Aspects on optimisation of High Dose Methotrexate treatment in children with Acute Lymphoblastic Leukaemia

Jönsson, Peter

Published: 2008-01-01

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Aspects on optimisation of
High Dose Methotrexate treatment
in children with
Acute Lymphoblastic Leukaemia

Peter Jönsson, M.D.

Clinical and Experimental Pharmacology
Department of Laboratory medicine
Faculty of Medicine

Lund University
Sweden, 2008
To Sofia,

Holger and Ebbe
“The pendulum of mind oscillate not between right and wrong but between sense and non-sense”

Carl Jung
(1875 - 1961)
CONTENTS

ORIGINAL PAPERS ........................................................................................................... VII

ABBREVIATIONS .......................................................................................................... IX

GENERAL BACKGROUND ............................................................................................. 1

INTRODUCTION ............................................................................................................. 1

METHOTREXATE ............................................................................................................ 2

History..................................................................................................................... 2

Pharmacology........................................................................................................... 3

Pharmacokinetics................................................................................................. 3

Clinical use and side effects.................................................................................. 4

CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)................................. 5

TREATMENT OF CHILDHOOD ALL .......................................................................... 5

Treatment overview............................................................................................. 5

The NOPHO-92 ALL treatment protocol............................................................... 6

SPECIFIC BACKGROUND........................................................................................... 9

HIGH DOSE METHOTREXATE (HDMTX)................................................................. 9

Evolution towards HDMTX.................................................................................... 9

HDMTX in contemporary treatment protocols...................................................... 10

ASPECTS ON DOSE OPTIMISATION OF HDMTX TREATMENT .................. 11

Introduction............................................................................................................ 11

Dose modification............................................................................................... 12

Adaptive strategies.............................................................................................. 12

Population Pharmacokinetics............................................................................. 13

AIMS OF THE THESIS............................................................................................... 15

METHODS ..................................................................................................................... 17

SUBJECTS ..................................................................................................................... 17
This thesis is based on the following papers, referred to by their Roman numerals:


III. Jönsson, P., Skärby, T., Heldrup, J., Schrøder, H., Höglund, P.
High dose Methotrexate treatment in children with acute lymphoblastic leukaemia may be optimised by a weigh-based dose calculation. 
(Manuscript)

Published papers are reprinted with kind permission from the copyright holders.
ABBREVIATIONS

6-MP  6-mercaptopurine
7-OHMTX  7-hydroxymethotrexate
ALL  acute lymphoblastic leukaemia
ALT  alanine aminotransferase
AUC  area under the curve
BSA  body surface area
CL  clearance
CNS  central nervous system
CR  creatinine ratio
CSF  cerebrospinal fluid
DAMPA  2,4-diamino-N10-methylpteric acid
EMIT  enzyme multiplied immunoassay technique
FPIA  fluorescence polarisation immunoassay
GFR  glomerular filtration rate
HDMTX  high dose methotrexate, > 1g/m2
HR  high risk
IgG  immunoglobulin G
IR  intermediate risk
LSA2L2  treatment protocol for childhood NHL
LV  leucovorin (5-formyltetrahydrofolate, folinic acid)
MTX23  methotrexate concentration 23 hours after start of infusion
NHL  non-Hodgkin lymphoma
NONMEM  non-linear mixed effect models
NOPHO  nordic society of paediatric haematology and oncology
NOPHO-92 ALL  NOPHO treatment protocol for childhood ALL (1992)
S  serum
SE  standard error
SR  standard risk
VHR  very high risk
WBC  white blood cell count
GENERAL BACKGROUND

INTRODUCTION

Already in the 16th century Paracelsus (1493-1541) said, “It is the dose that makes the poison”. This relationship is now known as the dose-response relationship. The relationship may be found with most drugs but oncology drugs have in general a narrower therapeutic index than drugs in common. This means that a minor change in dose may either result in severe toxicity, if increased, or poor anti neoplastic effects, if decreased. In both instances the consequences may be life threatening.

A way to delve further into the event that follows the drug administration is to study the concentration-response relationship in pharmacokinetic models. Pharmacokinetic models are however only means to an end as the survival with minimal toxicity is the ultimate aim with the treatment.

Childhood ALL has a unique place in the history of oncology, as it was the first cancer to be cured by drugs. It is therefore an important model upon which concepts of chemotherapy in other malignancies have been developed. Methotrexate is a major component of most contemporary ALL treatment protocols. The studies included in this thesis have investigated how concentration time data obtained after high dose methotrexate intravenous infusions (HDMTX) relate to renal toxicity and relapse risk in childhood ALL.
**METHOTREXATE**

**HISTORY**

Methotrexate (Figure 1) has been in clinical use for decades but the history of methotrexate begins with Aminopterin.

The first synthesis of Aminopterin was reported by Seeger in 1947 with the aim to develop a folate agonist. It was short after shown that Aminopterin had cytotoxic effects in mice and by Farber that it induced remission in childhood leukaemia. The success in the treatment with Aminopterin was however associated with severe toxicity such as stomatitis. In 1948 methotrexate, which is chemically related to Aminopterin, was described and shown to be more effective in animal studies and the clinical interest in methotrexate soon arose.

Although, the first synthesis of methotrexate was done over 50 years ago it is still a clinically widely used drug. It is effective in both autoimmune diseases such as rheumatoid arthritis and psoriasis and in the treatment of malignant diseases such as leukaemia, breast cancer and head and neck carcinoma. Intravenous administration of HDMTX with delayed LV rescue is given in the treatment of e.g. childhood ALL, osteosarcoma and malignant lymphoma of the CNS.

![Figure 1. Structure of methotrexate](image-url)
**PHARMACOLOGY**

Methotrexate is an antimetabolite inhibiting dihydrofolate reductase blocking the reduction of dihydrofolate to tetrahydrofolic acid. Depletion of tetrahydrofolic acid leads to a decreased thymidylate and purine biosynthesis, resulting in a decreased DNA synthesis. Accordingly, the cytotoxic effects occur primarily during the S-phase of the cell cycle.

The main metabolic effects come from methotrexate being polyglutamated inside the cell, where up to five glutamates are added. The relative difference in polyglutamate formation in normal versus malignant cells may account for the selective activity of the drugs. Methotrexate polyglutamates are retained longer in the cell and may provide a mechanism by which methotrexate polyglutamates may produce greater toxicity. HDMTX treatment achieves higher methotrexate polyglutamate concentrations than lower doses and is associated with higher antileukaemic effect.

