Methotrexate concentrations in cerebrospinal fluid and serum, and the risk of central nervous system relapse in children with acute lymphoblastic leukaemia

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The aim of the study was to characterize the relationship between the pharmacokinetics of methotrexate in serum and concentrations in the cerebrospinal fluid, and to analyse the association to risk of a central nervous system relapse in children with acute lymphoblastic leukaemia. In this retrospective study, 353 children with acute lymphoblastic leukaemia treated with high-dose methotrexate according to the Nordic Society of Pediatric Haematology and Oncology-92 acute lymphoblastic leukaemia protocol were studied. Data from 18 patients with a subsequent central nervous system relapse and 335 event-free patients were available. In 34 patients the methotrexate concentrations were monitored repeatedly during each 24-h methotrexate intravenous infusion and a cerebrospinal fluid sample was taken at the end of the infusion. Using statistics separating interindividual and intraindividual variability, methotrexate concentration in cerebrospinal fluid was found to be significantly dependent upon both serum concentrations at the end of infusion and the area under the concentration curve in serum ($P<0.0017$ and <0.002, respectively). The logistic regression analysis revealed that high patient median serum methotrexate concentrations at the end of infusion were significantly associated with decreased risk for a central nervous system relapse in the standard risk group ($P=0.02$) and the number of courses with a calculated cerebrospinal fluid concentration of more than 1 μmol/l ($P=0.048$) with a decreased risk of a central nervous system relapse in the combined (standard risk and intermediate) risk group. In conclusion, methotrexate concentrations in cerebrospinal fluid are dependent on methotrexate concentrations in serum and serum area under the concentration curve. Multivariate analysis indicates that an increased exposure to methotrexate is related to decreased risk for central nervous system relapse. *Anti-Cancer Drugs* **18:941–948 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Methotrexate (MTX) is a cytotoxic drug that has been in clinical use for decades in the treatment of many forms of neoplastic diseases, including leukaemia. The pharmacokinetics and pharmacodynamics of MTX have been studied extensively, and a concentration of 1 μmol/l MTX has been proposed as a minimum effective antileukaemic concentration [1,2]. Several factors such as urine pH (U-pH), emesis, MTX clearance, urinary output and kidney function have been found to be associated with high concentrations of MTX [3,4]. Owing to the poor penetration of MTX over the barrier between blood and the cerebrospinal fluid [CSF; in this paper called the blood–brain barrier (BBB)], the concentrations may be insufficient to eliminate leukaemic cells. This is clinically important, as the CSF MTX concentrations have been reported to be only a few percent of that in serum in children treated with systemic MTX for acute lymphoblastic leukaemia (ALL) [2,5–7].

High-dose MTX (HDMTX) was introduced, over 20 years ago, as prophylaxis against central nervous system (CNS) relapses in children with ALL [2,8] and it is generally considered able to offer antileukaemic concentrations in the CNS. Thus, HDMTX treatment is one of the most effective contemporary ALL treatment protocols; over 75–80% of children with ALL are cured [9] with a low frequency of CNS relapses [8].

The aim of this study is to characterize the relationship between MTX concentrations in serum and CSF in a subpopulation, and to apply statistics that adequately separate the interindividual and intraindividual variability, and to study the relationship between calculated

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CSF–MTX concentrations and the risk of a CNS relapse in a larger population.

Methods

The Lund subpopulation with measured concentrations of methotrexate in cerebrospinal fluid

Thirty-four children, diagnosed with ALL, participated in this open and prospective study. The children were between 1.4 and 17.7 years (median 4.4, mean 6.2 years) of age at the start of the first HDMTX treatment, 10 were females and 24 were males. Patients were included from 1994 until 1997 at the paediatric oncology unit at the University Hospital in Lund, Sweden.

According to the Nordic Society of Pediatric Haematology and Oncology (NOPHO) risk group classification [9] nine patients were classified as having standard risk (SR), nine had intermediate risk (IR), 10 had high risk (HR) and the final six had very HR (VHR) ALL. No patient had evidence of CNS manifestations at diagnosis or during the study period. Two patients had CNS relapses, approximately 3 years after the diagnosis. A CNS relapse is defined as lymphoblasts $> 5 \times 10^6$/l in the CSF. Data on MTX concentration in CSF ($MTX_{CSF\text{-}24\,h}$) and serum were available in 160 treatment courses (for details, see Table 1).

