NEUROTOXICITY IN CHILDREN AFTER TREATMENT FOR ACUTE LYMPHOBLASTIC LEUKAEMIA AND METHOTREXATE NEUROTOXICITY IN A CONTROLLED ANIMAL MODEL

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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium of the Department of Paediatrics, on June 13th, 2003, at 12 noon.

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Oulu, Finland
2003

Abstract
In the Nordic countries, event-free survival (EFS) exceeds 80% in certain groups of children treated for acute lymphoblastic leukaemia (ALL). With the improved cure rates, however, there are more children suffering from neurological late effects, especially due to therapy directed at the central nervous system (CNS). The aim of this study is to examine the changes taking place in the nervous system after leukemia treatment and to evaluate the role of treatment in these changes in patients and in an animal model.

Twenty-seven ALL survivors and healthy controls were examined by means of motor evoked potentials (MEPs). ALL survivors were also examined clinically. The children with ALL continued to show decreased motor nerve conduction in the peripheral nerves, but not within the CNS, five years after the cessation of treatment. Clinical neurological findings were obtained in 33% of the cases. The MEP results indicated reversibility of the motor injury due to CNS effects.

Nineteen patients underwent perfusion magnetic resonance imaging (MRI) at the cessation of treatment or 4-8 years after the treatment. Seventeen of them also underwent single-photon emission computed tomography (SPECT). The studies showed small perfusion defects in SPECT, which were not visible by perfusion MRI.

Methotrexate (Mtx) neurotoxicity was studied in a swine model using functional MRI, brain perfusion SPECT, iodine-123 labelled 2β-carbomethoxy-3β-(4-iodophenyl) tropane ([123I]β-CIT) SPECT and whole-hemisphere autoradiography with [125I]β-CIT in ten Mtx-treated animals and five control animals. Mtx-related changes in the brain could be detected as reduced or negative blood-oxygen-level-dependent (BOLD) responses to somatosensory activation in BOLD contrast MRI, which indicates changes in flow metabolism coupling. Perfusion defects in brain SPECT were seen in the Mtx group and the control group, which suggests that the perfusion defects seen in brain SPECT are probably multifactorial. The change in dopamine transporter (DAT) density in the Mtx group was not different from that in the controls.

The abnormalities in nerve conduction after treatment in survivors of ALL were partly reversible years after the treatment. The patients had perfusion defects in SPECT imaging which were not seen in perfusion MRI. The clinical significance of these defects remains obscure. The animal model suggested perfusion defects to be multifactorial.

Keywords: acute lymphoblastic leukaemia, autoradiography, brain, cerebrovascular disorders, follow-up studies, magnetic resonance imaging, methotrexate, motor evoked potentials, single-photon emission computer tomography
To my family
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Oulu, May 2003

Satu Lehtinen
Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>ADC&lt;sub&gt;z&lt;/sub&gt;</td>
<td>apparent diffusion coefficient</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td>BFM</td>
<td>Berlin-Frankfurt-Munster</td>
</tr>
<tr>
<td>BOLD</td>
<td>blood-oxygen-level-dependent</td>
</tr>
<tr>
<td>CBF</td>
<td>cerebral blood flow</td>
</tr>
<tr>
<td>CBV</td>
<td>cerebral blood volume</td>
</tr>
<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CRT</td>
<td>cranial radiation therapy</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DF-ROM</td>
<td>dorsiflexion range of motion</td>
</tr>
<tr>
<td>ECD</td>
<td>ethyl-cysteinate-dimer</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>EFS</td>
<td>event-free survival</td>
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<tr>
<td>ERP</td>
<td>event-related potential</td>
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<tr>
<td>FDG</td>
<td>fluorodeoxyglucose</td>
</tr>
<tr>
<td>FSE</td>
<td>fast spin-echo</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>Gy</td>
<td>Gray (1Gy = 100 cGy = 100 rad)</td>
</tr>
<tr>
<td>HR</td>
<td>high risk of relapse</td>
</tr>
<tr>
<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>IR</td>
<td>intermediate risk of relapse</td>
</tr>
<tr>
<td>MEP</td>
<td>mean evoked potential</td>
</tr>
<tr>
<td>MMN</td>
<td>mismatch negativity</td>
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<tr>
<td>MRD</td>
<td>minimal residual disease</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MTT</td>
<td>mean transit time</td>
</tr>
<tr>
<td>Mtx</td>
<td>methotrexate</td>
</tr>
<tr>
<td>NEX</td>
<td>number of excitations</td>
</tr>
<tr>
<td>NLE</td>
<td>necrotising leukoencephalopathy</td>
</tr>
<tr>
<td>Acronym</td>
<td>Term and Definition</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>NOPHO</td>
<td>Nordic Society for Paediatric and Haematology and Oncology</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PD</td>
<td>proton density</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEP</td>
<td>somatosensory evoked potential</td>
</tr>
<tr>
<td>SE-EPI</td>
<td>spin echo-echo-planar imaging</td>
</tr>
<tr>
<td>SPECT</td>
<td>single-photon emission computed tomography</td>
</tr>
<tr>
<td>SR</td>
<td>standard risk of relapse</td>
</tr>
<tr>
<td>SPSS</td>
<td>statistical package for social sciences</td>
</tr>
<tr>
<td>TdT</td>
<td>terminal deoxynucleotidyl transferase</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>VHR</td>
<td>very high risk of relapse</td>
</tr>
<tr>
<td>VEP</td>
<td>visual evoked potential</td>
</tr>
<tr>
<td>VMI</td>
<td>visual motor integration</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell</td>
</tr>
<tr>
<td>$[^{123}]\beta$-CIT</td>
<td>iodine-123 labelled 2$\beta$-carboxy-3$\beta$-(4-iodophenyl) tropane</td>
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List of original papers

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (I–IV).


IV Lehtinen S, Kolehmainen E, Torniainen P, Ahonen A, Tupala E, Harila-Saari A, Vainionpää L & Lanning M. Brain perfusion, $^{123}$I$\beta$-CIT SPECT and $^{125}$I$\beta$-CIT whole-hemisphere autoradiography after intravenous methotrexate administration to swine in a controlled animal model. Submitted for publication.
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1 Introduction

The increase in the average life expectancy of children treated for acute lymphoblastic leukaemia (ALL) represents an important advance in cancer therapy. Childhood leukaemia is among the success stories in cancer treatment during the past few decades. One of the reasons for the good results is the development of central nervous system (CNS) treatment along with advanced treatment protocols and improved supportive treatments, which have changed childhood leukaemia from a fatal to a curable disease for over 80% of the standard-risk (SR) and intermediate-risk (IR) patients (Gustafsson et al. 2000). Along with the better survival rates, the quality of life is more and more important for ALL survivors. Among the late effects, we should especially consider the neurological sequelae (van Der Does-van den Berg et al. 1995), neuropsychological defects (Espy et al. 2001), endocrine dysfunction (Morgan & Haugen 1997) and secondary malignancies (Garwicz et al. 2000).

Children treated for ALL often develop motor dysfunction related to the treatment (Vainionpää 1993). Gross and fine motor dysfunctions appear during the treatment and are believed to disappear gradually after the cessation of therapy. However, fine motor difficulties are reported to persist for two years or more after the cessation of treatment (Reinders-Messelink et al. 1996).

Vincristine is widely used as a chemotherapeutic agent in treatment protocols, and it is considered to be the main cause for the peripheral neuropathy detected in clinical and neurophysiological examinations. Abnormal motor evoked potentials (MEPs) have been recorded within both the CNS and the peripheral motor nervous tract at the end of therapy (Harila-Saari et al. 2001), and a decrease in sensory amplitudes in the peripheral nerves has been shown to persist even after two years (Harila-Saari et al. 1998).