**PHARMACOKINETICS**

Methotrexate is well absorbed after oral doses up to 40 mg/m² via the reduced folate active-transport system. At higher doses the gastrointestinal absorption is decreased. As doses of over 1000 mg/m² is implicated in the HDMTX treatment of ALL the drug is then administered as an intravenous infusion.

Methotrexate is a weak acid and 60% is bound to plasma proteins. The volume of distribution is approximately that of total body water. Methotrexate enter cell via an energy dependent folate transport process. At high systemic concentrations a passive diffusion of methotrexate into cells may also take place. Only a minor fraction distribute into the CSF, reaching a few percent of systemic concentrations.

Methotrexate is primarily excreted unchanged in the urine via glomerular filtration and by active secretion in the renal tubule. Studies in monkeys suggest that clearance is chiefly determined by renal tubular function at lower concentrations (0.1 - 3.7 μM) and by glomerular filtration rate at higher levels (13 - 70 μM). At least two elimination phases can be identified. In patients treated with HDMTX no
less than two metabolites (7-OHMTX and DAMPA) have been identified. During the initial elimination phase 7-OHMTX is the main metabolite identified.

**CLINICAL USE AND SIDE EFFECTS**

Methotrexate is used in non-oncology diseases such as severe psoriasis, rheumatoid arthritis and severe juvenile chronic arthritis. In these indications methotrexate is mostly used as a single agent administered as oral tablets. In general oral doses between 7.5 and 25 mg/m²/week are used in these conditions.

Methotrexate is a key drug in the treatment of ALL but is also effective, as previously mentioned, in the treatment of high-grade non-Hodgkin’s lymphoma, breast carcinoma, gastric carcinoma, choriocarcinoma as well as in head and neck carcinoma. Methotrexate is then often used in combination therapy with other drugs and can be administered through a variety of routes: oral, intravenous and intrathecal. The doses that are used in the treatment of neoplastic disease are often significantly higher than in the treatment of autoimmune disease. Thus, both route of administration and doses may differ significantly between oncology and non-oncology indications.

The toxicity seen during treatment with methotrexate is dose dependent. After low dose therapy the most common adverse reactions are haematopoietic and hepatotoxic. After HDMTX the toxicity is potentially life threatening especially in patients with renal dysfunction. HDMTX are routinely followed by a delayed intravenous infusion of LV that will rescue the cells from the impact of methotrexate. The most common adverse reactions after HDMTX treatment are myelosuppression, oral and gastrointestinal mucositis. Renal toxicity may be seen after HDMTX treatment and a suggested mechanism is precipitation of 7-OHMTX in the renal tubuli. After intrathecal administration neurotoxic reactions can occasionally be seen.
CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

The precise pathological events leading to the development of ALL are unknown, but they are likely to affect genes that control lymphoid cell homeostasis, resulting in deregulated clonal lymphoid cell expansion of immature progenitor cells. The diagnosis of ALL is based on immunophenotyping. There is no consensus on how to use known risk factors for relapse such as age, leukocyte count and genotype, for treatment stratification. Low systemic methotrexate exposure has been associated with poor treatment outcome, indicating under-dosing as the cause of treatment failure in some patients rather than drug resistance.

The annual incidence of childhood ALL in the Nordic countries is approximately 4.0 cases per 100,000 child year. This makes ALL the most common cancer in children and adolescents. There is a peak incidence around 2-5 years of age with most cases being B-precursor ALL. It is slightly more common in boys than in girls.

The cure rate is about 80%, with the Nordic countries having the highest survival rate in Europe: 85%. This is most likely related to the NOPHO initiative, starting in 1981, to use uniform treatment protocols and monitor effects. Patients who have not received radiation therapy and have attained 10 or more years can expect a normal long-term survival. Of note is that the term “cure”, in the context of childhood ALL, first appeared in 1971.

TREATMENT OF CHILDHOOD ALL

TREATMENT OVERVIEW

In the 1960s it was discovered that the combination of prednisone and vincristine could induce remission in children with ALL. Relapses of the CNS were however common but it was later shown that 6-MP and methotrexate were able to prolong the duration of complete remission. In an attempt to prevent late relapses of the CNS cranial irradiation and prophylactic administration of intrathecal methotrexate were added to the treatment. L-asparaginase and adriamycin were also
included in the treatment during the first 6 months of remission. Contemporary treatment protocols often cover 2 to 3 years of treatment\textsuperscript{74}.

Most treatment protocols stratify the patient treatment according to risk groups but there is no universal agreement on the definition of the different risk groups. The treatment protocols are usually complex but can generally be divided in an initial remission induction phase, followed by consolidation therapy and then long-term maintenance treatment\textsuperscript{75}. HDMTX treatment is a part of the consolidation and maintenance therapy.

The design of the treatment protocols are rather empirically based than a result of a truly scientific process\textsuperscript{22}. It is, however, the fine-tuning of the use of available drugs and the continuous improvement in supportive care that has enabled the constant improvement of outcome\textsuperscript{35,59,78}. Psychosocial support to patients and their families are also important parts of the management\textsuperscript{8}.

**The NOPHO-92 ALL Treatment Protocol**

Data on practically all children with ALL in the Nordic countries are registered in a central NOPHO database. This registration was started in 1981 and includes all children with ALL below 15 years of age\textsuperscript{43}. In this database, basic demographic and prognostic information and follow up data are gathered. Repeated comparisons with the mandatory Swedish Cancer Register have shown that the voluntary paediatric registration is virtually complete\textsuperscript{47}. In the Nordic countries, NOPHO has implemented common treatment protocols.

In the NOPHO-92 ALL treatment protocol patients were classified in risk groups determined by age and WBC (SR, 2-10 years, WBC < 10\textsuperscript{9}/L; IR 1 - < 2 years or \geq 10 years and/or WBC = 10-49 \times 10\textsuperscript{9}/L; HR/VHR, WBC \geq 50 \times 10\textsuperscript{9}/L.)\textsuperscript{44}. Treatment for each patient was determined by the risk group classification. A comprehensive treatment overview has been published elsewhere\textsuperscript{44}. The main features of the protocol were as follows:
According to the risk group classification the SR patients received 8 courses and IR 9 courses with methotrexate 5 g/m$^2$, HR 4 courses and VHR 2 courses with 8 g/m$^2$. An age-adjusted dose of methotrexate was administered intrathecally at the end of each methotrexate infusion. For the SR group intrathecal methotrexate was given on 12 occasions, IR on 17 occasions, HR on 16 occasions, and VHR on 12 occasions.