Patients and/or parents or their guardians gave informed consent. The Ethics Committee at Lund University approved the study.

The entire study population

The entire study population was subject to the same treatment schedule and risk classification as previously described in the Lund subpopulation. The major differences were that $MTX_{CSF\text{-}24\,h}$ was not assessed and that serum concentrations were not measured during the HDMTX infusion in the rest of the study population except before the end of the 24-h MTX infusion ($MTX_{24\,h}$). Data were available from 18 patients who had a CNS relapse (isolated or in combination with a relapse at another location) and from 335 patients who stayed in remission (for details see Table 2). Patients were included between January 1992 and December 1997, and were followed until 31 December 2000.

The 353 patients had similar characteristics as the rest of the patients diagnosed with ALL in Sweden, Denmark, Norway and Finland during the same period of time regarding age, sex, white blood cell count (WBC) at diagnosis, immune phenotype, number of CNS relapses and risk grouping (Table 3). Data on practically all children with ALL in the Nordic countries are registered in the central NOPHO database.

Patients in this study are from the same NOPHO-92 cohort from which results regarding kidney function and risk of delayed MTX elimination has been reported [4], and leucovorin doses and cure rate [10].

Treatment protocol

Patients were treated according to the NOPHO-92 ALL protocol.

According to the risk group classification [9] the SR patients received eight courses with 5 g MTX/m^2, IR nine courses with 5 g MTX/m^2, HR four courses with 8 g MTX/m^2 and VHR two courses with 8 g MTX/m^2. An age-adjusted dose of MTX was administered intrathecally at the end of each MTX infusion. For the SR group

Table 1 Number of patients and treatment courses in each risk group in the Lund subpopulation

Risk group	Patients		Treatment courses	
	n	%	*n*	%
SR (8)	9	27	63	39
IR (9)	9	27	54	34
HR (4)	10	29	32	20
VHR (2)	6	18	11	7
All	34		160	

HR, high risk; IR, intermediate risk; SR, standard risk; VHR, very HR. Numbers within brackets refer to number of HDMTX.

Table 2 Distribution of patients in the entire study population, in relation to risk group and relapse status

Risk group	Relapse free		CNS relapse	
	n	%*	*n*	%**
SR	125	35	6	5
IR	129	37	2	2
HR	50	14	4	7
VHR	31	9	6	16
All	335		18	

CNS, central nervous system; HR, high risk; IR, intermediate risk; SR, standard risk; VHR, very HR.
*Percentage related to the entire study population, **percentage within risk group.

Table 3 Comparison of patient characteristics for relapse-free patients and patients with a CNS relapse between all NOPHO patients and patients in this study

	NOPHO cohort (*n*=873)	This study (*n*=353)
Sex (male/female)	458/415 (52/48%)	198/155 (56/44%)
Age, median at diagnosis (p25–p75)	4 (2–7)	4 (3–7)
WBC at diagnosis	9.2 (3.9–29)	9.1 (4.0–28.9)
Immunphenotype (T/Pre B)	81/430 (9/49%)	36/192 (10/54%)
Number of CNS relapses	39 (4%)	18 (5%)
Risk group (SR/IR/HR/VHR)	300/313/154/96 (34/36/18/11%)	131/131/54/37 (37/37/15/10%)

CNS, central nervous system; HR, high risk; IR, intermediate risk; NOPHO, Nordic Society of Pediatric Haematology and Oncology; SR, standard risk; VHR, very HR; WBC, white blood cell count.

intrathecal MTX was given on 12 occasions, IR on 17 occasions, HR on 16 occasions and VHR on 12 occasions.

The entire treatment protocol has been described previously by Gustafsson *et al.* [9].

As part of the NOPHO-92 ALL maintenance therapy study, 258 of the children were randomly assigned to have their oral 6MP/MTX maintenance therapy adjusted by WBC and red blood cell levels of 6-thioguanine nucleotides (the cytotoxic metabolites of 6MP) and MTX (pharmacology group) or by WBC only (control group) as reported previously [11].