Several imaging methods have been used to visualise the CNS changes caused by ALL treatment. Magnetic resonance imaging (MRI) is the most sensitive method for structural evaluation. The white matter changes reported in MRI studies may be transient and disappear during follow-up. (Wilson et al. 1991.) Other possible structural abnormalities include haemorrhages, cerebral infarction, calcifications, atrophy and secondary neoplasms (Vázquez et al. 2002). The neuropsychological findings have had only minor or no significant correlations with the MRI abnormalities (Kingma et al. 2001, Luvone et al. 2002). Single-photon emission computed tomography (SPECT) imaging has shown small perfusion defects, which have not correlated with the MRI findings (Vera et al. 2006).
Positron emission tomography (PET) studies have shown the overall glucose metabolism to be reduced after leukaemia treatment (Phillips et al. 1991), and in a controlled clinical study, decreased glucose utilisation was reported in the thalamus and cerebellum of survivors of ALL (Kähkönen et al. 1999).

The functional methods of MRI are some of the new functional methods in medicine. Perfusion imaging, diffusion imaging and blood-oxygen-level-dependent (BOLD) contrast MRI may allow an early detection of functional brain injury before clear anatomic abnormalities (Le Bihan et al. 1995). Perfusion MRI is a method to study brain perfusion at the capillary level. Perfusion MRI has been used in cerebral ischemia with good correlation with SPECT results. Diffusion MRI shows the ischemic brain damage earlier than regular MRI or computed tomography (CT). (Sorensen et al. 1996.) Defects in the local increase of blood flow during neural activation can be demonstrated in BOLD contrast MRI (Le Bihan et al. 1995).

The aim of the work reported here has been to assess the functional brain changes and lesions of the entire motor pathway by modern brain imaging, neurophysiological and clinical neurological methods in survivors of ALL. Methotrexate (Mtx) is thought to be one of the main causes of treatment-related CNS neurotoxicity. By using a controlled animal model with all other chemotherapy excluded, Mtx-related changes in the brain dopamine transporter (DAT) density were evaluated using iodine-123 labelled 2β-carbomethoxy-3β-(4-iodophenyl) tropane ([123I]β-CIT) SPECT and whole-hemisphere autoradiography with [125I]β-CIT as a tracer. Better understanding of the mechanisms underlying Mtx-induced neurotoxicity would enable efficient treatment with fewer adverse effects.
2 Review of the literature

2.1 ALL in childhood

2.1.1 Epidemiology

Leukaemia is the most common malignancy among children aged under 15. ALL accounts for 76–85% of all diagnoses of childhood leukaemia (Gurney et al. 1995, NOPHO 2000), representing 30% of all childhood malignancies (NOPHO 2000). The Nordic registers of childhood malignancies constitute a good source for epidemiologic analyses, being based on reliable and standardised data collection. Each year, approximately 150–200 children are diagnosed with ALL in the Nordic countries, with an overall incidence of 3.9 cases per 100 000 children aged under 15. The incidence of childhood ALL has been stable in the Nordic countries during the past years. (NOPHO 2000.) In contrast, it has been shown that the incidence of ALL has been increasing in the United States (McNeil et al. 2002) and England (McNally et al. 2001). The increase has been more pronounced amongst young children in the age group of two to four years (McNally et al. 2002, McNeil et al. 2002).

There are substantial geographic differences in the incidence of childhood leukaemia. In the developed countries, the incidence rates for childhood ALL are two- to fourfold compared to the rates in the underdeveloped countries, which could represent differences in environmental factors, genetic factors and diagnostic accuracy. (Greaves et al. 1993.) In evaluating the epidemiologic literature on acute leukaemia, it should be kept in mind that changes over time in the diagnostic practices and registration may account, in part, for any observed trends.

In the developed countries, there is a significant peak in the incidence of childhood ALL between the ages of two and five years, and one subtype, referred to as common ALL, accounts for the high incidence in this age group (Greaves 1999). Overall, boys have a higher leukaemia risk than girls, but leukaemia diagnosed in the first year of life is more common in girls than in boys. Throughout childhood, the incidence of ALL in blacks is consistently about half of that in whites. (Gurney et al. 1995.)
2.1.2 Causal factors of childhood ALL

Although the cause of most acute leukaemias is not known, certain major factors have been implicated in some cases. Ionising radiation is the best known causal mechanism for acute leukaemia (Greaves 1997), but it is unlikely to be the major causal pathway. Epidemiological evidence suggests the presence of certain chemicals (such as benzene), viruses (human T-cell leukaemia/lymphoma virus 1/Epstein-Barr virus) and bacteria (Helicobacter pylori) in the development of leukaemia and lymphoma in children and in adults (Greaves 2002). In case-control studies, maternal exposure to low-dose radiation has been associated with a small but significant increase in the risk (about 1.4-fold) of subsequent childhood acute leukaemia (Doll & Wakeford 1997). An epidemiological study of infant leukaemia has implicated transplacental chemical exposure to pesticides and a drug (dipyrone) during pregnancy (Alexander et al. 2001). Electromagnetic fields were ruled out as a major factor in leukaemia etiology in an English study (UK Childhood Cancer Study Investigators 2000) opposite to other reports (Wertheimer & Leeper 1979, Schuz et al. 2001).

The two- to five-year age peak in leukaemia incidence, which was firstly noted in the white population of the United States in the 1960s (Pierce et al. 1969), has been related to an unusual response to a childhood infection (Greaves & Alexander 1993, Greaves 1997). One hypothesis suggests that ALL in children is caused by a failure of the immune system in infancy, and that the aberrant response to infection promotes the crucial, second postnatal event (Greaves 1997). The other hypothesis proposes that the transiently increased rates of leukaemia are due to population-level mobility and mixing, which result in infections in previously unexposed or susceptible individuals (Kinlen 1995).

Children with Down’s syndrome have a 10- to 20-fold risk for ALL (Berger 1997). Other genetic disorders associated with an increased risk of ALL are Klinefelter’s syndrome, neurofibromatosis, ataxia telangiectasia and Shwachman’s syndrome (Sandler & Ross 1997). There have been reports of familial aggregation of leukaemia (Farwell & Flannery 1984). There is now compelling evidence that chromosome translocations are often the initiating events in leukaemia (Greaves 2002). Studies on identical twins show that ALL is frequently initiated by an intrauterine genetic event (Wiemels et al. 1999) or possibly metastasis through shared placental circulation (Ford et al. 1993). The most common structural genetic abnormality in childhood leukaemia is a fusion of two genes, TEL and AML1 (Greaves 2002). This is generated by a chromosome translocation between the chromosomes 12 and 21. Studies on identical twins show, however, that such an event is not a sufficient precondition for the onset of clinical leukaemia, and some additional event or exposure is required postnatally (Wiemels et al. 1999). On the other hand, the high concordance rate for leukaemia in monozygotic twins in infancy and the very short latency (around 18 months) suggest than an MLL gene fusion in the appropriate fetal hematopoietic stem cell may be sufficient for leukaemogenesis (Greaves 1999).
2.1.3 Development of therapy for ALL

The 20th century witnessed remarkable progress towards better understanding and treatment of leukaemia. Before any specific therapy was introduced, the median survival was approximately two to three months, and leukaemia was considered a fatal disease. At the beginning of the 20th century, there was only palliative treatment in the form of arsenic trioxide and ionising radiation. (Brenner & Pinkel 1999.)

The first attempts to treat leukaemia were made in the 1940s using alkylating agents. Nitrogen mustard, which was the first alkylating agent used clinically, produced temporary remission, but with considerable toxicity. (Goodman et al. 1946.) The first effective agents used to treat childhood ALL were two antifolates, 4-aminopteryl-glutamic acid (Aminopterin) and Mtx, which emerged from nutrition research (Spies 1946). In 1948, Farber and colleagues reported remissions lasting for several months in patients receiving a folinic acid antagonist Aminopterin (Farber et al. 1948). This progress was followed by the development of corticosteroids (Stickney et al. 1950) and synthetic antipurines (Burchenal et al. 1953) in the 1950s.