Induction therapy consisted of prednisone (60 mg/m$^2$/day divided into three doses on days 1-36, then tapering), vincristine (2.0 mg/m$^2$ days 1, 8, 15, 22, 29, and 36), doxorubicin (40 mg/m$^2$ days 1, 22, and 36 (+day 8 for HR and VHR-ALL)), asparaginase (30,000 IU/m$^2$ days 37 to 46), and intrathecal methotrexate (days 1, 8, 15, and 29).

Consolidation therapy depended on risk group and included 3 times HDMTX for SR-ALL, whereas patients with IR- and HR-ALL received alternating series of (a) cyclophosphamide (total cumulative dose: 3 g/m$^2$) with low-dose cytarabine and oral 6-MP or 6-thioguanine, (b) 4 times HDMTX with oral 6-MP (only IR) or 2 times HDMTX and high-dose cytarabine (12 g/m$^2$, only HR and VHR), (c) 4 weeks of reinduction with dexamethasone (10 mg/m$^2$/day divided in three doses for 4 weeks then tapering), weekly vincristine (2.0 mg/m$^2$/day times 4), weekly daunorubicin (30 mg/m$^2$/day, times 3 (HR and VHR) or 4 (IR)), and 4 times asparaginase (30,000 IU/m$^2$ at 3-4 days intervals), and (d) (only HR and VHR) two 2 months interval periods of oral weekly methotrexate and daily 6-MP with 2 vincristine/prednisone reinductions per period.

Maintenance therapy with starting methotrexate doses of 20 mg/m$^2$ and 6-MP doses of 75 mg/m$^2$ was initiated 13 weeks (SR), 32 weeks (IR), or 63 weeks (HR) after diagnosis and continued until 2 years (IR, HR and VHR) or 2.5 years (SR) after diagnosis. The doses of methotrexate and 6-MP were titrated to obtain a WBC of 1.5-3.5x10$^9$/L. During the first year of maintenance therapy, patients with SR- and IR-ALL received alternately either pulses of vincristine (2.0 mg/m$^2$ x1) and prednisone (60 mg/m$^2$/day for 1 week) or HDMTX (5 g/m$^2$) every 4th week until 5 courses of each had been administered. Patients with HR received vincristine (1.5 mg/m$^2$x1) and prednisone (40 mg/m$^2$/day for 1 week) pulses with intrathecal methotrexate and
2 HDMTX courses. As maintenance therapy, patients with VHR received a maximum of 6 courses of truncated LSA2L2 regime, however, until 2 years after diagnosis\textsuperscript{34}.
SPECIFIC BACKGROUND

HIGH DOSE METHOTREXATE (HDMTX)

EVOLUTION TOWARDS HDMTX

Both the methotrexate concentration and time of exposure seem to be of importance for the effect of methotrexate. The rational of administering high doses of methotrexate are both to overcome drug resistance and to reach out to sanctuary sites, such as the testis and CNS. The suggested mechanism is that methotrexate at high doses enter cells not only by active transport but also by passive diffusion. Another reason to introduce HDMTX has been that cranial radiation, used as prophylactic treatment, caused serious adverse effects on the brain and alternative treatments were looked for.

In the mid 60s Goldin, in pioneering studies, developed the idea of methotrexate administration with a delayed administration of LV. This prevented the methotrexate induced toxicity without diminishing the antileukaemic effect of the drug. This concept made it possible to administer much higher doses of methotrexate than previously without unacceptable toxicity.

In the late 60s doses up to 240 mg/m²/24h were administered and in the 70s doses of 500 mg/m² were explored. In the 80s Borsi et al administered dose up to 33.6 g/m²/24 h in an attempt to increase methotrexate exposure to the CNS in ALL patients with relapse.
**HDMTX IN CONTEMPORARY TREATMENT PROTOCOLS**

High dose methotrexate is generally considered able to offer antileukaemic concentrations in the CNS. Thus, HDMTX treatment has been a part of most contemporary ALL protocols\(^44,78\) with a low frequency of CNS relapses\(^46\). Most contemporary treatment protocols contain HDMTX treatment in the dose range of 5-8 g/m\(^2\)\(^66\).

Several factors such as urinary pH, emesis, methotrexate clearance, urinary output and kidney function have been found to be associated with high concentrations of methotrexate\(^58,82\). A suggested mechanism for delayed elimination is precipitation of 7-OHMTX in the renal tubule causing renal dysfunction\(^54,55,98\). To prevent tubular precipitation of 7-OHMTX most HDMTX protocols stipulate alkalisation and standardised hydration to keep urinary pH and diuresis high. Methotrexate concentrations achieved are related to the level of hydration\(^34,39,85\). An increased urinary pH significantly elevates renal clearance of methotrexate\(^86\). Reduced methotrexate concentrations, more vigorous hydration and alkalisation may reduce toxicity\(^20,82\). A general feature of many HDMTX protocols is therefore to augment sodium bicarbonate and hydration treatment upon indications that methotrexate elimination will become delayed.

Due to the poor penetration of methotrexate over the barrier between blood and the CSF, the concentrations may occasionally be insufficient to eliminate leukemic cells. This is clinically important, since the CSF methotrexate concentrations have been reported to be only a few percent of systemic concentrations in children treated with HDMTX\(^13,64,90,102\).

The definition of HDMTX has varied over time. In earlier studies doses of 5 - 50 mg/m\(^2\) were regarded as normal and > 100 mg/m\(^2\), as high. Today it is reasonable to consider >1 g/m\(^2\) as HDMTX. These doses result in high systemic methotrexate concentrations and require administration of LV to prevent life threatening toxicity\(^2\). In addition to LV therapy, the HDMTX treatment requires intravenous fluid hydration, alkalisation of urine and monitoring of methotrexate and creatinine concentration to prevent life threatening toxicity\(^66\).
ASPECTS ON DOSE OPTIMISATION OF HDMTX TREATMENT

INTRODUCTION

Since ancient times physicians have adjusted the dose of a drug according to the characteristics of the individual being treated and the response obtained. Dose adjustments are extra difficult when toxic effects cannot be detected until they are severe or irreversible. Accordingly, the optimal way to dose most cytotoxic drugs remains to be defined and the widespread use of BSA for normalisation of these drugs have been questioned. This is also true for HDMTX in the treatment of childhood ALL. Population pharmacokinetics and the evaluation of biomarkers for delayed elimination of methotrexate offer a more scientific approach to optimise HDMTX therapy.

Although death due to acute toxicity from HDMTX is nowadays extremely rare high systemic methotrexate concentrations have been associated with increased toxicity and delayed elimination. Not only methotrexate concentration but also time of exposure is of importance for both toxicity and antileukaemic activity. A concentration of $1\mu$M methotrexate has been proposed as a minimum effective antileukaemic concentration.

