestinal toxicity and the greater need for methotrexate dose reductions in patients with Down syndrome. Hence, these problems are most likely explained by differential pharmacodynamic effects in the tissues between children with and without Down syndrome. Although the number of patients was limited to draw conclusions, we feel that it may be safe in children with Down syndrome to start with intermediate dosages of methotrexate (1-3 g/m^2) and monitor the patients carefully.

Key words: metrotrexate pharmacokinetics, Down syndrome, acute lymphoblastic leukemia, methotrexate.

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Introduction

Down syndrome (DS) is one of the most common congenital chromosome abnormalities, with a prevalence of 16 per 10,000 live births in the Netherlands.[1] Children with DS have an increased risk of developing both acute myeloid leukemia, as well as acute lymphoblastic leukemia (ALL).[2]

DS-ALL patients differ in presenting characteristics from ALL patients without DS (non-DS-ALL). For instance, a lower frequency of T-cell ALL[2-5] and CD10-negative ALL (pro-B-cell ALL) is found in DS-ALL.[4] Moreover, there are differences in the distribution of genetic abnormalities, with lower frequencies of unfavorable characteristics such as *MLL-AF4* and the Philadelphia-chromosome, as well as lower frequencies of favorable characteristics such as high hyperdiploidy and *TEL-AML1* in DS-ALL cases.[2,3,6]

Several studies indicate that the prognosis of children with DS-ALL is poorer than that of non-DS-ALL patients.[2,3,7] Whitlock *et al.* reported that children with DS-ALL treated according to the National Cancer Institute's standard-risk arm in Children's Oncology Group protocols had a worse outcome compared to children with non-DS-ALL.[2] In contrast, DS-ALL and non-DS-ALL patients stratified in the National Cancer Institute's high-risk arm did not show significant differences in outcome. This suggests that DS-ALL cells are relatively resistant to chemotherapy and that intensification of therapy for DS-ALL patients may be warranted.[2] Furthermore, this study suggests that the National Cancer Institute classification may not be appropriate for risk-group stratification in DS-ALL. Preliminary results of the ALL-BFM 2000 study showed no significant differences in minimal residual disease levels in the first 3 months of treatment between DS-ALL and non-DS-ALL children, nor in relapse risk (6.1% in DS patients *versus* 11.4% in non-DS patients).[8,9] Of interest, the risk of serious adverse events was significantly higher in DS-ALL patients (23.4%) than in non-DS-ALL (6%) patients, as was the cumulative incidence of treatment-related deaths (9% *versus* 2%).[8,9] These data suggest that treatment intensification in DS-ALL patients needs to be carefully balanced against the risk of enhanced toxicity and a potential excess in treatment-related mortality.

One of the key agents used in the treatment of ALL is methotrexate. Methotrexate inhibits dihydrofolate reductase, leading to inhibition of DNA synthesis. Methotrexate polyglutamylation increases the intracellular retention of the drug, which is an important parameter of methotrexate's efficacy.[10-13] Methotrexate is associated with side effects, especially mucositis, liver toxicity and myelosuppression. Patients can be rescued from excessive toxicity with leucovorin, which is routinely administered following the infusion of higher dosages of methotrexate. It is well-known that DS-ALL patients are more susceptible to methotrexate-induced side-effects than non-DS-ALL patients,[14,15] which is due to the higher cellular sensitivity of tissues affected by methotrexate, such as the mucosa and bone marrow. This vulnerability often results in reductions of the methotrexate dose. It is not known whether the differences in toxicity between DS and non-DS children also reflect differences in methotrexate pharmacokinetics. The only available study, by Garré *et al.*, showed that methotrexate plasma concentrations 42 h after the start of infusion were significantly higher in five DS-ALL patients than in three non-DS-ALL patients.[16]

We performed a retrospective case-control study of 44 children with DS-ALL and 87 non-DS-ALL controls, enrolled in Dutch Childhood Oncology Group (DCOG) studies. The aim of this study was to identify differences in methotrexate pharmacokinetics between DS and non-DS children, and whether any differences found were related to side effects.

Design and Methods

Patients

We identified all DS-ALL patients enrolled in three consecutive DCOG treatment protocols: DCOG ALL-8, -9 and -10 studies, conducted between November 1991 and December 2006. The children were enrolled in the eight participating University Hospitals in the Netherlands. Only children who were treated according to the protocols and in complete remission after induction therapy were included. For each DS-ALL case, we selected two non-DS-ALL controls who were matched for treatment protocol, sex and body surface area.