The use of combination chemotherapy with a corticosteroid, an antifolate and an antipurine became standard therapy for childhood ALL. The combination chemotherapy resulted in longer survival and also improved the patients’ quality of life, but eventually almost all patients still experienced relapse and death. (Brenner & Pinkel 1999.) The search for diabetic drugs resulted in the discovery of vincristine, which belongs to the group of vinca alkaloids and is capable of producing remission of ALL (Noble et al. 1958, Karon et al. 1962).

In the early 1960s, a four-phase treatment plan, called “total therapy”, for ALL was developed. The first phase consisted of prednisone and vincristine. After remission had been achieved and the child was free of infection and bleeding and in a better nutritional condition, the second phase was administered. This consisted of high doses of antimetabolite compounds injected intravenously every day for one week, while the third phase consisted of cerebrospinal irradiation. The last phase consisted of prolonged chemotherapy for two to three years. (Pinkel 1971.)

Along with the longer survival, the CNS became the most common site of initial relapse, and by the early 1970s, the incidence of CNS leukaemia was over 80% among the children who remained in bone marrow remission (Evans et al. 1970). The observation of CNS as a possible nest of leukaemic cells led to the development of CNS treatment. Most systemically administered drugs do not penetrate the blood-brain barrier, which hence protects leukaemic cells from the cytotoxic effects of the drug, and the CNS serves as a store of relapse (Balis & Poplack 1989).

Radiotherapy was first administered as palliation to patients with overt CNS disease in the early 1960s, but it was soon noted that craniospinal irradiation or cranial irradiation combined with intrathecal Mtx in adequate doses was able to inhibit relapse in the CNS (Aur et al. 1969, Pinkel et al. 1972). The most widely used approach was a combination of intrathecal Mtx and cranial irradiation in doses of 24 or 18 Gray (Gy) (Chessells 1994). Cranial radiation therapy (CRT) may have serious late effects, and to reduce these effects, lower radiation doses have been applied. The use of 18 Gy dose of cranial irradiation combined with intrathecal Mtx is equally effective as 24 Gy. (Nesbit et al. 1981.)
As survival rates improved, concerns about the potential deleterious delayed effects of therapy also increased. Meadows and colleagues were the first to report in 1981 declines in the intelligence quotient (IQ) scores and cognitive dysfunction of children with ALL treated with cranial irradiation in a prospective evaluation of intelligence by using standardised tests in a random population of children with ALL (Meadows et al. 1981). Currently, most treatment protocols rely on intrathecal and systemic chemotherapy, with cranial irradiation reserved for selective groups of patients (Pui et al. 2001).

Our increasingly sophisticated knowledge of the genetics and biology of leukaemia opens up hopes of developing better treatments for leukaemia. Immunotherapy and gene therapy are being investigated for their potential in leukaemia treatment (Brenner & Pinkel 1999).

### 2.1.4 Risk factors and prognosis

Investigators agree that careful evaluation of the risk of relapse is needed at the time of diagnosis, to avoid under- or over-treatment. However, there has been no consensus on the most useful criteria worldwide. Age, white blood cell (WBC) count, leukaemic cell genotype, phenotypic characterisation and treatment response to early induction of remission are commonly used factors in risk classification. (Gaynon et al. 1997, Gaynon et al. 2000, Harms & Janka-Schaub 2000.)

The type of treatment programme chosen is the most important determinant of outcome (Pui et al. 2001). Age and leukocyte count have been powerful prognostic indicators in the B lineage (Hammond et al. 1986), but not in the T lineage of ALL (Eden et al. 2000, Maloney et al. 2000). Their value, however, is limited even in B-lineage ALL, because up to a third of patients with SR (age 1–9 years and leukocyte count < 50 x 10⁹/L) may relapse, and the patients at very high risk of relapse (VHR) cannot be reliably distinguished from the high-risk (HR) patients by these measurements (Pui et al. 2001). Patients aged under one year have a very poor prognosis (Biondi et al. 2000, Chessells et al. 2002).

For reasons that are still unknown, male sex is an unfavourable prognostic factor (Hammond et al. 1986), and boys fare significantly worse than girls in many treatment protocols (Pui et al. 1999). In studies carried out in the United States, children of African-American and Hispanic ancestry have been reported to have significantly worse outcomes than white children, after adjustment for other prognostic factors (Pollock et al. 2000), but one single institute reported no difference in outcome, which was due to equal access to effective treatment for all patients (Pui et al. 1995).

The genetic features of leukaemic cells influence the aggressiveness of the disease and the response to therapy, but do not solely predict the outcome (Pui et al. 2001). TEL-AML1 fusion and hyperdiploidy (> 50 chromosomes per cell) have been related to a favourable outcome (McLean et al. 1996), but up to 20% of these patients will eventually relapse (Borkhardt et al. 1997, Rubnitz et al. 1997). However, a third of the HR patients with the Philadelphia chromosome with BCR-ABL fusion and the t(4;11) with MLL-AF4 fusion can be cured with chemotherapy only (Pui & Evans 1998). Age of 1–9 years has been associated with a favourable outcome in cases with the Philadelphia chromosome or
the t(4;11), while a high leukocyte count conferred a poor prognosis to those with the former genetic feature (Arico et al. 2000). The mechanisms by which genetic abnormalities result in differences in disease aggressiveness or drug sensitivity are only partially known (Pui et al. 2001).

In the Nordic countries, cases have earlier been classified as SR, intermediate-risk (IR) and HR of relapse according to NOPHO (Gustafsson et al. 1989). In the recent treatment protocols, patients have been classified to receive standard, intermediate or intensive/very intensive/extra intensive therapy according to NOPHO (unpublished), as shown in table 1. In addition to these treatment groups, there are special groups including infants under the age of one year and B-cell ALL.

Table 1. Criteria for the treatment groups of children with ALL.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Age 1–9 years, WBC ( \leq 10 \times 10^9/\text{L} ), no unfavourable features</td>
</tr>
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</table>
| Intermediate                  | Age 1–9 years, WBC \( > 10 - < 50 \times 10^9/\text{L} \), no unfavourable features  
|                               | or age \( \geq 10 \) years, WBC \( < 50 \times 10^9/\text{L} \), no unfavourable features |
| Intensive, very intensive or  | WBC \( \geq 50 \times 10^9/\text{L} \)                                       |
| extra intensive               | Mediastinal mass                                                         |
|                               | Chromosomal translocation \((9;22), (4;11), (1;19)\)                        |
|                               | Hypodiploidy \((< 45)\) chromosome status                                |
|                               | T-cell leukaemia                                                         |
|                               | CNS or testis involvement                                                |
|                               | Slow responder                                                           |
|                               | – day 15 M3 \((> 25\% \text{ blasts, bone marrow not hypoplastic})\)     |
|                               | – day 29 M2 or M3 \((> 5\% \text{ blasts, bone marrow not hypoplastic})\) |
| Interfant-99 treatment protocol | Age under 1 year                                                         |
| Own treatment protocol        | B-cell leukaemia                                                         |

The change in leukaemia treatment from palliation to cure in the 1970s has resulted in more advanced treatment modalities. The proportion of children who will survive in complete continuous remission out of all children with ALL is expressed as event-free survival (EFS). The development of combination therapies consisting of cytotoxic drugs with or without stem-cell transplantation has increased the survival rates of patients with childhood ALL to over 70% in many centers (Gaynon et al. 2000), ranging within 74–83% in the United States (Silverman et al. 2000, Silverman et al. 2001). In the Nordic countries, EFS at 7 years for SR/IR patients diagnosed in 1992–1998 exceeds 80%. For HR patients, EFS at 7 years varies between 60% (patients < 5 years at diagnosis) and 66%. The patients with the most difficult prognostic factors in the VHR group have an EFS of 63% at 7 years. (Gustafsson et al. 2000.)
2.1.5 CNS leukaemia