Several studies have indicated that ALL patients with a higher systemic clearance of methotrexate or a low systemic methotrexate exposure have worse outcome. Furthermore, it has been shown that with an individualised treatment approach it was possible to improve the outcome in children B-lineage ALL. Thus, the inter-individual variation in exposure to methotrexate is clinically important and attempts to individualise the dose are a key issue.

In man a transient decrease of GFR may be induced by HDMTX. With longer time to follow up GFR may, however, not be attenuated. Although methotrexate clearance has been reported to be correlated to GFR, glomerular function, at start of the methotrexate infusion, may only explain a small part of the variability in methotrexate clearance.
High LV doses increase the risk for relapse despite the fact that doses were correlated with high methotrexate levels and longer methotrexate elimination time. The choice of methotrexate and LV doses may be regarded as an intricate balance between effect and counter effect and studies determining the minimal necessary dose of LV to counteract the methotrexate toxicity are needed.

**DOSE MODIFICATION**

Traditionally the dosing of most anti cancer drugs, including methotrexate, is individualised according to body surface area (BSA). Modification according to body size is especially indicated when it varies greatly, as during growth, but the quest for other ways to tailor doses is also desired since much of the variability is still unexplained. Although BSA dose modifications may be useful to predict a safe starting dose in the first human studies with a new chemical entity it is not clear why this approach has been extended to patients. Furthermore, very few physiological factors relevant to pharmacokinetics are related to BSA.

There are however examples where BSA based dosing strategies have been abandoned. As an illustration dosing of Carboplatin are adjusted to GFR. This evolution has also been true for intrathecal methotrexate administration where dose normalisation according to BSA has been abandoned in favour of an age adjusted dosing. In addition, WBC guides oral methotrexate therapy during maintenance treatment for childhood ALL.

**ADAPTIVE STRATEGIES**

**THERAPEUTIC DRUG MONITORING**

After the introduction of LV rescue high doses of methotrexate could be administered with reduced toxicity. A factor complicating the use of HDMTX is that the elimination of methotrexate can be profoundly delayed. The methotrexate levels are routinely used to guide dosing of LV and are therefore regularly assessed in clinical practice.

In the NOPHO-92 protocol serum methotrexate level of 3 μM at 36 hours after start of methotrexate infusion is a ”cut-off” level for increasing hydration and
alkalisation. In the ALL-BFM-95 protocol (Berlin-Frankfurt-Münster) it is suggested that the hydration and alkalisation should be augmented already 24 hours after infusion start if the steady state level of methotrexate is above 150 μM. In addition, if urinary output is low furosemide is administered intravenously and additional sodium bicarbonate is given if urinary pH is low.

**RENAL FACTORS**

Renal function plays a key role in methotrexate pharmacokinetics and a delayed elimination of methotrexate appears to be related to elevated S-creatinine. Predictions of delayed elimination may enable an early treatment intervention preventing the development of profound toxicity. It therefore seems logical trying to find better markers for renal function to guide the treatment.

S-creatinine is an indicator of GFR but a number of markers for renal function have been suggested to describe various aspects of renal function better, and in more detail: Urinary protein HC (α1-microglobulin) is freely filtered in glomeruli and normally reabsorbed in the renal tubules. Increased urinary levels of protein HC has been suggested as a sensitive and reliable indicator of tubular dysfunction. Increased excretion of urinary albumin and IgG indicate glomerular damage with an impaired barrier function. These parameters have previously been used to assess renal function after cisplatin therapy and reference ranges have been proposed for adults and children. Serum cystatin C has been claimed to estimate GFR better than S-creatinine. This has also been shown in children with and without renal dysfunction and age independent reference limits have been proposed. Iohexol-clearance is an established method for estimation of GFR.

**POPULATION PHARMACOKINETICS**

Pharmacokinetics may be description as “what the body does to the drug”. A more formal description is “the quantification of the time course of a drug [and its metabolites] in the body and the development of appropriate models to describe observations and predict outcome in other situations”. A pharmacokinetic model describes the relationship between dose and concentration in an individual.

13
Pharmacokinetic investigation is often performed in healthy subjects or in selected patient populations (e.g. patients with hepatic or renal failure). Although the estimated pharmacokinetic parameters are accurate they may not reflect the true patient population. In an attempt to make it possible to study pharmacokinetic parameters in clinical practice Sheiner and Beal introduced a nonlinear mixed effects model approach coined NONMEM to solve the problem. With this method pharmacokinetic estimates can be obtained from sparse data. The obtained results on average values of pharmacokinetic parameters are very similar to those obtained by traditional means.

Population pharmacokinetics may be defined as the study of the variability in systemic drug concentration between individuals when standard dosage regimen are administered. In contrast to classical pharmacokinetic, population pharmacokinetics use sparse data and covariates from many individuals to obtain estimates. From a methodological point of view a wide distribution within covariates is an advantage. The nonlinear mixed effects model in NONMEM is based on the assumption that the kinetic parameters are normal distributed in the population or that they can be transformed into a normal distribution. The method is therefore parametric. This is an ideal method for estimating population pharmacokinetic parameters in the paediatric population, where frequency and blood volume of sampling are important considerations.

The program estimates the population mean pharmacokinetic parameters as well as inter- and intra individual variability. It is also capable of evaluating quantitative relationships between pharmacokinetic parameters and patient- and study specific variables. In the mathematical model, two sources of variability are handled, the explained part (fixed) and the unexplained part (random). The explained part is the population average values and usually a function of known covariates, such as patient age, weight, sex, etc, whereas the unexplained part are treated as random.
AIMS OF THE THESIS

The general aim of this thesis was to investigate factors contributing to the optimisation of HDMTX treatment in children with ALL. More specifically, the objectives of the studies were to:

- Determine the relationship between methotrexate elimination time and various aspects of renal function and to evaluate the ability for elevated serum creatinine and/or methotrexate to predict a delayed methotrexate elimination (paper I).
- Characterise the relations between systemic and cerebrospinal fluid concentrations of methotrexate by using statistics separating the inter- and intra-subject variability and to analyse the association between the methotrexate concentrations and the risk of a CNS relapse (paper II).
- Estimate methotrexate population pharmacokinetic parameters in children treated with HDMTX, to identify and evaluate covariates (e.g. body surface area) contributing to the inter-patient variability and to relate the pharmacokinetic parameters to outcome (paper III).
METHODS

SUBJECTS

In all studies subjects were administered HDMTX according to NOPHO-92 ALL protocol. In addition, 24 and 13 patients treated according to the ALL BFM 90 protocol were included in study I and the pharmacokinetic part of study II, respectively. The HDMTX treatments are identical in the two protocols.