Treatment protocols and methotrexate administration

From 1991 until January 1997, children with newly diagnosed ALL were enrolled in the BFM-based treatment protocol DCOG-ALL-8.[17] Patients were stratified into three risk groups: standard, medium and high risk. Patients with standard and medium risk received high-dose methotrexate courses (5 g/m^2/course in 24 h), given every 2 weeks for a total of four courses, combined with intrathecal triple therapy consisting of methotrexate/Di-Adreson F-aquosum/cyatrabine, and oral 6-mercaptopurine (25 mg/m^2/day) given once daily for 8 weeks. Patients in the medium-risk group were randomized to receive this block with either oral low-dose 6-mercaptopurine or intravenous high-dose 6-mercaptopurine (1300 mg/m^2, directly following the methotrexate infusions) every 2 weeks. High-risk patients received high-dose methotrexate (5 g/m^2/course in 24 h) in two of the three high-risk blocks. Three doses of leucovorin rescue (standard-risk group: 15 mg/m^2; medium and high-risk groups: first dose 30 mg/m^2; subsequent doses 15 mg/m^2) were given every 6 h, starting 36 h after the start of the methotrexate infusion for standard-risk patients, and at 42 h for medium and high-risk patients.

The DCOG-ALL-9 protocol (1997–2004) stratified children into two risk groups; non-high-risk and high-risk. Non-high-risk patients received three high-dose methotrexate courses (2 g/m^2/course in 24 h) given once weekly and high-risk patients received four high-dose methotrexate courses (3 g/m^2/course in 24 h), given every 2 weeks. High-dose methotrexate courses were combined with intrathecal therapy at the start of every methotrexate infusion. Children in the non-high-risk group were not given 6-mercaptopurine; those in the high-risk group received oral 6-mercaptopurine (50 mg/m^2, once daily) for 8 weeks. Leucovorin rescue therapy (15 mg/m^2) was initiated 36 h after the start of the infusion, and was administered every 6 h for three doses.[18]

From November 2004 onwards, children with ALL were treated according to the DCOG-ALL-10 protocol, which is ongoing. Patients were stratified into three risk groups; standard, medium and high-risk. Standard- and medium-risk patients received high-dose methotrexate courses (5 g/m^2/course in 24 h), given every 2 weeks for a total of four courses. These high-dose methotrexate courses were combined with intrathecal therapy and oral 6-mercaptopurine (25 mg/m^2, once daily) for 8 weeks. Leucovorin rescue (15 mg/m^2) was given every 6 h starting 42 h after the beginning of the methotrexate infusion, for a minimum of three doses. High-risk patients received three blocks with high-dose methotrexate (5g/m^2/course in 24 h) after which they eligible for stem cell trans-

plantation or three further high-risk courses if they did not have suitable donors. Leucovorin rescue therapy (15 mg/m^2) was initiated 42 h after the start of the infusion and was given every 6 h.

All protocols used similar supportive care guidelines for administration of high-dose methotrexate, including hyperhydration (2.5-3.0 L/m^2/day), and urine alkalinization (using sodium bicarbonate infusion, aiming at producing urine with a pH between 7 and 8). If methotrexate plasma levels were 0.4 μmol/L or higher 48 h after the start of the methotrexate infusion, hyperhydration, alkalinization and leucovorin rescue were continued for at least another 24 h. The plasma level of methotrexate required to discontinue these measures was 0.25 μmol/L or below at 72 h or later. No specific guidelines regarding methotrexate administration for DS patients were provided in any of these protocols. The methotrexate and leucovorin dosages are specified in detail in Table 1.

Methotrexate toxicity and plasma levels

The data were extracted from patients' files, and included the number of methotrexate courses, the dose of methotrexate that was prescribed, the methotrexate plasma levels, the leucovorin rescue that was given, the hyperhydration and urine alkalinization procedures, as well as side-effects during and after the methotrexate infusion until the next block of chemotherapy. Toxicity data were graded according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0. Methotrexate plasma levels were recorded 48 h after the start of the methotrexate infusion, as well as at additional time-points in the case of high levels, or in case the hospital routinely determined plasma levels at other time-points. Other items that were tabulated included co-medication, delays in starting subsequent therapy elements, creatinine and liver function tests. In a few cases the exact time of the methotrexate plasma level determination was missing; in these cases the assumption was made that the physicians followed the treatment protocol, and that samples had been taken at the prescribed time-points.

Pharmacokinetic analysis

The pharmacokinetic model was fitted to the data from all individuals simultaneously, using non-linear mixed effect modeling (NONMEM).[19] The population parameters, intra- and inter-patient and residual variances were estimated using the NONMEM software program (double precision; version VI, level 1.0). The first-order conditional estimate method was used throughout the analysis.