High-dose methotrexate treatment

Depending on the risk group classification the stipulated doses of MTX were 5 g/m^2 (SR, IR) or 8 g/m^2 (HR, VHR) of which 1/10 was infused over the first hour and the remaining (9/10) over the following 23 h. Intravenous hydration, glucose 5% containing 40–42 mmol/l $NaHCO_3$ and 20 mmol/l KCl, was stipulated to 3000 ml/m^2 over 24 h. The hydration was increased to 4500 ml/m^2 over 24 h if MTX 36 h after infusion start was $\geq$ 3 μmol/l.

U-pH was measured at every voiding. $NaHCO_3$ (20 mmol in the courses with 5 g MTX/m^2 and 2 mmol/kg in the courses with 8 g MTX/m^2) should be administered intravenously if U-pH was less than 7. Furosemide (0.5–1 mg/kg, maximum 20 mg) should be administered intravenously if diuresis was less than 100 ml/m^2/h. In the 5- and 8-g courses racemic folinic acid (N^5-formyltetrahydrofolic acid) was administered i.v. 36 h after infusion start in the doses 15 and 50 mg/m^2, respectively. At 39 (only 8-g courses) and 42 h additional doses of 15 mg/m^2 were given and thereafter every 6 h but increased if MTX exceeded 1 μmol/l at 42 h. Folinic acid was to be administered every 6 h until the serum MTX level went below 0.2 μmol/l.

Laboratory methods

In all patients serum MTX was measured approximately 1 h before the end of infusion ($MTX_{24\,h}$). Thereafter MTX was measured every 6 h starting 36 h after start of infusion (12 h after end of infusion), until serum MTX was below 0.2 μmol/l.

In addition to the above the study protocol for the Lund subpopulation stated that serum MTX should be measured also at 1, 4 and 6 h after start of infusion. Furthermore, a sample for analysis of MTX concentration in CSF was drawn through a lumbar puncture ($MTX_{CSF\text{-}24h}$). This was to be done at the end of the infusion, between 20 and 24 h after the start of infusion. The CSF sample was taken immediately before the intrathecal MTX administration.

MTX was analyzed using EMIT (enzyme multiplied immunoassay technique; Behring Diagnostics, Syva Business, San Jose, California, USA) or FPIA (fluorescence polarization immunoassay; Abbott Scandinavia, Solna, Sweden).

Pharmacokinetic analysis and statistical procedure

On the basis of *a priori* knowledge serum MTX concentrations were analysed using a two-compartment model [12] in WinNonlin (version 1.5; Scientific Consulting, Cary, North Carolina, USA). For each patient weighted least-squares estimations were performed using the reciprocals of the observed concentrations as weighting factor. Using the fitted model the pharmacokinetic parameters were derived.

To analyse the relationship between the pharmacokinetics of MTX in serum and concentrations in the CSF, a linear model with a mixed procedure with fixed and random effects was used to allow for dependence of multiple observations from the same patient. This approach also handles the fact that patients contribute with different numbers of courses. Furthermore, the intraindividual and interindividual variability can be handled.

The association between serum concentration of MTX and the risk of CNS relapse was analysed using logistic regression analysis with an iterative maximum likelihood approach. The analyses were carried out by risk group, as the risk for relapse, the number of treatment courses, the MTX dose and the treatment protocol differ between risk groups. In patients with CNS relapse only treatment courses before the date of relapse were included. As the relapse risk is unlikely to be constant over time, we chose to analyse the material using a logistic regression approach. The logistic regression equation defines the probability of a CNS relapse at a given concentration. First, a stepwise multiple logistic regression (with backward elimination) eliminated the least significant variables (i.e. those with a P value greater than 0.05) in subsequent steps. Second, the multiple logistic regression process was repeated with a stepwise forward selection (inclusion of variables with a P value less than 0.05). Statistical calculations were performed using the software SAS (version 6.02; SAS Institute, Cary, North Carolina, USA).

Results

The Lund subpopulation (with measured concentrations of methotrexate in cerebrospinal fluid)

Thirty-four patients received a total of 160 treatment courses. Eighteen patients in 117 courses were treated with 5 g/m^2 and 16 patients (43 courses) were administered 8 g/m^2. The MTX concentrations in both serum and CSF were highly variable with considerable interindividual and intraindividual variability. MTX concentrations in CSF at the end of infusion ranged from 0.29 to

10.5 μmol/l (median, 1.35 μmol/l) and in serum from 35.6 to 186 μmol/l (median, 86 μmol/l).