Depending on the data needed for the analysis the number of subjects differed: In study I the relationship between kidney function and elimination time of methotrexate was studied in 264 Swedish children. In addition, renal function was studied in more detailed in 11 consecutive children at one centre.

In study II the relationship between achieved methotrexate concentrations in CSF and systemic exposure were studied in 34 children at one centre. The relationship was then applied to 353 patients from Norway, Denmark and Sweden in order to estimate the risk of a relapse of the CNS.

In study III a population pharmacokinetic analysis was performed with patient characteristics from 304 children. The relationship between the final pharmacokinetic parameter estimates and the risk of relapse was investigated in 340 patients from Denmark and Sweden with ALL.

HDMTX TREATMENT IN THE NOPHO-92 PROTOCOL

Depending on the risk group classification the stipulated doses of methotrexate were 5 g/m² (SR, IR) or 8 g/m² (HR, VHR) of which 1/10 was infused over the first
hour and the remaining (9/10) over the following 23 hours. Intravenous hydration, glucose 5% containing 40-42 mM NaHCO3 and 20 mM KCl, was stipulated to 3000 ml/m² over 24 hours. The hydration was increased to 4500 ml/m² over 24 hours if methotrexate 36 hours after infusion start was ≥3 μM.

Urinary pH was measured at every voiding. NaHCO3 (20 mmol in the courses with methotrexate 5 g/m² and 2 mmol/kg in the courses with 8 g/m²) should be administered intravenously if urinary pH was <7. Furosemide (0.5 – 1 mg/kg, maximum 20 mg), should be administered intravenously if diuresis < 100 ml/m²/hour. In the 5 and 8-gram courses racemic folinic acid (N5-formyl-tetrahydrofolic acid) was administered intravenously 36 hours after infusion start in the doses 15 and 50 mg/m², respectively. At 39 (only 8 gram courses) and 42 hours additional doses of 15 mg/m² was given and thereafter every 6 hours but increased if methotrexate exceeded 1 μM at 42 hours. Folinic acid was to be administered until 6 hours after methotrexate went below a level of 0.2 μM.

LV, or tetrahydrofolic acid, is an antidote to methotrexate. The exact mechanism of LV rescues, after methotrexate treatment, is not known², but thought to involve repletion of intracellular reduced folates²⁰. The end result is a resumption of DNA synthesis. Reduced folates, such as LV, prevent the toxic effects of methotrexate.

MEASUREMENTS

In all subjects systemic methotrexate concentrations was measured approximately 1 hour before the end of infusion (MTX23). Thereafter methotrexate was measured every six hours starting thirty-six hours after start of infusion (12 hours after end of infusion), until methotrexate was below 0.2 μM. In a subpopulation serum methotrexate was measured also at 1, 4 and 6 hours after start of infusion (Study I-III).

A sample for analysis of methotrexate concentration in CSF was drawn through a lumbar puncture, at the end of the 24 h HDMTX infusion. The CSF sample was taken immediately before the intrathecal methotrexate administration (Study II).
S-creatinine (Study I,III) and S-cystatin C (Study I) were measured prior to each course. S-creatinine was thereafter followed daily during the course. Protein HC, IgG, albumin and creatinine were analysed in spot-urine before start and during the second day of treatment (Study I). Iohexol clearance was measured during the initial phase of HDMTX infusion (Study I).

Data regarding methotrexate dose, age, height, weight, BSA, sex, serum creatinine, ALT and diagnosis were entered into an especially designed database. All measurements were obtained before start of each treatment course (Study III).

LABORATORY METHODS

Methotrexate was analyzed using EMIT (Behring Diagnostics, Syva Business, San Jose, CA, USA) or FPIA (Abbott Scandinavia AB, Solna, Sweden). Both assays are commercially available and in worldwide use.

Cystatin C was determined by an immunoassay on a Cobas Mira Plus Instrument (Roche, Stockholm, Sweden). Urinary Protein HC, IgG and albumin were analysed using immunoturbidimetry as previously described (Study I). GFR was estimated by Iohexol clearance measuring the concentration at two time points during the elimination phase, (for details see) (Study I). Creatinines in serum (Study I-III), and urine (Study I) as well as ALT (Study III) were determined at the local hospital by the use of commercially available analysis kit.

DATA HANDLING

In a subcohort of the NOPHO register, detailed data on treatment (e.g. methotrexate concentrations, LV doses, dates and times) and patient characteristics was collected. This started after a decision in SBLG (Svenska barnläkargruppen) and NOPHO, and the data was entered into a data base application (Microsoft Access 97) especially designed for the purpose. Permission to use the generated database was granted by the Data Inspection Board of Sweden (Study I-III).
PHARMACOKINETICS

Based on a priori knowledge systemic methotrexate concentrations were analysed using a two-compartment model\textsuperscript{12} in WinNonlin (version 1.5, Scientific Consulting Inc., Cary, NC, USA). For each subject 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 (Study II).

Methotrexate concentration-time data was analysed by a population pharmacokinetic method using the software package NONMEM version V. The data was randomly split into two parts containing 152 patients each. One part was considered an index set and the other a validation set. The final model was validated using the validation set and in a last step the two data sets were merged for calculation of the final population estimates. Seven covariates (age, height, weight, BSA, serum creatinine, ALT measured before start of each treatment course and sex) were evaluated regarding their potential influence on the pharmacokinetic parameters. NONMEM produces a minimum value of the objective function based on a log likelihood function. A reduction by 7.88 (corresponding to a p value < 0.005) was considered significant when determining whether a model with one additional parameter gave a better fit. Plots were also used to find the best model (Study III).

STATISTICS

Data analysis was performed using Graph Pad Prism, version 3.0, GraphPad software, inc., San Diego, USA (Study I). Statistical calculations were performed using the software SAS (version 6.02 and 8.2) from SAS institute, Cary, NC, USA (Study II, III).

Mean elimination times with 95% confidence intervals and multiple linear regressions to assess differences between means were calculated. For serum parameters (Iohexol clearance, S-cystatin C and S-creatinine) both multiple linear regressions and correlations were performed. Sensitivity and specificity were calculated for creatinine ratios. The predictive value for a positive and negative value as well as risk ratios were also computed. Linear regression for correlation analyses
between number of days and elimination time was performed. Two-tailed Student’s t-test was used if not otherwise stated. A value of $p < 0.05$ was regarded as statistically significant (Study I).