Methotrexate pharmacokinetics was described according to a two-compartmental model with a first order elimination from the central compartment. The following parameters were estimated: the volume of distribution of the central compartment (V1), clearance from the central compartment (CL), volume of distribution of the peripheral compartment (V2) and inter-compartmental clearance (Q). In the structural model pharmacokinetic parameter values were standardized for a body weight of 70 kg using an allometric model.[20] For instance CL and V1 were standardized as CLpop = CLstd • (WT / 70)0.75 and V1pop = V1std • (WT / 70), where CLpop and V1pop are typical population parameter values in individuals with a certain weight (WT) and CLstd and V1std are the standard values for patients with a weight of 70kg. Inter-and intra-patient variability of the pharmacokinetic parameters was estimated using an exponential error model. For instance, inter- and intra-individual variability in CL was estimated using: CLi = CLpop x exp (ηi + κi) where CLi represents the clearance of individual i, and η and κ are the respective inter- and intra-patient random effects with a mean of zero and a variance ω2. The covariance between inter-patient variability was estimated as well. The residual variance in a NONMEM model corresponds to the difference between the observed concentration (Cobs) and predicted concentration (Cpred). The latter is predicted on the basis of individual parameters (CLi, V1i, etc.). Residual variance was modeled with a combined additive and proportional error model: Cobs, i = ε1 + Cpred, i (1 + ε2), where ε1 and ε2 are independent random variables with zero mean and common variances of σ2. The adequacy of the developed model was evaluated by examination of the precision of the parameter estimates, the values of random-effect variances and various diagnostic plots.[20-23]

In order to explain the pharmacokinetic variability between and within the patients, relationships were investigated between pharmacokinetic parameters and various characteristics of the patients. Covariates were introduced in a multiplicative way. Categorical variables, such as DS, were modeled as: CLi = CLpop x θ^{DOWN}, where CLpop is the population value for methotrexate clearance in non-DS patients (exponent DOWN=0) and θ is the fractional change in clearance in DS patients (DOWN = 1). Continuous variables, such as creatinine clearance (CRCL), were modeled centered around the median value in the population: CLi = CLpop * (CRCL/142) $^{\theta}$, where CLpop is the methotrexate clearance in individuals with a CRCL of 142 mL/min and θ is an exponential. The objective function value was used for comparison of the models. Discrimination between hierarchical models was based on the objective function value using the log-likelihood ratio test. A *P* value of 0.05, representing a decrease in the objective function value of 3.8 units, was considered statistically significant (df=1).[19]

Individual pharmacokinetic parameters were generated by Bayesian analysis. On the basis of these parameters, individual plasma concentration-time profiles were generated for the assessment of the area under the plasma concentration *versus* time curve (AUC), and the plasma concentration 48 h after the start of the methotrexate infusion.

Table 1. Methotrexate and standard leucovorin rescue dosages in the three Dutch Childhood Oncology Group ALL treatment protocols.

Protocol	Methotrexate	Leucovorin rescue
DCOG ALL8		
Standard risk group	4x2 g/m^2 every 14 days	15 mg/m^2, every 6 h from T=36 for 3 dosages
Medium risk group	4x5 g/m^2 every 14 days	30 mg/m^2 at T=42; followed by 15 mg/m^2 at T=48 and T=54
High risk group	2x5 g/m^2 every 21 days	30 mg/m^2 at T=42; followed by 15 mg/m^2 at T=48 and T=54
DCOG ALL9		
Non-high risk	3x2 g/m^2 every 7 days	15 mg/m^2, every 6 h from T=36 for 3 dosages
High risk	4x3 g/m^2 every 14 days	15 mg/m^2, every 6 h from T=36 for 3 dosages
DCOG ALL10		
Standard risk	4x5 g/m^2 every 14 days	15 mg/m^2 , every 6 hours from T=42 for 3 dosages
Medium risk	4x5 g/m^2 every 14 days	15 mg/m^2, every 6 hours from T=42 for 3 dosages
High risk	5 g/m^2 every 50 days, for 3-6 courses	15 mg/m^2, every 6 hours from T=42 for 3 dosages

T: time-point (in h) after start of methotrexate-infusion; DCOG: Dutch Childhood Oncology Group.

Statistics

The Statistical Package for the Social Sciences (SPSS) analysis system (v.15.0, SPSS Inc., Chicago, IL, USA) was used for statistical comparisons. To analyze differences between DS-ALL and non-DS-ALL patients, non-parametric matched paired analysis was applied. The non-parametric Cochran test for k related samples was used for toxicity parameters with binary values. Analyses were two-tailed and the level of statistical significance was set at less than <0.05.

Results

Patients' characteristics

In total 47 DS-ALL patients, enrolled in protocols DCOG ALL-8, -9 and -10, were identified in the DCOG database. Three patients died during induction therapy and could not, therefore, be evaluated. For the remaining 44 DS-ALL patients (25 boys, 19 girls), 87 matched non-DS-ALL controls (50 boys, 37 girls) were selected. One patient with DS was matched to one instead of two non-DS-ALL patients, because no other appropriate control could be identified.