2.1.5.1 Pathogenesis and symptoms of CNS disease in ALL

Leukaemic involvement of the CNS was observed as early as 1823 by the anatomist Burns referred by Moore and colleagues (Moore et al. 1960). Price and Johnson described the pathological features of CNS leukaemia in 1973. Infiltration results from proliferation of cells in the walls of superficial arachnoid veins, which seed at the time of diagnosis, when there is a large amount of leukaemic cells. These cells, which remain inaccessible to systemic chemotherapy, proliferate and destroy the arachnoid trabeculae by penetrating into the channels of cerebrospinal fluid (CSF) circulation. The brain lesions include gliosis, necrosis, cerebral hemorrhage and nonhemorrhagic degenerative encephalopathy. (Price & Johnson 1973.) Another histopathological study showed leukaemic infiltration in the arachnoid to result in obstruction of CSF flow, increased intracranial pressure and hydrocephalus (Moore et al. 1960).

The symptoms of CNS leukaemia are most often due to a raised intracranial pressure, which causes headache, vomiting, papilledema, nuchal rigidity and sometimes diplopia. Cranial nerve palsies may also occur (Bleyer & Poplack 1985, Burger et al. 2003). CNS leukaemia is detected in less than 5% of the children with ALL at diagnosis (Schrappe et al. 2000b). With the use of CNS treatment and routine surveillance lumbar punctures, CNS leukaemia is most often asymptomatic when it is diagnosed (Ochs 1989, Burger et al. 2003).

2.1.5.2 Risk factors for CNS disease

ALL is a heterogenous disease with several risk factors. There have been attempts to define groups of patients at high risk of CNS leukaemia. It has been shown that the patients with a high WBC count at the time of diagnosis have an early risk of CNS relapse, and the risk of CNS leukaemia correlates inversely with the platelet count (West et al. 1972). Other risk groups consist of infants (Silverman et al. 1997) and patients with B-cell leukaemia (Hann et al. 1990). Patients with a high WBC count, high hemoglobin, organomegaly, male sex and an age older than 10 years have been shown to be at a high risk of CNS relapse (Steinherz et al. 1991). The expression of CD56 has been examined in haematological malignancies, and the expression in leukaemic cells in ALL may be associated with an increased risk of CNS disease (Ravandi et al. 2002).

2.1.5.3 Definition and diagnosis of CNS leukaemia

A clear definition of CNS disease is essential, although there is still some controversy as to the most widespread definition of CNS leukaemia. The usual clinical definition is a finding of more than five leukocytes per microliter of CSF in the presence of lymphoblasts after cytocentrifugation (Mastrangelo et al. 1986). Exact diagnosis might be
difficult, especially when the CSF contains less than five leukocytes per microliter or is contaminated by peripheral blood. Some clinicians have reported that these patients have an increased risk of CNS relapse among children with ALL (Mahmoud et al. 1993), but some others have disputed this assessment (Gilchrist et al. 1994, van den Berg et al. 1995a). Since any number of identifiable leukaemic cells in CSF at diagnosis had conferred a poor prognosis, a new classification of CNS status at diagnosis was proposed: CNS1 denotes the absence of identifiable leukaemic blast cells in CSF; CNS2 the presence of leukaemic cells in a sample that contains fewer than five WBC per microliter; and CNS3 a nontraumatic sample that contains \( \geq 5 \) WBC per microliter with identifiable blasts or the presence of a cerebral mass or cranial nerve palsy with leukaemic cells in CSF (Mahmoud et al. 1993). Traumatic lumbar puncture \( \geq 10 \) erythrocytes per microliter of CSF at the time of diagnosis can adversely affect the treatment outcome of children with ALL (Gajjar et al. 2000). A recently published study reported CNS2 patients to have the same prognosis as patients with CNS1 status, whereas the 5-year EFS of patients after traumatic lumbar puncture at diagnosis is inferior to CNS1 (73% compared to 80%), but superior to CNS3 patients (73% compared to 50%). The patients with traumatic lumbar puncture had an increased incidence of CNS relapses (8%). (Burger et al. 2003.)

Patients with cranial neuropathy or cerebral mass usually have blasts in their CSF, but it may be necessary to make the diagnosis with these symptoms in the absence of abnormal CSF findings, particularly in B-ALL (Chessells 1994, Krishnamurthy et al. 2002). Although repeated analyses of CSF are considered sensitive method of documenting infiltration of the meninges by malignant cells, CNS leukaemia may exist without any detectable leukaemic cells in the CSF (Chessells 1994). In up to 50% of the cases, it is seen at autopsy following the natural course of the disease (Price & Johnson 1973).

As an additional method of cytological assessment of cells in CSF samples, staining for terminal deoxynucleotidyl transferase (TdT) in the CSF has been suggested to correlate strongly with the occurrence of CNS leukaemia in patients with TdT-positive leukaemia (Hooijkaas et al. 1989). Some authors have tested soluble factors (Jeffery et al. 1990) and immunocytochemical (Dagdemir et al. 1998) and molecular techniques (Galoin et al. 1997) in the detection of CNS involvement. Flow cytometry has also been used to confirm CNS leukaemia and to eliminate other conditions (Subira et al. 2002).

### 2.1.6 Current treatment of leukaemia

The improved rate of cure of ALL can be largely attributed to the development of more effective chemotherapeutic regimens in well-designed clinical trials (Eden et al. 2000, Harms & Janka-Schaub 2000). The basic approach to therapy consists of a remission induction phase followed by intensification (consolidation) treatment and then by prolonged maintenance therapy. Treatment of subclinical leukaemia of the CNS is initiated early and continued for variable lengths of time, depending on the treatment protocol. (Pui et al. 2001.)
2.1.6.1 Remission induction phase

The goal of the first month of therapy is to induce remission. Patients who achieve immunological or molecular remission (i.e. leukaemic involvement of < 0.01% of nucleated bone marrow cells at the end of remission induction therapy) are predicted to have a better clinical outcome than patients whose remission is defined solely by morphological criteria (Pui & Campana 2000). Morphological remission is defined as no clinical symptoms or signs of disease, a normal blood cell count and normocellular bone marrow with less than 5% blast cells (Pui & Crist 1994). Morphological examination is, however, subjective and quite limited in sensitivity. To be detected with certainty, leukaemic blast cells must constitute 1–5% of the total nucleated cell population (van Dongen et al. 1998).

The backbone of induction therapy in many treatment protocols consists of vincristine and daily corticosteroid, often combined with L-asparaginase and/or an anthracycline (Crist et al. 1992). The induction regimen in the Nordic protocol includes intravenous prednisolone, vincristine and doxorubicin, and intramuscular L-asparaginase as well as intrathecal Mtx. ALL patients with unfavourable features also receive intravenous cyclophosphamide and cytosine arabinoside and oral 6-mercaptopurine. With improvements in chemotherapy and supportive care, the rate of complete remission is 98% in the Nordic countries (Gustafsson et al. 2000).

Especially concerning the group of HR patients, the intensification of induction therapy has been under investigation. The aim of more intensive induction therapy is more rapid and profound reduction of the leukaemic cell burden, to prevent the development of drug resistance in leukaemic cells (Pui et al. 2001). More intensive induction therapy may also lead to increased early morbidity and mortality (Hurwitz et al. 2000) and may not be necessary if the patient receives post-induction intensification therapy (Harms & Janka-Schaub 2000).