To analyse the relationship between the pharmacokinetics of systemic methotrexate concentrations 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 (Study II).

The association between calculated CSF methotrexate concentration and the risk of CNS relapse (Study II), and between the pharmacokinetic parameters and the risk of relapse (Study III) were both analysed using logistic regression. The analyses were carried out by risk group, as the risk for relapse, the number of treatment courses, the methotrexate dose and the treatment protocol differ between risk groups. In patients with CNS relapses only treatment courses before the date of relapse were included. Since the relapse risk is unlikely to be constant over time we choose to analyse the material using a logistic regression approach. The logistical regression equation defines the probability of a relapse at a given concentration (Study II) / pharmacokinetic parameter estimate (Study II).
RESULTS AND DISCUSSION

Within oncology, drugs are often given at maximum tolerated doses with the aim to have maximal effect without causing unacceptable toxicity. A complicating factor is that present dose algorithms are imperfect resulting in variable systemic concentrations. Understanding the drivers of the pharmacokinetic variability may open for a more individualised approach and potentially an improved outcome. Good therapeutic practice should always be based on an understanding of pharmacokinetic variability\textsuperscript{105}.

Another way to advance treatment may be to look for biomarkers that predict toxicity which make it possible to intervene early in order to prevent overt toxicity. In the context of HDMTX, markers of renal function may serve this purpose.

The aim of pharmacological therapy is to give the right patient the right dose at the right time. A move in this direction would be to increase the knowledge on how to predict pharmacokinetics. If more about the different sources of pharmacokinetic variability are known the risk for over- and underdosing might be reduced.

PREDICTIONS OF PHARMACOKINETICS

PREDICTIONS FROM PRE DOSE INFORMATION (PAPER III)

Traditional dosing based on BSA within oncology has been questioned\textsuperscript{81} as it fails to standardise the marked interpatient variations in exposure of most cytotoxic drugs\textsuperscript{42}. Further, very few organ functions important to pharmacokinetics are related to BSA\textsuperscript{87}. In study III a population pharmacokinetic model was built with data from 1284 HDMTX courses in 304 children. Weight improved the model fit to the data
significantly better than BSA or any of the other potential explanatory covariates tested. Others have come to similar conclusions\textsuperscript{4,73}. The results indicate that the methotrexate concentrations would be more predictable if HDMTX dosing were based on weight instead of BSA. This is clinically important as it may potentially improve the outcome without increasing toxicity.

The traditional gold standard to estimate pharmacokinetic parameters is a clinical study with intense sampling in few subjects. Due to the inconvenience causes it may not be feasible in a paediatric clinical setting. Therefore a population pharmacokinetic method may be more appropriate\textsuperscript{40}. The advantage is that accurate estimates of pharmacokinetic parameters may, on the group level, be obtained with less data points from each patient making it feasible in a clinical setting\textsuperscript{105}. This is supported in study III were CL 0.185 l/h/kg (5.3 l/h/m\textsuperscript{2}) is well in agreement with other reported values\textsuperscript{4,14,27,32,73}.

Furthermore, body surface area is merely calculated from height and weight, and obtaining accurate and reproducible measurements of height in children is difficult\textsuperscript{93}. Moreover, according to ICH guideline E11 “Clinical Investigation of Medicinal Products in the Paediatric Population” dose adjustments in the paediatric population should be based on mg/kg rather than mg/m\textsuperscript{2}, unless the benefit to the patient outweighs the increased risk of calculation errors\textsuperscript{53}. Thus, for both practical and pharmacokinetic reasons the current dose calculation for HDMTX, based on BSA, may be questioned.

\textbf{CSF CONCENTRATIONS FROM SYSTEMIC CONCENTRATIONS (PAPER II)}

Due to the poor penetration of methotrexate over the barrier between blood and the brain, the concentrations may occasionally be insufficient to eliminate leukaemic cells from the brain. A methotrexate concentration of 1 μM has been proposed as a minimum effective antileukaemic concentration from in vitro experiment\textsuperscript{62} and has been acknowledge by others\textsuperscript{9,13}. Increased knowledge about the relationship between systemic and CNS concentrations are clinically important as the CNS may be a sanctuary site for leukemic cells\textsuperscript{77}. Although the methotrexate concentrations in the CSF are not the same as in the CNS the correlation is most likely
acceptable. This is supported by the fact that the CNS relapse rate has declined considerably after the introduction of the current methotrexate dosing regimen\(^9,13\).

In study II we identify a relationship (Figure 2) between systemic and CSF methotrexate concentrations, using statistics that handle the inter- and intra patient variability. This method allows separation of the two sources of variability and the fact that patients contribute with different numbers of courses. Methotrexate concentration in CSF was found to be significantly dependent upon both systemic concentrations at the end of infusion and the AUC (\(p<0.0017\) and \(p<0.002\), respectively). The following relationships were found:

\[
\text{[methotrexate in CSF]} \mu M = 0.78 \mu M (SE 0.26) + 0.0091 (SE 0.0027) \times \text{MTX23},
\]

\[
\text{[methotrexate in CSF]} \mu M = 0.63 \mu M (SE 0.25) + 0.0043 (SE 0.0010) \text{h-1} \times \text{AUC} \
\]

There are conflicting reports in the literature regarding whether methotrexate levels in the systemic circulation and CSF are correlated\(^{13,31,90}\) or not\(^{60,64}\). The mean CSF to serum ratio in study II of 0.018 (1.8\%) are however in good agreement with other studies\(^{13,31,64,90,102}\).
We used a mixed linear model with fixed and random effects in order to adequately quantify the relationship and separate the two sources of variability. In previous studies on the relationship between CSF level and systemic concentrations of methotrexate have either studied inter-individual variability alone or studied both sources of variability, using statistics not separating the two.

In the NOPHO ALL 92 protocol methotrexate is administered both as an intravenous infusion and as an intra lumbar injection. Methotrexate concentrations in the CSF have been show to be as high as 100 μM and highly variable (0.6 - 22 μM) after intra lumbar injection of 6.25 or 12.5 g/m². This is orders of magnitude above CSF methotrexate concentrations after HDMTX, but the duration above the putative cytotoxic concentrations is shorter. Intra CSF drug administration results in a non-uniform distribution throughout the subarachnoidal space. Lumbar administration gives high lumbar drug concentrations but low ventricular and vertex concentrations. Accordingly, the need for lumbar administration of methotrexate has been questioned in protocol including HDMTX.