The patients' characteristics are shown in Table 2. All DS-ALL patients had B-cell-precursor ALL, and 7/87 (8%) non-DS-ALL patients had T-cell ALL. DS-ALL patients were slightly older than the non-DS-ALL patients (3.4 *versus* 5.4 years, respectively; *P*=0.02), which was the result of matching according to body surface area. There was a difference in median presenting white blood cell count between DS and non-DS children (8.8×10^9/L *versus* 26.9×10^9/L, respectively; *P*=0.005). Five DS-ALL patients had significant comorbidity, including complex congenital heart failure (n=3; surgically corrected before the diagnosis of ALL in all of them), hypothyroidism (n=1), and diabetes mellitus (n=1). However, all patients were in a clinically good condition before they were diagnosed with DS-ALL, and all were treated with curative intent.

Table 2. Characteristics of the syndrome's DS-ALL patients and their non-DS-ALL matched controls.

Parameter	DS-ALL	Non-DS-ALL	*P* value
Number	44	87	
Matching parameters			
Sex, n (%)			
Male	25 (56.8)	50 (57.5)	
Female	19 (43.2)	37 (42.5)	1.00
BSA, median (range), m^2	0.70 (0.3-1.6)	0.74 (0.5-1.6)	0.87
Treatment protocol.			
ALL 8, n (%)	10 (22.7)	20 (23)	
ALL 9, n (%)	24 (54.5)	48 (55.2)	
ALL 10, no n (%)	10 (22.7)	19 (21.8)	0.99
Patients' characteristics			
Age, median (range), years	5.4 (2.0-17.1)	3.6 (1.3-14.7)	0.03
Initial WBC, median$x10^9$/L	8.8 (1.2-460)	27.0 (0.8-684.0)	0.005
Immunophenotype			
Pro B-ALL, n (%)	0	4 (4.6)	
BCP-ALL, n (%)	44 (100)	76 (87.4)	
T-ALL, n (%)	0	7 (8)	0.05
DNA index			
<1.16	29/32 (90.6)	45/53 (84.9)	
≥ 1.16	3/32 (9.3)	8/53 (15.1)	0.52
Cytogenetic abnormalities			
t(9;22), n (%)	2/44 (4.5)	1/87 (1.1)	0.261
t(12;21), n (%)	4/44 (9.1)	6/87 (6.9)	0.732
Methotrexate courses			
Number of methotrexate courses (n)[#]	152	316	
Administered at 2 g/m^2	89 (58.6)	126 (39.8)	
Administered at 3 g/m^2	15 (9.9)	71 (22.5)	
Administered at 5 g/m^2	39 (25.6)	119 (37.7)	
Administered other	9 (5.9)	0	<0.001
Courses not received, n.	3	1	0.68
Reduced dosage, n (%)*			
All courses, n (%)	26 (17.1)	0 (0)	<0.001
Dose reduction below the prescribed 2 g/m^2	2/74	0/126	
Dose reduction below the prescribed 3 g/m^2	0/15	0/71	
Dose reduction below the prescribed 5 g/m^2	24/63	0/119	

*WBC: white blood cell count; BSA: body surface area; BCP-ALL: B-cell precursor acute lymphoblastic leukemia. #This is the actual dose that was given to patients, which sometimes differed from the prescribed dose in the protocol. *Dose reductions were made empirically before the first course of methotrexate, or dosages were adapted based on toxicity experienced in the first course.*

Methotrexate treatment

In total, 468 high-dose methotrexate courses were administered to the 44 DS-ALL children (n = 152 courses) and 87 non-DS-ALL children (n = 366 courses). Dose reductions were applied in DS-ALL patients in 26 of the 152 (17.1%) methotrexate courses, in 9 out of the 44 (20.5%) patients; in contrast, none of the non-DS-ALL patients had a dose reduction. Three DS-ALL patients received one course less than required per protocol, and one non-DS-ALL patient received three instead of four courses because of severe methotrexate-induced side effects (*P*=0.68). Dose reduction was electively initiated from the first course onwards, in anticipation of possible greater toxicity, in 18/26 courses in five DS children. In 8 of 26 courses in four DS patients, dose reductions were applied from the second or subsequent courses onwards because of documented excessive toxicity in earlier courses of high-dose methotrexate. Dose reductions occurred in protocol DCOG ALL-9 at the 2 g/m^2 methotrexate dose (2 courses in 1 patient), and in protocol DCOG ALL-10 at the 5 g/m^2 dose (6 courses in 3 patients). Of interest, the number of DS patients requiring dose reductions in the second or subsequent courses due to excessive toxicity in earlier courses was 1/27 (3.7%) patients when treated at the 2-3 g/m^2 dose level, and 3/12 (25%) patients when treated at the 5 g/m^2 dose level (*P*=0.046).