Early response to therapy has been considered a consistent independent prognostic factor in childhood ALL, and it has also been used to prescribe treatments (Gaynon et al. 1997). Assessment of the early response to treatment by measuring minimal residual disease (MRD) is one of the most powerful and independent prognostic indicators (Coustan-Smith et al. 1998). Persistence of lymphoblasts (even at a level of 1%–4%) on day 15 of remission induction was associated with a poor prognosis, and residual disease of this extent on the days 22 and 25 signified a particularly dismal outcome, suggesting a need for more intensive treatment (Sandlund et al. 2002). In contrast to the report of Coustan-Smith and colleagues (Coustan-Smith et al. 1998), the data reported by van Dongen and colleagues indicated that analysis of MRD at a single time point is not sufficient for the recognition of either patients with a poor prognosis or patients with a good prognosis (van Dongen et al. 1998). This result is in agreement with the recent report pointing out the importance of detecting residual disease even at the end of therapy, which provides additional prognostic information independent of that obtained at the end of induction (Marshall et al. 2003).

Several methods have been developed to detect submicroscopic levels of leukaemia in patients with ALL (Campana & Pui 1995). Flow cytometric detection of aberrant immunophenotypes, polymerase chain reaction (PCR) analysis of breakpoint fusion regions of chromosome aberrations, and detection of clone-specific immunoglobulin and
T-cell receptor gene arrangements by PCR amplifications appear to be the most reliable (Campana & Pui 1995, Campana & Coustan-Smith 1999, de Haas et al. 2001). MRD studies provide direct measurements of leukaemic cell responses to chemotherapy in individual patients. This information can be used to improve the strategies of risk assessment and the choice of treatment in the management of children with ALL. Patients with less than 0.01% leukaemic cells at the end of remission induction are likely to have an excellent treatment outcome, whereas more intensive treatments should be considered for patients with high levels (≥ 1%) of MRD at the end of the induction phase or persistent disease during early continuation therapy. (Coustan-Smith et al. 2000.)

2.1.6.2 Consolidation and intensification phase

Following the restoration of normal hematopoiesis, patients in remission enter the next phase, called consolidation therapy. This treatment, which is administered shortly after the induction of remission, includes several drugs, most often intrathecal and high-dose intravenous Mtx with or without oral 6-mercaptopurine and intravenous cytosine arabinoside (Gustafsson et al. 2000). The regimen called delayed intensification includes a combination of intravenous dexamethasone, vincristine and daunorubicin/doxorubicine, and intramuscular L-asparaginase and oral thioguanine given with or without intravenous cyclophosphamide in addition to intravenous cytosine arabinoside and intrathecal Mtx. Childhood ALL patients with standard therapy do not receive delayed intensification treatment. CNS consolidation for ALL patients with unfavourable features consists of two alternating courses of high-dose Mtx and high-dose cytosine arabinoside intravenously before a delayed intensification phase. This group of patients receive other alternating courses of high-dose Mtx and high-dose cytosine arabinoside before interim maintenance with intravenous vincristine, oral dexamethasone, oral 6-mercaptopurine and weekly oral Mtx. A fourth course of high-dose Mtx and high-dose cytosine arabinoside consolidation is administered before starting maintenance therapy according to the Nordic protocol.

2.1.6.3 Maintenance phase

With the exception of patients with mature B-cell leukaemia, children with ALL require prolonged continuation of treatment for reasons that are poorly understood. The general rule is to continue the treatment for 2.0–2.5 years after the diagnosis. (Pui et al. 2001.) The combination of weekly oral Mtx and daily 6-mercaptopurine constitutes the backbone of the continuation regimen. The dose is tailored to the limits of tolerance measured by neutrophil counts (Chessells et al. 1997). In addition, intermittent pulses of intravenous vincristine and intrathecal and intravenous Mtx are also given. Dexamethasone has been substituted for prednisolone in many clinical trials because of its better clinical efficacy (Gaynon et al. 2000), and it is given orally in five-day pulses together with intravenous vincristine in the Nordic protocol.
As maintenance therapy, the patients with intensive treatment receive oral Mtx, hydroxyurea and thioguanine, intravenous cyclophosphamide, daunomycin, carbustine, cytosine arabinoside and vincristine and intrathecal Mtx. After two cycles of this combination, the patient continues on classic maintenance consisting of intravenous vincristine and oral dexamethasone pulses and continuous oral 6-mercaptopurine and weekly oral Mtx. The maintenance therapy is discontinued in this patient group two years after the initial diagnosis. The total duration of treatment in standard and intermediate therapy is 2.5 years in the Nordic protocol.

Patients with CNS disease at diagnosis receive therapeutic craniospinal irradiation with doses of 24 Gy cranial and 12 Gy spinal, which is optional. Patients stratified to receive very intensive treatment receive prophylactic CRT of 18 Gy, which is only given to children five years of age or older.

Philadelphia-chromosome-positive ALL and early haematological relapse are clear indications for haematopoietic stem cell transplantation (Arico et al. 2000). In the Nordic countries, allogeneic stem cell transplantation is a part of the extra intensive treatment protocol targeted to patients with leukocyte count > 200 x 10^9/L or very slow response, chromosomal translocation (4;11) or (9;22) or hypodiploidy < 34.

2.1.6.4 Subclinical treatment of CNS

The presence of overt CNS disease at the time of diagnosis negatively affects the EFS of children with ALL (Hammond et al. 1986). The effect of a small number of leukaemic blasts in the CSF at diagnosis on EFS is controversial (Mahmoud et al. 1993, Gilchrist et al. 1994). Patients with high-risk genetic features, T-lineage ALL, a large leukaemic-cell burden and leukaemic cells in the cerebrospinal fluid (even from iatrogenic introduction from a traumatic lumbar puncture) are at an increased risk of CNS relapse and require more intensive CNS-directed therapy (Gajjar et al. 2000).

High-dose intravenous Mtx generally has a marginal effect on the control of CNS leukaemia (Pui et al. 2001). High-dose Mtx and intrathecal Mtx together, however, reduced the risk of CNS relapse in one study, but did not affect other types of relapse or overall survival (Eden et al. 2000). Dexamethasone has been shown to improve CNS control (Gaynon et al. 2000), but it has been related to increased early morbidity and mortality (Hurwitz et al. 2000).

CRT is the most effective CNS-directed therapy. With regard to the late effects of CRT, i.e. secondary brain tumours and neurotoxicity, many treatment protocols use intensive intrathecal and systemic chemotherapy for 80–90% of patients (Pui et al. 2001). By using this combination and administering CRT as CNS-directed therapy to a selected group of patients, a CNS relapse rate of less than 5% has been attained in most studies (Pui et al. 1998, Schrappe et al. 2000a). The reduction of preventive CRT to 12 Gy in patients at higher risk within the medium risk group of relapse (large cell load, no initial CNS involvement) did not increase the rate of CNS-related relapse when effective systemic chemotherapy was used (Schrappe et al. 2000a).
2.2 Neurological side effects of different treatment modalities

2.2.1 Vincristine

Vincristine is an alkaloid derived from the periwinkle plant, Vinca Rosea. Vincristine kills cells by inhibiting the formation of the mitotic spindle, which causes the proliferating cells to die, thereby terminating morbid cell growth. Vincristine blocks mitotic cell division by bonding to tubulin molecules and inhibiting their polymerisation to microtubules, while the previously formed microtubules depolymerise. Microtubules are the primary structural element of the mitotic spindle. (Gidding et al. 1999.)

Vincristine, however, affects more than just leukaemic cells. It is predictably neurotoxic, which is the limiting factor in the use of the drug (Casey et al. 1973). Neurotoxic effects may be divided into four groups: peripheral neuropathy, autonomic neuropathy, cranial nerve neuropathy and encephalopathy (Tuxen & Hansen 1994).