Among those treated with 5 g/m², nine out of the 18 patients had a CSF concentration of methotrexate over the proposed cytotoxic concentration (1 μM) in all their treatment courses, whereas this was the case for 14 out of 16 of the patients treated with 8 g/m². Of the 34 patients in the study only one patient failed to achieve 1 μM in at least one treatment cycle. For ethical consideration in this paediatric cohort only one CSF sample per treatment course was drawn. Thus, it is not possible to estimate methotrexate time course (AUC) in the CSF in this study.

With the relationship identified in study II concentration levels in the CSF can be calculated from the systemic exposure. This may be clinically important in order to further optimise the pre symptomatic CNS directed therapy in childhood ALL.
In order to safely administer HDMTX, monitoring of systemic concentrations of methotrexate, urinary flow and urinary pH is of paramount importance. Changes in these parameters are indicative of upcoming toxicity enabling appropriate treatment to be started before profound and deleterious changes may be seen. In Study I the performance of a number of biomarkers for renal functions were studied regarding their ability to predict a prolonged methotrexate elimination.

In study I we found that HDMTX induced significant elevations in S-creatinine (Figure 3). None of the markers for renal function measured in a subgroup before start of HDMTX correlated to time of methotrexate elimination. Neither were any of the urinary parameters (U-albumin, U-IgG, U-Protein HC) measured the second day of treatment related to methotrexate elimination time. These findings indicate that the elimination time of methotrexate during HDMTX is related to a decrease in glomerular filtration in contrast to a decreased tubular function as was previously suggested.

![Figure 3. Serum creatinine ratios day 1 and methotrexate elimination time. Squares, Circles and Triangles represents: <4 , ≥ 4 and < 7 , and ≥ 7g methotrexate /m² respectively. Open symbols are MTX23 ≤150 μM and filled symbols are MTX23 >150 μM. Number of courses in each region of the figure is given.](image-url)
The clinical relevance of pre-treatment GFR estimation is limited and it has been suggested that routine estimations of GFR, do not contribute to the clinical management of HDMTX. Interestingly, in study I methotrexate elimination time correlated significantly \( (p = 0.04) \) to pre-treatment estimations of GFR, if a course with diclofenac was excluded. Diclofenac is an NSAID and concomitant medication with NSAIDs is known to prolong the methotrexate elimination.

Study I showed that 99% of creatinine ratios on day 1 in courses with normal elimination time \(< 72 \text{ h}\) are \(< 1.5\). An increase creatinine of 50% on day 1 may therefore be regarded as a reasonable approximation for a pathological elevation in S-creatinine. This is in line with clinical observations but to our knowledge study I is the first study to quantify the relationship.

In the ALL-BFM-95 protocol it is suggested that alkali-sed hydration should be augmented if the level of methotrexate sampled just before end of infusion is higher than 150 \( \mu\text{M} \). Creatinine ratio measured during the first 12-24 hours after start of methotrexate infusion seems to be a better predictor for delayed elimination than is the methotrexate concentrations, especially if information on previous creatinine measurements is used to reduce the impact of an occasionally low creatinine value before start of methotrexate infusion.

In conclusion, in study I an increase in S-creatinine of 50% from baseline could identify patient at risk for a delayed methotrexate elimination. The sensitivity and specificity for the test is better than methotrexate concentration at the end of the HDMTX infusion. The result also indicates that a glomerular impairment develops during the HDMTX treatment, but the barrier and tubular function remains unaffected as suggested by the lack of elevation in U-albumin, U-IgG and U-Protein HC.

**Predictions of relapse risk**

Although knowledge on pharmacokinetics is important the ultimate goal with the treatments is to improve outcome. In study II and III we therefore explored the relationship between pharmacokinetics of HDMTX and relapse risk.
To extend the pharmacokinetic analysis in study II we explored the risk of a CNS relapse in relation to the CSF methotrexate concentration. We calculated the number of courses estimated to be above 1 μM in CSF based on the relationship identified between MTX23 and CSF methotrexate concentration in the subpopulation. In addition, the subjects’ minimum, maximum, median and average systemic methotrexate concentrations at the end infusion were calculated for each individual. These five factors together with MTX23 in the first treatment course were considered in the logistic regression analysis.

Increased median methotrexate 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). Furthermore, an increased number of courses with a calculated CSF level > 1μM was significantly associated (p=0.048) with a decreased risk of a CNS relapse in the combined lower (SR + IR group). Thus, the findings in the present study suggest that patients with a high methotrexate exposure have a decreased risk of a CNS relapse. The result is in line with a meta-analysis investigating the importance of CNS directed therapies for childhood lymphoblastic leukaemia21. Protocols that instead use a more frequent administration of prophylactic intrathecal methotrexate may, however, not see the same benefit from the addition of intravenous HDMTX.

In the treatment of HR or VHR only four and two HDMTX courses, respectively, were administered compared with the eight in SR and nine courses in IR. This may partly explain why no significant association between methotrexate concentrations and risk of CNS relapses was seen in HR or VHR despite a relatively high incidence of events. Considering the size of the cohort studied, the number of CNS relapses is still low making the type of calculations performed challenging due to a low statistical power. In addition, the CSF concentrations in the entire material are only calculated from the equation identified in a subpopulation. Thus, 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 may be generalised to a larger population.
Patients with a higher systemic clearance of methotrexate or a low systemic methotrexate exposure have worse outcome. In addition, individualised treatment has been shown to improve the outcome in children with B-lineage ALL. This is in line with the results of study III where an increased clearance and volume of distribution, were associated with an increased relapse risk.

By logistic regression it was shown that the central and peripheral volume of distribution as well as expressions of systemic and inter compartment clearance could be considered predicable of the risk of relapse. This relationship was absent in the HR and VHR group. The presence of the relationship in the SR and IR groups indicates, however, that these patients may need higher exposures to ensure a sufficient antileukaemic effect from methotrexate. The NOPHO 92 protocol stipulate 8 g/m² for HR and VHR, whereas patients in the SR and IR groups are administered 5 gram/m².

To expand the analysis further, the influence of body weight was tested and an increase in body weight was, unexpectedly, found to be significantly associated with an increased relapse risk in the total, SR (p=0.00186) and IR groups (p=0.0121) groups. Although, the most important determinant of outcome is the treatment protocol itself, the findings may not be explained by the increasing likelihood of associated risk factors with increasing age, as patients in the SR and IR group lack high-risk features. A possible explanation may instead be that older (and heavier) children are under-treated with the current dose modification related to BSA.