Toxicity of high-dose methotrexate courses

We first evaluated the frequency of grade 3-4 toxicities after the first high-dose methotrexate course only (after excluding the five DS-ALL patients with initial dose reductions in anticipation of greater toxicity), as toxicity in later courses was influenced by dose reductions and cumulative toxicity. DS patients experienced a significantly higher frequency of grade 3-4 gastrointestinal toxicity than did non-DS-ALL patients (DS: 13/38 patients (34.2%) *versus* non-DS: 3/76 patients (3.9%); *P*=0.001), as shown in Table 3.

We next compared the cumulative frequencies of grade 3-4 toxicities, in this analysis including methotrexate courses 2, 3 and 4 in all patients. Despite dose reductions, children with DS still had a higher risk of cumulative grade 3-4 gastrointestinal toxicity than had non-DS-ALL patients (27/102 patients (26.5%) *versus* 8/204 patients (3.9%), respectively; *P*=0.001).

DS patients did not experience enhanced hematologic toxicity. Moreover, no difference in hematologic toxicity

was found between DS-ALL patients who did or did not receive 6-mercaptopurine during methotrexate therapy (P=0.58). The same lack of difference in hematologic toxicity was observed when comparing the non-DS-ALL controls who did or did not receive 6-mercaptopurine (P=0.74).

Neurological toxicity (grades 1-4) was reported in three DS (in 5 courses) and in three non-DS patients (in 4 courses). Grade 4 methotrexate encephalopathy, consisting in seizures, unconsciousness, and/or transient hemipareses, occurred in one DS patient (in 2 courses) and in two non-DS patients (in 1 course per patient).

Methotrexate pharmacokinetics

Figure 1 shows the observed methotrexate plasma concentrations and the diagnostic plots of the developed population pharmacokinetic model in DS-ALL and non-DS-ALL patients. The population pharmacokinetic parameters are given in Table 4. Predicted concentrations are evenly distributed around the line of unity, indicating the 'goodness of fit' of the model. For each patient the individual estimates of CL, Q, V1 and V2 were obtained by Bayesian analysis. On the basis of these parameters, individual plasma concentration-time profiles were calculated.

The two-compartment pharmacokinetic model adequately described the data, and the parameters were generally well estimated as indicated by their standard errors. Allometric normalization of clearances for weight reduced the inter-patient variability from 45% to 31%. Both inter- and intra-patient variability in clearance was moderate with values of 31% and 15%, respectively. Covariate analysis revealed that methotrexate clearance was 5% lower in DS-ALL patients than in non-DS-ALL patients (P=0.001). Median (range) post-hoc values for clearance were 4.7 (2.4–11.9) L/h and 4.9 (1.3–10.4) L/h in DS-ALL and non-DS-ALL patients, respectively; standardized values were 12.3 (7.3–18.9) L/h/70 kg and 13.0 (4.6–25.2) L/h/kg, respectively. No relationship was found between the pharmacokinetic parameters and the treatment center or treatment protocol, methotrexate dose, hyperhydration (L/m^2), number of leucovorin dosages, creatinine clearance, age, white cell