2.2.1.1 Peripheral neuropathy

Therapeutic doses cause symmetrical peripheral sensory-motor neuropathy in nearly all patients treated for ALL (Bradley et al. 1970). In the peripheral nervous system, the drug rapidly induces alterations in the cellular micro-tubuli structure, which leads to oedema of the fast and slow conducting axons (Quasthoff & Hartung 2002). Pathological studies in vitro have shown vincristine-induced dose-related partial blockage of fast axoplasmic transport with concurrent disappearance of microtubules and appearance of paracrystals (Green et al. 1977). In an experiment, Cho and colleagues found giant axonal swellings and secondary demyelination of the paranodal type mainly in the proximal portions of the peripheral nerves outside the spinal canal (Cho et al. 1983). These proximal swellings were so profound that it was suggested that they could lead to secondary distal axonal lesions by blockage of axoplasmic transport subsequent to the structural changes in microtubules and neurofilaments exposed to vincristine (Sahenk et al. 1987).

The studies involving adult patients show that the earliest sign of vincristine-induced peripheral neuropathy is the depression of Achilles tendon reflexes, which might be asymptomatic in many cases (Kaplan & Wiernik 1982). The severity of peripheral neuropathy is related to the total dose and duration of therapy. With continued therapy, paresthesias, motor weakness and generalised depression of deep tendon reflexes have been reported. (Gidding et al. 1999.) Eventually, more than half of the patients have been reported to lose all reflexes. Paresthesia in the fingers and toes was the most common subjective complaint, occurring in about 50% of patients. (Sandler et al. 1969.) Due to muscular weakness, patients may develop clumsiness of the hands, slapping gait and foot drop. Muscle cramps and muscle weakness up to high-degree paresis of the distal muscles are characteristic of the advanced stage of this type of neuropathy. (Casey et al. 1973.)

Vincristine neurotoxicity is cumulative; the higher the drug concentration per dose, the shorter the intervals between the doses and the longer the therapy is continued, the greater is the degree of neurotoxicity (Gidding et al. 1999). Secondly, the patient’s age is
believed to be related to the degree of neurotoxicity; children are less susceptible than infants, adolescents and adults (Allen 1978), although different results have been reported (Hussain et al. 1993). Other possible predisposing factors include a poor nutritional condition, impaired performance status, liver dysfunction (Allen 1978) and prior disorders of the peripheral nervous system (Naumann et al. 2001). The authors of a pharmacokinetic study of vincristine concluded that administration of a standard dosage of vincristine to children with ALL resulted in highly variable systemic drug exposure, which may have implications for neurotoxicity (de Graaf et al. 1995). The combined use of vincristine and other chemotherapeutic agents, such as Mtx and L-asparaginase, may lead to synergistic neurotoxicity (Kaplan & Wiernik 1982).

2.2.1.2 Autonomic neuropathy

Vincristine-induced neurotoxicity in the autonomic system is mostly manifested as gastrointestinal dysfunction, such as colicky abdominal pain and constipation, which are the earliest signs occurring after a few days of drug administration (Holland et al. 1973). Paralytic ileus may develop later in the affected patients (Casey et al. 1973, Holland et al. 1973). Transient autonomic neuropathy, measured as reduced heart rate variability, has also been reported to be a frequent complication of vincristine treatment (Hirvonen et al. 1989). Other signs of autonomic nervous system dysfunction are orthostatic hypotension and urinary bladder dysfunction (Sandler et al. 1969, Bradley et al. 1970).

2.2.1.3 Cranial nerve findings and encephalopathy

Neurotoxicity in the cranial nerves is manifested as bilateral ptosis and reduced facial expressivity during therapy (Sandler et al. 1969). Transient cortical blindness (Byrd et al. 1981), diplopia with ophthalmoplegias and photophobia may also occur (Sandler et al. 1969).

Vincristine therapy may also lead to encephalopathy with seizures (Hurwitz et al. 1988) and to a syndrome involving inappropriate antidiuretic hormone secretion (Slater et al. 1969). These side effects of vincristine therapy are uncommon.

2.2.1.4 Findings in evoked potentials of the nervous system

Nerve conduction studies have shown normal or slightly abnormal distal motor latencies and conduction velocities in motor or sensory nerves. The amplitude is decreased in both motor and sensory nerves (Casey et al. 1973, Caccia et al. 1977). Electroneuromyographic examinations have identified vincristine-related neuropathy in the distal parts of peripheral nerves, indicating axonal neuropathy and only slight reduction in conduction velocity (McLeod & Penny 1969, Bradley et al. 1970). A histological study
of sural nerves showed vincristine-related damage in fibres of both large and small diameter (McLeod & Penny 1969).

In a study of 38 children with somatosensory evoked potentials (SEPs), lesions were seen in the entire nervous system, suggesting that demyelination may be more important in the pathogenesis of vincristine neuropathy than is commonly thought (Vainionpää et al. 1995). The study based on SEPs shortly after treatment with intrathecal Mtx also showed disturbed nerve conduction within the spinal cord in children with ALL (Vainionpää et al. 1997). SEPs have shown long-standing axonal loss throughout the nervous system and demyelination within the spinal cord two years after treatment (Harila-Saari et al. 1998).

In a previous investigation of MEPs at the end of therapy of children treated for ALL, significantly prolonged latencies were found within the entire motor pathway as well as significantly decreased MEP amplitudes in the peripheral motor nerves, indicating both demyelination and a loss of descending motor fibers or muscle fibers (Harila-Saari et al. 2001). Prolongation of visual evoked potential (VEP) latencies has been observed after radiation, although it may also be induced by chemotherapy only (Russo et al. 1985, Russo & Schiliro 1987).

### 2.2.2 Methotrexate

Mtx is an analogue of folic acid. Its primary mechanism is the inhibition of dihydrofolate reductase, which results in deprivation of cells of tetrahydrofolic acid necessary for cellular reproduction (Shuper et al. 2000).

The increasing use of Mtx has been accompanied by increased neurotoxicity (Mahoney et al. 1998). This neurotoxicity is even more severe in combination with CRT, probably due to the interruption of the blood-brain barrier by radiation (Griffin et al. 1977). Mtx is used together with other drugs, such as cytosine arabinoside, which also has a significant toxic feature (Ochs 1989). Still, Mtx is usually assumed to be the most important causative factor of the neurotoxicity related to cancer treatment, while the other treatments contribute an additional effect (Shuper et al. 2000).

Mtx can influence the CNS through several metabolic pathways. Mtx may interfere with adenosine, homocysteine and bioppterin metabolism (Quinn & Kamen 1996). Dihydrofolate reductase is required to maintain the cellular pool of tetrahydrofolate during thymidylate synthesis by methylation from deoxyuridylate (Shuper et al. 2000). Long-term administration of intramuscular Mtx 4 mg/kg to monkeys weekly for one year resulted in a significant decrease in the folate content of especially the brain (Winick et al. 1987). Folate deficiency is associated with a consequent reduction of S-adenosylmethionine concentrations. S-adenosylmethionine plays a role in many transmethylation reactions needed in transmitter metabolism. (Bottiglieri et al. 1994.) S-adenosylmethionine is known to be important in the maintenance of the myelin sheath, and its deficiency is presumed to cause the demyelination observed during the treatment of childhood ALL (Shuper et al. 2000). Some researchers have suggested that the S-adenosylmethionine deficiency might cause demyelination by reducing the methylation of the myelin basic protein and thereby leading to CNS damage (Surtees et al. 1998).
Elevated adenosine concentrations in the CSF have been demonstrated in children receiving Mtx, who also show elevated plasma homocysteine (Refsum et al. 1991, Sciotti & Van Wylen 1993, Bernini et al. 1995). Adenosine has the capacity to dilate cerebral blood vessels, slow down the release of neurotransmitters at the presynaptic junction and slow the neuronal discharge. It is plausible that adenosine accumulation could be neurotoxic. (Quinn & Kamen 1996.) Subacute Mtx neurotoxicity may be mediated by adenosine and relieved by aminophylline (Bernini et al. 1995). Folate and cobalamin deficiencies lead to hyperhomocystinemia (Refsum et al. 1991). Homocysteine is believed to be directly toxic to the vascular endothelium and a potential cause of vascular disease resulting in stroke, myocardial infarction and venous thromboembolism (van den Berg et al. 1995b, Shuper et al. 2000). Mtx can cause neurological deficits (Meadows et al. 1981), mineralising microangiopathy (Vazquez et al. 2002) and ischemic white matter changes (Wilson et al. 1991), which are visible in radiography and suggestive of a vascular disease.