Pharmacokinetic parameters not adjusted for weight were able to predict the risk of relapse in patients treated with 5 g/m² but not in patients treated with 8g/m². Considerably less treatment courses are given to patients treated with 8 g/m² than 5 g/m², which may partly explain the discrepancy. In addition, pharmacokinetic parameters in patients treated with 5 g/m² normalised to body weight were not significantly correlated to relapse risk. This indicates that body weight is a suitable anthropomorphic measurement to dose individualise HDMTX. The use of body weight instead of BSA for dose individualisation is also advocated in the ICH guideline.
Although the present study is fairly large there are certain limitations. Firstly, part of the study material is collected retrospectively, which potentially may impact on the validity of the data. Secondly, the material is not complete regarding all the required information. The fact that only courses with a complete set of covariates were included in the analyses may potentially introduce a bias. A comparison regarding the distribution of age and risk groups with the entire NOPHO material did, however, not reveal any differences. Further, the patient characteristics and exposure data were registered in the patient records before the follow-up was performed.

To sum up, heavier (older) patients with an increased clearance and volume of distribution in the SR and IR groups had an increased relapse risk. A body weight based dose calculation may improve the outcome but the study findings should be tested in a controlled trial before they are implemented into clinical practise.
CONCLUSIONS

- Elevation of serum creatinine by more than 50% is a better predictor of delayed elimination than the systemic level of methotrexate at the end infusion. Markers of tubular function did not relate to a delayed elimination.

- Algorithms separating between inter- and intra-patient variability in systemic and cerebrospinal fluid concentrations were described. Applying this relationship to a larger population indicated that an increased methotrexate exposure of the CNS was related to a decreased risk of a CNS relapse.

- The population pharmacokinetics parameters were estimated and body weight improved the model fit significantly better than any of the other covariates (e.g. body surface area). Patients with an increased clearance and volume of distribution in the SR and IR groups had an increased relapse risk, irrespective of location. The use of body surface area for dose calculation in HDMTX treatment in this patient population may therefore be questioned.

An overall aim with this thesis has been to better understand the variability of HDMTX and ultimately improve the outcome childhood ALL. Although caution should be exercised when extending these retrospective findings to clinical practise, these results extend the current knowledge around potential ways to optimise the HDMTX treatment in childhood ALL.
Metotrexat är ett läkemedel som har använts i mer än ett halvt sekel i behandlingen av olika former av cancer och autoimmuna sjukdomar (t.ex. reumatism). Höga doser metotrexat ges intravenöst vid behandlingen av barn med akut lymfatisk leukemi (ALL). Trots att prognosen för dessa barn förbättrats radikalt så återstår cirka 15-20% som inte blir botade.

Huvudsyftet med denna avhandling har varit att försöka identifiera möjliga sätt att förbättra den intravenösa methotrexate behandlingen. Detta har gjorts genom att studera koncentrationerna av methotrexate i blodet och ryggmärgsvätskan med hjälp av matematiska modeller och statistiska metoder.

I det första delarbetet studeras njurfunktionens betydelse för hur lång tid det tar för kroppen att göra sig av metotrexat. Resultaten antyder att den passiva filtrationen i njuren är av större betydelse än den aktiva sekretionen. Markörer som pekar på risk för skada identifieras (serum kreatinin) och dess förmåga att förutsöka risken för fördröjd utsöndring av metotrexat kvantifieras i 264 barn med akut lymfatisk leukemi.

I det andra delarbetes undersöks hur koncentrationer av methotrexate i centrala nervsystemet relaterar till de nivåer som finns i systemkretsloppet. Dessutom undersöks vilken betydelse som de uppnådda nivåerna i ryggmärgsvätskan har för risken för återfall i ALL som drabbar hjärnan. Resultaten pekar på att ju högre nivåer av läkemedel i ryggmärgsvätskan en patient har desto mindre är risken för återfäll i hjärnan.
I det tredje delarbetet studeras vilka egenskaper hos individer som kan förklara att olika nivåer av methotrexate uppmätts i systemkretsloppet trots att dosen individualiseras till kroppsvikt. Resultaten visar att skillnader i kroppsvikt är den faktor som bäst förklarar de uppmätta skillnaderna. Det visar sig också att det är de tyngsta barnen som har den största risken för återfall. Tillsamman gör detta att vi föreslår att man bör överbära att överge den nuvarande doseringen som baseras på kroppsvikt till förmån för en viktjusterad anpassning av dos.

Sammanfattningsvis indikerar resultaten att en viktjusterad dosering och/eller högre dosering av intravenöst givet metotrexat minska risken för återfall samt att en ökning i serum kreatinin relaterar till förlängsammad utsöndring av metotrexat. Fynden bör utvärderas i en framtida klinisk prövning för att slutligen fastställa värdet av våra resultat.
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to:

Tor Skärby, my mentor and tutor, for introducing me into the field of research, continues coaching, fruitful scientific discussions and for great friendship.

Peter Höglund for generously sharing his vast experience in pharmacokinetics and statistics, and for unravelling the mysteries of population pharmacokinetic.

Karl-Erik Andersson and Edward Högestätt for giving me the possibility to do my PhD work at the section of Clinical and Experimental Pharmacology.

Tomas Senderovitz and Lars Borgström, my former and present superior, for giving the possibility to complete this thesis while employed at Ferring A/S and AstraZeneca R&D, respectively.

Past and present colleagues at Clinical and Experimental Pharmacology at Lund University, who have contributed each in their own way to this work. A special thanks to Britt Olsson and Anette Person for skilful database handling.

All co-authors and the NOPHO organisation, especially Göran Gustafsson, who made theses studies possible.

Kimi, Ron, Bernie and the other at the Formula 1 circus for great entertaining, reminding me every other Sunday that it takes a team effort to reach one’s goal.

Gunnar Jönsson, my father, and Ruth, my late mother, for always being there, giving me the best possible support.

And to the most important people of my life: Sofia, Holger and Ebbe, thank you for your love and patience. Sofia, without you I would have been lost.
This work was supported by grants from the Children’s Cancer Foundation of Sweden, the Swedish Cancer Society, Crafoord’s, B. Kamprad’s, Gyllenstiern’s Krapperup, Lund University Hospital Foundations and MSD (Sweden).
REFERENCES


13 Borsi JD, Moe PJ. A comparative study on the pharmacokinetics of methotrexate in a dose range of 0.5 g to 33.6 g/m² in children with acute lymphoblastic leukemia. Cancer 1987;60(1):5-13.


70 Pinedo HM, Chabner BA. Role of drug concentration, duration of exposure, and endogenous metabolites in determining methotrexate cytotoxicity; 1977.
72 Pizzo PA, Poplack DG. Principles adn Practice of Pediatric Oncology. 5 ed. Philadelphia: Lippincott Williams & Wilkins; 2006.