The distribution of the CSF levels is shown in Fig. 1. The MTX concentration in CSF was above the proposed concentration for effect of 1 μmol/l in 131 (82%) of the treatment courses. Mean CSF concentrations were significantly higher in treatment courses with 8 g/m^2 than in those with 5 g/m^2, 2.05 and 1.45 μmol/l, respectively ($P < 0.001$). For the CSF concentration of 10.5 μmol/l, the original data about the handling in the laboratory were traced, but no reason to withdraw the sample was found and it was therefore not excluded.

Among those treated with 5 g/m^2, nine out of the 18 patients had a CSF concentration of MTX over 1 μmol/l in all their treatment courses, whereas this was the case for 14 out of 16 of the patients treated with 8 g/m^2. Of the 34 patients in the study only one patient failed to achieve 1 μmol/l in at least one treatment cycle. This patient did, however, not have a CNS relapse.

The mean ratio between the MTX concentration in CSF and serum at the end of infusion was 0.018 (range 0.002–0.12). Trend to a decreasing (or increasing) ratio with increasing number of treatment courses was not found (data not shown).

Through a pharmacokinetic fitting procedure the area under the concentration time curve (AUC) for MTX in serum was estimated. The AUCs were found to be highly variable (median, 215; range, 81–479 μmol/l × h), but were strongly correlated to the MTX concentration in serum at the end of infusion ($r^2 = 0.80$, $P < 0.0001$).

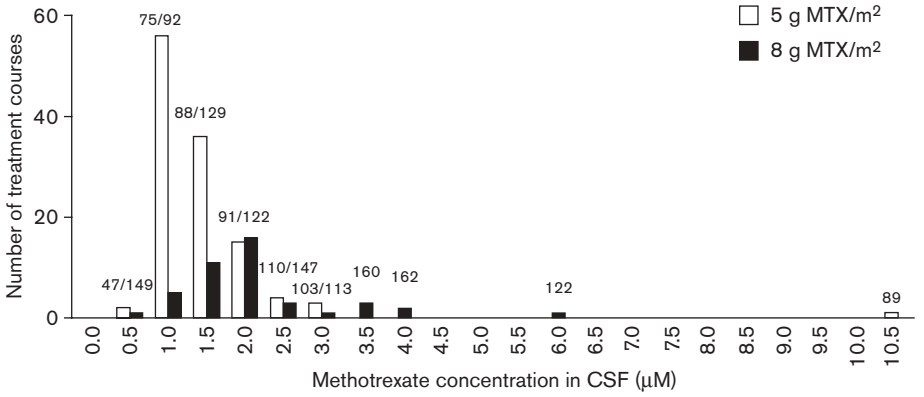
Analyses with a mixed procedure (fixed and random effect) demonstrated that the MTX concentration in CSF was significantly dependent upon both the serum concentration of MTX at the end of infusion ($MTX_{24\,h}$) and serum AUC ($P < 0.0017$ and < 0.002, respectively). The following relationships were found: [$MTX_{CSF\text{-}24\,h}$] μmol/l = 0.78 μmol/l (standard error 0.26) + 0.0091 (standard error 0.0027) × $MTX_{24\,h}$ and [$MTX_{CSF\text{-}24\,h}$] μmol/l = 0.63 μmol/l (standard error 0.25) + 0.0043 (standard error 0.0010)/h × AUC μmol/l × h. For details see Fig. 2. The results were similar and remained significant when individual mean MTX concentrations in CSF and serum (MTX-end) for all the patients were included in a linear regression analysis (data not shown). Correlation between the mean CSF concentrations and age was not found (data not shown).

The entire study population

To explore the risk of a CNS relapse in relation to the CSF MTX concentration we calculated the number of courses estimated to be above 1 μmol/l in CSF based on the relationship identified between $MTX_{24\,h}$ and $MTX_{CSF\text{-}24\,h}$ in the Lund subpopulation. In addition, the participants' minimum, maximum, median and average MTX serum concentration at the end infusion were calculated for each individual. These five factors together with the serum concentration at the end of infusion in the first treatment course were considered in the multiple logistic regression analysis.