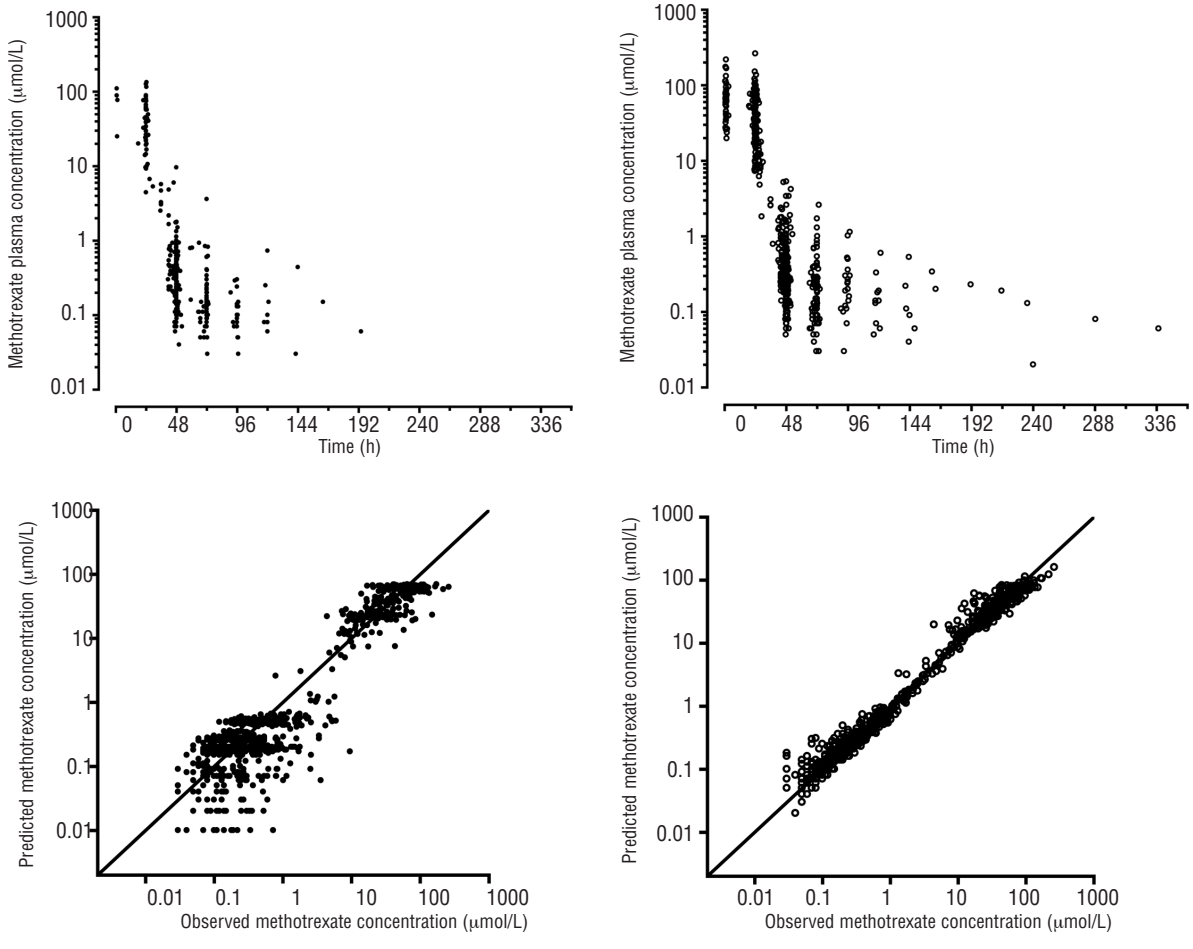

Figure 1. Methotrexate plasma levels and diagnostic plots of the population pharmacokinetic model. Methotrexate plasma levels at various time-points following the start of a 24 h methotrexate-infusion in DS-ALL: (A) n=152 courses in 44 patients and non-DS-ALL (B) n=316 courses in 87 patients. Each dot represents a plasma level in a patient measured at a given time-point. Plasma concentrations at 48 h were available for all patients whereas levels at 1 h and 24 h were only determined in some of the hospitals. In case of methotrexate levels of 48 h > 0.4 µmol/L, monitoring of methotrexate levels was continued. (C) The predicted methotrexate concentrations calculated by the NONMEM two-compartment model *versus* observed concentrations for all patients. The points are evenly distributed around the line of unity indicating the goodness of fit of the model. Deviations from the line are caused by intra- and inter-patient variability and residual variability. (D) Individually (Bayesian) predicted methotrexate concentrations *versus* observed concentrations for all patients. All dots are close to the line of unity indicating limited residual variability.

count, bilirubin or aspartate aminotransferase levels.

The 5% difference in methotrexate clearance between DS-ALL and non-DS-ALL patients is small, which is further demonstrated by the fact that no significant differences were detected in the plasma concentration of methotrexate 24 and 48 h after the start of the methotrexate infusions. At 24 h, the median methotrexate level in DS-ALL patients was 38.74 μmol/L (range, 0.38-133.11 μmol/L; 25th and 75th percentiles: 19.7–66.3 μmol/L) and that in non-DS patients was 36.49 μmol/L (range, 7.62-261.49 μmol/L; 25th and 75th percentiles: 22.8-63.5 μmol/L) (P=0.51). At 48 h, the median methotrexate level in DS-ALL patients was 0.28 μmol/L (range, 0.04-9.57 μmol/L; 25th and 75th percentiles: 0. 15–0.51 μmol/L), while that in non-DS patients was 0.27 μmol/L (range, 0.06-14.63 μmol/L; 25th and 75th percentiles 0.17–0.41) (P=0.41). After stratification for the various dosages of methotrexate that were administered to the patients (either 2, 3 or 5 g/m^2/course), again methotrexate plasma levels did not differ significantly between DS-ALL and non-DS-ALL children.

The methotrexate plasma levels were 0.4 μmol/L or higher at 48 h, which is the cut-off value used in DCOG centers for additional leucovorin rescue, in 36.4% of the methotrexate courses in DS-ALL patients, compared to in 27.7% of the courses in non-DS-ALL patients (P=0.14). No correlation was found between the methotrexate AUC (range, 276-2603 μmol/L*h) of the first course of methotrexate and grade 3-4 toxicity in the DS-ALL patients, although the number of patients in the 5 g/m^2 group was limited (Figure 2). We also did not observe a clear correlation when all subsequent courses were included. Grade 3-4 toxicity occurred both at low and high AUC, and was even seen at the lowest AUC of 276 μmol/L*h in one DS-ALL patient.