Mtx has been reported to influence cerebral biopterin metabolism by inhibiting dihydropteride reductase and thereby tetrahydrobiopterin synthesis, which is needed at the initial steps of biogenic amine synthesis, and to reduce the synthesis of dopamine and serotonin (Millot et al. 1995, Quinn & Kamen 1996).

Mtx neurotoxicity and its effects can be categorised as immediate, acute to subacute or delayed neurologic symptoms (Bleyer 1981). While some patients have been reported to suffer from merely temporary neurological abnormalities, such as headache, nausea, vomiting, fever, back pain and CSF abnormalities, including clinical symptoms of arachnoiditis (also known as chemical meningitis) (Jaffe et al. 1985), some others may experience quite severe neurotoxicity leading to permanent neurological deficits (Ch'ien et al. 1981).

The immediate CNS dysfunction after intravenous administration of high doses of Mtx usually occurs within one day after the administration. The symptoms and signs are similar to chemical meningitis after an intrathecal injection of Mtx (Bleyer 1981). An unusual complication of intrathecal Mtx is spinal cord myelopathy and transient or permanent paraplegia (Gagliano & Costanzi 1976, Ochs 1989).

The acute to subacute syndrome occurs within days to several days after the administration of Mtx. The symptoms of this disorder include seizures, affective disturbance or sudden onset of focal neurological deficits, which are usually transient and manifested as paresis, blurred vision, aphasia and pseudobulbar palsy (Ochs 1989, Mahoney et al. 1998). Hemiparesis associated with facial nerve palsy and dysarthria as well as ischemic lesions seen in imaging studies have also been reported (Yim et al. 1991). Elevated myelin basic protein in the CSF has been reported in association with this syndrome, indicating demyelination (Clark et al. 1982).

The delayed form of Mtx neurotoxicity appears weeks to months after therapy, and it varies in severity. It is characterised by leukoencephalopathy and a decrease in neuropsychologic and higher cognitive functioning (Ochs 1989, Copeland et al. 1996). The syndrome is much more severe if radiation is used as well (Bleyer 1981). Mainly background slowing in electroencephalogram (EEG) has been observed in association with the syndrome (Ueberall et al. 1997).
2.2.3 Corticosteroids

Glucocorticoid receptors have an important role in mediating the antileukaemic mechanism of corticosteroids. The proportionally higher free plasma levels of dexamethasone allow more extensive penetration into the CSF than prednisolone, of which over 90% is bound to transcortin or other plasma proteins. (Balis et al. 1987.) Dexamethasone also has a longer half-life and a longer duration of biologic action than prednisolone. These findings might explain the lower incidence of meningeal leukaemia in children receiving dexamethasone for the treatment of ALL. (Jones et al. 1991.)

Glucocorticoid receptors are widely expressed throughout the brain, especially in the hippocampal dentate gyrus, the periventricular nucleus of the hypothalamus, the amygdala and the prefrontal cortex. Hippocampal pyramidal neurons appear to be highly vulnerable to either hypercortisolemia caused by severe stress or to exposure to exogenous glucocorticoids. (Uno et al. 1994.) The hippocampus has been the main target in investigating glucocorticoid-related cognitive impairments because it contains large proportions of glucocorticoid receptors (Type I and II) and also plays an established role in declarative memory processes (Alderson & Novack 2002). Dexamethasone, which does not readily enter brain tissue, preferentially occupies pituitary receptors with very little occupancy of Type II hippocampal receptors. However, prednisolone is structurally more similar to cortisol than dexamethasone and therefore likely to have a high affinity for Type II glucocorticoid hippocampal receptors. (McEwen 1997.) The relationship between memory impairments and prednisolone, but not dexamethasone, appears to support a mediating role of Type II glucocorticoid hippocampal receptors in glucocorticoid-related memory impairments (Alderson & Novack 2002). Direct exposure to glucocorticoids may decrease dendritic branching, alter the synaptic terminal structure, increase extracellular glutamate accumulation and decrease the number of neurons in the CA3 (the part of hippocampus known as the CA region) hippocampal subfield. In addition, hippocampal neurons are affected by prolonged exposure to high circulating levels of corticosterone. (Loring & Meador 2000.) Even short-term elevations in glucocorticoid concentrations may result in cognitive changes in children (Bender et al. 1988).

Corticosteroids might increase the vulnerability of the striatum and therefore cause dopaminergic neurotoxicity (Johnson et al. 2002). Corticosteroids may also cause transient brain atrophy visualised by imaging studies (Bentson et al. 1978). In addition, corticosteroids are important in interactions with other treatments causing neurotoxicity (Mullenix et al. 1994).

2.2.4 Cytosine arabinoside

Cytosine arabinoside (Ara-C, cytarabine) is an effective drug, which can be used intravenously or intrathecally in treating leukaemia or lymphoma. This pyrimidine nucleoside analogue competitively inhibits DNA polymerase in replicating cells. Cytarabine neurotoxicity is a rare complication of treatment administered in systemic conventional doses (100–200 mg/m²/day for 5 to 7 consecutive days), but following more
frequent use of high-dose infusions of cytarabine (usually 2–3g/m² every 12 h for 6–12 doses), toxicity of a unique type has emerged. (Ochs 1989.) The predominant CNS toxicity after high-dose cytarabine treatment is cerebellar dysfunction characterised by ataxia, nystagmus and dysarthria within three to eight days after treatment (Lazarus et al. 1981, Winkelman & Hines 1983, Herzig et al. 1987). In addition, cerebral dysfunction causing somnolence, confusion, personality changes or seizures (Hwang et al. 1985) and peripheral neuropathy have been reported (Borgeat et al. 1986). The peripheral neuropathy may vary in severity. The symptoms of CNS dysfunction often resolve within two days to a few weeks after the manifestation of neurotoxicity, but long-term neurotoxicity has also been reported (Lazarus et al. 1981, Winkelman & Hines 1983).

EEG shows slow wave activity related to neurotoxicity (Hwang et al. 1985). CT and MRI scans of the brain are nearly always normal (Vera et al. 1999), but case reports have described high signal intensity lesions in the central white matter and the cerebellum as well as basal ganglia necrosis (Patel & Rao 1996, Sirvent et al. 1998, Vaughn et al. 1993). Diffuse heterogenous brain hypoperfusion identified by SPECT has also been reported in high-dose cytarabine neurotoxicity (Vera et al. 1999). In pathologic studies, loss of Purkinje cells and other morphological changes in the cerebellum have been demonstrated (Salinsky et al. 1983).

Neurotoxicity attributed to intrathecal cytarabine is manifested as paraparesis or seizures (Dunton et al. 1986). The complications may be reversible, but myelopathy after intrathecal administration of cytarabine might be incompletely reversible. Cytarabine in conjunction with CRT may lead to necrotising leukoencephalopathy. (Baker et al. 1991.) Most studies of cytarabine neurotoxicity are based on adult series, and there are relatively few case reports of neurotoxicity in children (Eden et al. 1978, Dunton et al. 1986, Shaw et al. 1991).