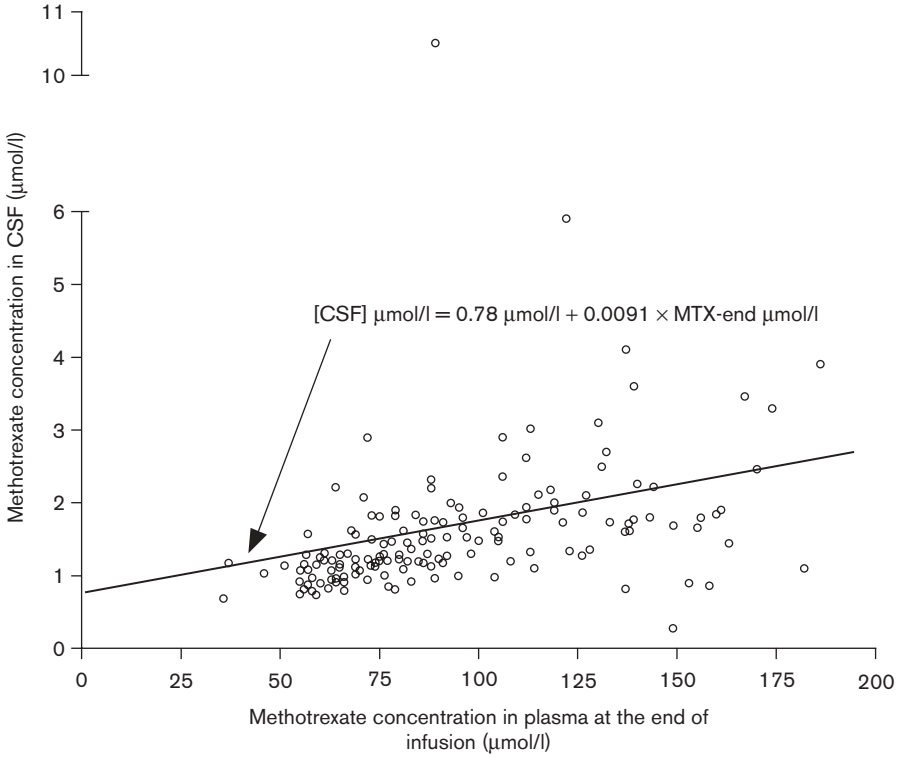
Increased median serum MTX concentration at the end of infusion was significantly correlated to a decrease in the risk of a CNS relapse in the SR group ($P = 0.02$); this association was not significant in any of the other risk groups (HR, $P = 0.6$; VHR, $P = 0.9$; IR was not tested as there were only two CNS relapses in this group), for more details, see Table 4. None of the other factors tested showed a significant correlation to the risk of a CNS relapse.

Fig. 1



Histogram showing the frequency distribution of the MTX concentrations in the CSF ($MTX_{CSF\text{-}24\,h}$) in 160 treatment courses in the Lund subpopulation. Numbers above bars depict mean serum MTX concentrations: 5 g/8 g MTX/m^2. CSF, cerebrospinal fluid; MTX, methotrexate.

Fig. 2



Multiple logistic regression analysis of the MTX concentrations in the CSF ($MTX_{CSF\text{-}24\,h}$) and serum at the end of infusion ($MTX_{24\,h}$) in the Lund subpopulation. CSF, cerebrospinal fluid; MTX, methotrexate.

To increase the power of the logistic regression, we combined the lower-risk patients (SR + IR group) and the higher-risk patients (HR + VHR) in a risk group stratified analysis. Multiple logistic regression with forward stepwise selection revealed that an increased number of courses with a calculated CSF level > 1 µmol/l was significantly associated ($P = 0.048$) with a decreased risk of a CNS relapse in the combined lower- but not in the higher-risk group. None of the other factors were significantly correlated to the CNS relapse risk.

With a stepwise backward elimination procedure, no significant correlations were found.

Discussion

This study has shown that in children treated with HDMTX for ALL the $MTX_{CSF\text{-}24\,h}$ is dependent on both serum $MTX_{24\,h}$ and serum AUC. Systemic exposure can thereby be connected in a quantitative manner to concentration levels in the CSF. Furthermore, findings indicate that an increased exposure to MTX is related to a decreased risk for a CNS relapse (isolated or in combination with a relapse at another location).

Table 4 Median S-MTX (µmol/l) in the entire study population, in relation to risk group and relapse status

Risk group	Relapse free		CNS relapse	
	S-MTX	SD	S-MTX	SD
SR	87	22	65	26
IR	78	21	83	16
HR	132	40	141	14
VHR	146	30	145	39

CNS, central nervous system; HR, high risk; IR, intermediate risk; SR, standard risk; VHR, very HR; S-MTX, serum methotrexate.