Table 3. Frequency of grade 3/4 toxicities in DS-ALL and non-DS-ALL patients after high-dose methotrexate therapy blocks.

Side effects	DS	Non-DS	*P* value
Including the 1st course only*			
Number of methotrexate courses	39	87	
Anemia	0/5	0/10	
Leukopenia	0/5	3/10	0.71
Neutropenia	0/4	4/7	0.37
Thrombocytopenia	0/4	0/8	
Neurological toxicity	1/38	1/76	0.60
Gastrointestinal toxicity (mucositis)	13/38	3/76	0.001
Cumulative toxicity –including courses 2-4			
Number of methotrexate courses	108	229	
Anemia	2/43	1/86	0.36
Leukopenia	10/43	9/86	0.06
Neutropenia	8/24	11/48	0.36
Thrombocytopenia	5/43	4/86	0.33
Liver toxicity (transaminases)	1/15	0/30	0.36
Neurological toxicity	1/102	1/204	0.60
Gastrointestinal toxicity (mucositis)	27/102	8/204	0.001

*Toxicity was graded according to the Common Toxicity Criteria for Adverse Events version 3.0. Not all toxicities could be evaluated in all subjects – as many centers did not routinely check blood values between courses. Number of grade 3-4 toxicities divided by number of measurements for the specific parameter. *Patients with dose reduction in anticipation of greater toxicity were excluded from the analysis.*

Discussion

Given the well-known reduced tolerance of methotrexate in children with DS, we performed a retrospective case-control study to determine whether the enhanced susceptibility for methotrexate-induced side-effects is not only due to the well-known difference in cellular sensitivity (for instance of the mucosa), but whether it is also the result of differences in pharmacokinetics between DS-ALL and non-DS-ALL patients.[2,16,24]

In our study, a significantly higher proportion of children with DS experienced methotrexate-induced gastrointestinal toxicity compared with the non-DS controls, which is consistent with other reports.[2,16,24,25] Dose reductions were applied both in anticipation of possible toxicity, and because of apparent excessive toxicity, and were restricted to DS patients only. However, due to excessive toxicity both DS-ALL patients (n=3) and one non-DS-ALL patient, each received one course less than required per protocol.

A two-compartment pharmacokinetic model was constructed to characterize the pharmacokinetics of methotrexate. The methotrexate clearance observed in this study was

Table 4. Population pharmacokinetic parameters for methotrexate in children with ALL.

	Estimate	SE (%)
Population parameter		
V1 (L/70kg	46	12
CL (L/hr/70kg)	13	8
θ^{Down}	0.95	5
θ^{Gender}	0.87	9
V2 (L/h/kg)	10	19
Q (L/h/kg)	0.3	31
Inter-patient variability		
V1 (%)	38	45
CL (%)	31	34
V2 (%)	74	38
Q(%)	57	51
Correlation		
V1 – CL	0.9	
V1 - V2	0.86	
CL - V2	0.63	
V1 – Q	0.72	
CL – Q	0.73	
V2 – Q	0.81	
Intra-patient variability		
V1 (%)	37	54
CL (%)	15	65
Residual variability		
Additive (μmol/L)	0.02	22
Proportion (%)	35	31

V1 and V2, central and peripheral volume of distribution, respectively; CL, clearance in male non-DS-ALL patients; θ^{DOWN}, fractional change in clearance in DS-ALL patients; θ^{GENDER}, fractional change in clearance in DS-ALL patients; Q, inter compartmental clearance; SE: standard error of the estimate.

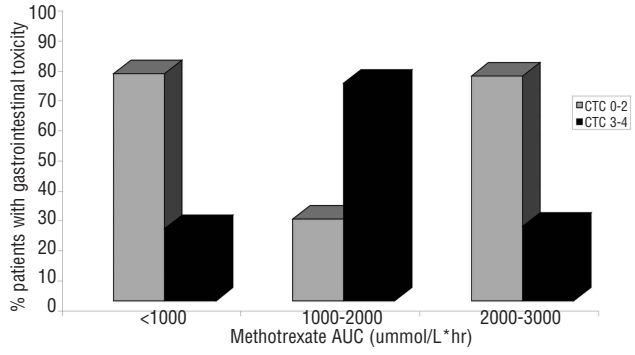

Figure 2. Correlation of methotrexate area under the curve (AUC) *versus* gastrointestinal toxicity in the first methotrexate course in DS-ALL patients only. CTC: Common Toxicity Criteria for Adverse Events. CTC grade 0-2 and grade 3-4 versus the methotrexate AUC. Number of patients per subgroup: <1000 μmmol/L*h: n=29, 1000-2000 μmmol/L*h: n=11, 2000-3000 μmmol/L*h: n=4.

concordant with that found in other studies.[26-29] For instance, Relling *et al.* reported a mean methotrexate clearance of 99.9 mL/min/m^2 (~0.149 L/h/kg) in 134 children enrolled in the St. Jude Total Therapy study XII for newly diagnosed ALL.[26] We found that methotrexate clearance was 5% lower in the DS-ALL patients than in the non-DS-ALL patients. This is only a marginal difference, and probably not clinically relevant, which is reflected by the fact that methotrexate plasma concentrations in DS-ALL and non-DS-ALL patients did not differ at either 24 h or 48 h after the start of the infusion. Altogether, we did not observe major differences in methotrexate pharmacokinetics between DS-ALL and non-DS-ALL children, which would explain the enhanced rate of side effects in DS children. The only other study regarding methotrexate pharmacokinetics in DS-ALL found significantly higher plasma concentrations in DS-ALL patients, but numbers were small (5 DS-ALL and 3 non-DS-ALL patients).[16]

We could not relate clinically severe toxicity to the methotrexate AUC, and toxicity was not restricted to DS-patients with higher plasma levels only. This suggests that the enhanced frequency of gastrointestinal side effects in the DS patients must have been related to pharmacodynamic differences of the gastrointestinal mucosa between DS and non-DS children. Several differences in methotrexate pharmacodynamics between DS and non-DS children have been reported in the literature. For instance, patients with DS have lower folate levels than control patients without DS, which may result in enhanced polyglutamylation and methotrexate-induced cell-killing.[16,30] Another plausible explanation for the observed methotrexate toxicity in DS patients could be a gene dosage effect for enzymes found on chromosome 21.[30,31] In particular, the reduced folate carrier gene (*RFC*), which is responsible for methotrexate transport over the cell-membrane, is localized on chromosome 21q22.[15,32] However, at higher concentrations passive diffusion of methotrexate across the cell-membrane may also occur.[33,34] This may explain why, in an earlier study, we could not demonstrate higher sensitivity of DS-ALL cells to methotrexate, compared to non-DS ALL cells.[32]

Furthermore, polymorphisms in genes linked to the pharmacodynamics of methotrexate, such as folate-metabolism related genes, could give rise to enhanced toxicity, as has been shown in previous studies by us and others.[35-38] Children harboring polymorphisms exhibited significantly more gastrointestinal toxicity. More knowledge on folate-related polymorphisms may contribute to further individualization of methotrexate treatment in ALL and specifically for DS-ALL patients.

It remains a challenge to advise clinicians on the right dose of methotrexate to use in DS patients. Even in non-DS-ALL patients, different protocols incorporate different dosages, and there seems to be no consensus on the best dose of methotrexate to use. In this retrospective study, a significantly higher number of DS patients were given a dose reduction in subsequent courses of methotrexate when treated with higher doses (5 g/m^2/course) than when treated with intermediate doses (1–3 g/m^2/course). Of interest, the number of DS patients requiring dose reductions due to excessive toxicity in earlier courses was 3.7% when treated with 2-3 g/m^2, and 25% when treated with 5 g/m^2. Although the number of patients in our series is limited, we feel that it may be safe to start with intermediate dosages of methotrexate, followed by careful monitoring. The fear of enhanced toxicity, however, needs to be balanced against efficacy, as DS-ALL cells are not more sensitive to chemotherapy than non-DS-ALL cells, a situation differing from that of myeloid leukemia in DS which is characterized by hypersensitivity to chemotherapy.[32,39-41]

In summary, we did not find evidence for differences in methotrexate pharmacokinetics between DS-ALL and non-DS-ALL patients, which might have explained the higher rate of grade 3-4 gastrointestinal toxicity and the greater need for methotrexate dose reductions in DS-ALL patients due to excessive toxicity in earlier courses. Hence, these differences are most likely explained by differential pharmacodynamic effects of methotrexate in tissues/organs between DS and non-DS children. Based on the clinical experience in this retrospective study, no major safety concerns were observed when using intermediate doses of methotrexate (1-3 g/m^2) in DS-ALL children, and hence this might be a safe dose to consider in future studies.

Authorship and Disclosures

TDB and CMZ designed the study. VdH contributed to the selection of patients and was responsible for the clinical data at diagnosis. TDB, RP and CMZ analyzed and interpreted the data. TDB and RAAM performed the statistical analyses. RP and VdH reviewed the manuscript. TDB and RAAM and CMZ wrote the manuscript.

The authors reported no potential conflicts of interests.

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