In this study 34 patients were included in the subpopulation. With this sample size the statistical power to detect important differences is of course low and it was therefore not considered feasible to correlate the CSF levels with physiological parameters or patients' characteristics. The nature of this retrospective study implies that the analyses are post-hoc analyses.

For ethical reasons, it is difficult to perform studies in a paediatric population. In the treatment protocol an intrathecal administration of MTX was stipulated at the end of HDMTX infusion and a sample from the CSF was taken immediately before this administration. It was not considered reasonable to do another lumbar puncture at a

later time point although it would have added valuable information. Therefore, it is not possible to estimate the time course (AUC) of MTX in the CSF in this study.

In this study the statistical analysis of the Lund subpopulation was performed using mixed linear regression with fixed and random effect. This method allows separation of the two sources of variability and the fact that patients contribute with different numbers of courses. Furthermore, individual regression lines can have different slopes and intercepts around a population mean. To our knowledge, this is the first study in which this kind of statistics has been applied in this patient population.

Although the included patients are of different age they represent a fairly homogeneous population as they are all treated for the same disease (ALL) according to the same protocol (NOPHO-92). Sources of variability in the Lund subpopulation are probably further reduced as all patients in the study are treated at the same centre.

The mean ratio between MTX concentrations at steady state in CSF ($MTX_{CSF\text{-}24\,h}$) and serum ($MTX_{24\,h}$) was 0.018 (1.8%) in this study. This is well in agreement with others who have found values between 1 and 3% [2,5–7,13].

Conflicting reports exist regarding whether MTX levels in the systemic circulation are correlated to concentrations in the CSF or not. Milano *et al.* [5] did not find a correlation when treating 58 patients with 5 g/m^2, neither did Lippens and Winograd [14] administering 3 g/m^2 to 25 children with lymphoid malignancies. Seidel *et al.* [7], however, found a weak but statistically significant correlation between steady-state MTX concentrations in serum and in CSF in the dose range of 6–8 g/m^2. In a much broader dose range (0.5–33.6 g/m^2), Borsi and Moe [2] found a highly significant correlation. In none of the studies was statistical analysis used that could separate the intrapatient and interpatient variability. An interesting observation is that in the two studies in which no correlation was found a fixed dose per square metre was applied to all patients. In the other two, and the present, the patients received doses over a dose range. With a dose range the MTX concentration, both in serum and CSF, will fall in a broader range and the likelihood of finding a statistical correlation will increase. Evans *et al.* found, however, a significant correlation in a study with 29 children receiving 1 g/m^2. Patients contributed with only one course each and the courses could therefore be considered independent from each other [13]. The relationship at serum MTX concentrations in the range of 5.4–33.7 μmol/l was calculated as follows, [CSF] μmol/l = 0.17 μmol/l + 0.006 × [MTX-end] μmol/l. In this study both the slope factor and the intercept were higher than in the study by Evans. A major difference between the studies is that the serum concentrations in this study were considerably higher and included a broader concentration range, 35.6–186 μmol/l.

To reach a MTX concentration over 1 μmol/l in CSF has been suggested as a goal for HDMTX by several authors [1,2,5]. In this study the proposed cytotoxic level was reached in 82% of the courses. Only one patient failed to achieve a CSF concentration of 1 μmol/l in at least one cycle. In the study by Borsi and Moe all children achieved the target concentration in at least one course. The doses were, however, slightly higher (6–8 g/m^2) than in this (5–8 g/m^2) study.

Some authors [2,7] have reported that the CSF/serum ratio for MTX is decreasing with increasing numbers of courses. A proposed explanation is that in the beginning of a treatment schedule MTX leaks into the CSF through subclinical leukaemic infiltrations in the BBB. With increasing numbers of treatment courses, the lesions will heal and diminish the leakage.

Tetef *et al.* [15] studied 13 patients with meningeal carcinomatosis from solid tumours that were treated with intravenous HDMTX and compared them with three patients without CNS manifestations. Levels of MTX in the CSF were found to be higher in the patients with CNS disease than in the three patients without meningeal engagement. These findings indicate that neoplastic engagement of the BBB can increase the amount of drug that enters the CSF. Milano *et al.* [5], however, found no trend indicating a change in the ratio with increasing number of courses. In contrast to patients in the study by Tetef *et al.*, there was no patient with evidence of CNS disease at the time of MTX treatment in the study by Milano *et al.* The findings in this study are in agreement with the study by Milano *et al.*, both in terms of absence of change in CSF/serum ratios and in patients lacking evidence of CNS involvement. In the Lund subpopulation one patient treated with 5 g/m^2 and one treated with 8 g/m^2 later had CNS relapse. Both patients had a slightly lower average MTX concentration in CSF (1.40 and 1.59 for 5 and 8 g/m^2, respectively) compared with the average in the cohort (1.45 and 2.05 for 5 and 8 g/m^2, respectively).

In this study MTX is administered both as an intravenous infusion and as an intralumbar injection. MTX concentrations in CSF have been show to be as high as 100 μmol/l [16] and highly variable (0.6–22 μmol/l) after intralumbar injection of 6.25 or 12.5 g/m^2 [17], this is orders of magnitude above CSF MTX after intravenous HDMTX. Intra-CSF drug administration, however, results in a non-uniform distribution throughout the subarachnoidal space. Intrathecal administration gives high lumbar drug concentrations but low ventricular and vertex concentrations [18]. Not unexpectedly, the need for intrathecal treatment with

MTX has been questioned [19]. In the study by Glantz *et al*., cytotoxic CSF levels were maintained longer after HDMTX treatment than after intrathecal administration but peaks were lower. Furthermore, the patients with neoplastic meningitis who were treated with HDMTX had a significantly longer median survival compared with those treated with intrathecal MTX.

In this study, we show that increased median serum MTX concentration at the end of infusion was significantly associated with a decrease in the risk of a CNS relapse in the SR group. The median is less susceptible to outliers than the average, and the minimum and maximum values are by definition the most extreme values observed. The median value is thereby perhaps the value that best reflects the consistency in MTX concentration in the CSF over the treatment courses. Furthermore, in the combined risk group SR + IR (same MTX doses) an increased number of courses with a calculated CSF level > 1 µmol/l was significantly associated ($P = 0.048$) with a decreased risk of a CNS relapse. This indicates that it is important to ensure an adequate MTX exposure in as many HDMTX courses as possible. The findings in this study are in agreement with previous results from NOPHO where the risk of a relapse, irrespective of site, was shown to decrease with increasing serum MTX concentrations in the SR group [10].

In the treatment of HR or VHR only four and two courses, respectively, were administered compared with the eight in SR and nine courses in IR. This may partly explain why no significant association between MTX concentrations and risk of CNS relapses was seen in HR or VHR despite a relatively high incidence of events.

It must, however, be pointed out that the CSF concentrations in the entire material are only calculated from the equation identified in the Lund subpopulation. This means that the actual concentration in the CSF is not known for the individuals in the entire population, but estimated. Still it is reasonable to assume that the equation is valid in both populations.

The findings in this study suggest that patients with a high exposure of MTX have a decreased risk of a CNS relapse. This is in line with the result from a recently published meta-analysis about CNS-directed therapies for childhood lymphoblastic leukaemia [20]. By adding intravenous MTX to long-term intrathecal therapy, or radiotherapy with intrathecal therapy, both CNS and non-CNS relapses were reduced although the effect on CNS relapse did not reach statistical significance ($P = 0.08$). Little information is, however, available about the effect of the different systemic doses and it is concluded that the available data are insufficient to demonstrate whether higher intravenous doses (increased systemic exposure) are of benefit to the patients. In the meta-analysis, studies with doses between 0.5 and 8 g/m^2 were included. As the meta-analysis points in the direction that the addition of intravenous MTX reduces the risk of a CNS relapse and this study suggests that an increased systemic exposure may do the same; it cannot be excluded that higher doses reduce the relapse rate more than lower. Protocols that instead use a more frequent administration of prophylactic intrathecal MTX may, however, not see the same benefit from the addition of intravenous HDMTX.

In this study, we have identified a relationship between MTX in serum and CSF by using statistics that handle the interpatient and intrapatient variability. Applying this relationship to a larger population has shown that an increased exposure to MTX is related to a decreased risk of a CNS relapse. This study provides information about the relationship between MTX concentration in serum and CSF, and effects of MTX exposure on CNS relapses. The clinical value of the findings need, however, to be confirmed in prospective clinical trials.

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