2.2.5 L-asparaginase

The enzyme L-asparaginase catalyses the hydrolysis of the amino acid L-asparagine to aspartatic acid and ammonia. Most tissues have asparagine synthetase activity and do not require exogenous sources of asparagines. Some tumour cells do not have this synthetic ability, and in such cases L-asparaginase may be therapeutic by depriving tumour cells of an essential amino acid. The enzyme has been useful in the treatment of children with ALL. (Muller & Boos 1998.)

A wide range of adverse effects have been related to L-asparaginase therapy. Acute encephalopathy associated with L-asparaginase is characterised by somnolence, lethargy, disorientation, seizures and coma, which may be related to hyperammonemia (Leonard & Kay 1986, Labs & Poplack 1989). L-asparaginase causes deficiencies and imbalance in the coagulation process and may lead to thrombotic and haemorrhagic complications within two to three weeks from the beginning of the therapy, including strokes and intracranial haemorrhages with typical changes on brain CT or MRI images reported in 1% to 3% of children with ALL (Priest et al. 1982, Labs & Poplack 1989, Kingma et al. 1993). Transient slowing in EEG, probably caused by metabolic disturbances, has been associated with L-asparaginase treatment (Moure et al. 1970, Korinthenberg et al. 1990).
L-asparaginase has been associated with reduced muscular strength (Hovi et al. 1993). In addition, the changes in the endocrine pancreatic function, especially in the form of an impaired glucose metabolism, are observed under L-asparaginase treatment (Muller & Boos 1998). A major complication of L-asparaginase therapy in ALL is pancreatitis, reported in 2–10% of the patients (Whitecar et al. 1970, Sahu et al. 1998).

Evidence of encephalopathy often appears during the first day after the administration of L-asparaginase (Pratt et al. 1971). Most frequently, the mild somnolence or confusion disappears within some days after the end of the course of treatment (Weiss et al. 1974).

Table 2. Main types of neurotoxicity due to chemotherapy used in the treatment of ALL.

<table>
<thead>
<tr>
<th>Chemotherapy agent</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine</td>
<td>autonomic neuropathy</td>
</tr>
<tr>
<td></td>
<td>encephalopathy</td>
</tr>
<tr>
<td></td>
<td>peripheral neuropathy</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>encephalopathy</td>
</tr>
<tr>
<td></td>
<td>chronic leukoencephalopathy</td>
</tr>
<tr>
<td></td>
<td>meningeal irritation</td>
</tr>
<tr>
<td></td>
<td>paraplegia</td>
</tr>
<tr>
<td></td>
<td>reversible stroke-like episodes</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>behavioural abnormalities</td>
</tr>
<tr>
<td></td>
<td>memory dysfunctions</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>encephalopathy and cerebellar dysfunction</td>
</tr>
<tr>
<td></td>
<td>peripheral neuropathy</td>
</tr>
<tr>
<td></td>
<td>paraplegia</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>encephalopathy</td>
</tr>
<tr>
<td></td>
<td>confusion and behavioural abnormalities</td>
</tr>
<tr>
<td></td>
<td>lethargy</td>
</tr>
<tr>
<td></td>
<td>muscle weakness</td>
</tr>
</tbody>
</table>

2.2.6 Cranial radiation therapy

The clinical neurotoxic reactions may be classified according to the time of onset after the initiation of CNS therapy. Acute reactions occur within hours to days after the commencement of treatment, subacute reactions begin days to weeks later, and delayed reactions are noted several months or years later. (Bleyer 1981.)

Acute reactions are mostly mild, consisting of nausea, vomiting, headache and drowsiness. The reactions are dose-dependent, and the symptoms usually subside spontaneously. (Bleyer 1981.)

More commonly, neurotoxic reactions are subacute, transient somnolence syndrome being the most common subacute neurotoxic reaction. Somnolence syndrome is primarily characterised by drowsiness, nausea and malaise, but may also include irritability, fever, ataxia, anorexia and severe tiredness. (Freeman et al. 1973.) EEG recordings nearly always show diffuse general slowing during the postirradiation syndrome (Garwicz et al. 1975), and CSF pleocytosis is occasionally present (Bleyer 1981). The pathophysiology of subacute changes has been attributed to both direct effects on the proliferating oligodendrocytes, resulting in transient demyelination, and temporary changes in the blood-brain barrier (Schultheiss et al. 1995). The symptoms usually disappear within one
to three weeks, but there is evidence that learning difficulties occur after CRT more often in patients who have had the somnolence syndrome than in patients without this syndrome (Ch'ien et al. 1980). Reduction of the total dose from 24 Gy to 18 Gy has lessened the neurotoxicity of CRT to acceptable levels, causing relatively limited late neurotoxicity in patients receiving 18 Gy of CRT (Halberg et al. 1992, Waber et al. 2001). Combination therapy including high-dose Mtx and CRT has been associated with IQ decline, especially in females (Waber et al. 1995).

Cerebral necrosis is a devastating form of delayed neurotoxicity, which only occurs in cases where the whole brain has been exposed to a cumulative radiation dose of more than 60 Gy (Bleyer 1981). The onset of a necrotic reaction typically occurs six months to three years post therapy. Symptoms range from mild confusion to motor, sensory and/or speech deficits, seizures, symptoms of increased cranial pressure and progressive dementia. (Schultheiss et al. 1995.) The condition is usually fatal, unless a focal area of radionecrosis can be surgically removed (Meister & Meadows 1993).

Necrotising leukoencephalopathy (NLE) presents with symptoms ranging from mild lassitude or personality change to marked dementia, spasticity, ataxia and pseudobulbar paresis 4 to 12 months after the completion of CRT (Packer et al. 1987). Pathologically, the disorder consists of multifocal areas of coagulation necrosis deep in the white matter (Price & Jamieson 1975). The etiology is most commonly multifactorial, and a relationship between the incidence of leukoencephalopathy and the radiation dose or intrathecal and intravenous chemotherapy has been reported (Rubinstein et al. 1975). Almost all patients in whom NLE developed after CRT had received doses higher than 20 Gy (Packer et al. 1987).

Radiation is also the prime cause of mineralising microangiopathy with dystrophic calcification of brain tissue (Packer et al. 1987), which has been related to intracranial calcifications correlating with memory deficits and reduction of IQ (Brouwers & Poplack 1990). Furthermore, ALL survivors have an increased risk of secondary brain tumours (Garwicz et al. 2000).

2.3 Late neurological effects

2.3.1 Impaired motor competence

Late effects of cancer treatment have been reported in several organ systems. Neurological late effects in the CNS and peripheral nervous system may be manifested as encephalopathy, peripheral neuropathy or neuropsychological and intellectual dysfunction (Byrd 1985).

Peripheral neuropathy due to vincristine is a fairly common unwanted effect manifested in children treated for ALL as impaired motor function during treatment (Vainionpää 1993).

Visual motor integration (VMI) is used to assess visual perceptual skills and fine motor coordination. In a study by Whitt and colleagues, no differences were found between radiated and non-radiated children, but both groups performed worse than the population norms in fine motor coordination and visuo-perceptual organisation. (Whitt et al. 1984.)
These results are in agreement with another study, in which one third of both groups had slight impairment in psychomotor skills (Harten et al. 1984). Radiated children have, however, been reported to perform worse than non-radiated children on the VMI and on tests for fine motor skills (Copeland et al. 1985). In another study, a group of children with leukaemia treated with vincristine were compared to a group of children with solid tumours treated without vincristine. Neither group had received irradiation. The fine motor problems found in the leukaemia group were therefore interpreted as vincristine neuropathy. (Copeland et al. 1988.)

In neurological evaluation of 40 children with ALL, 18–30% of the entire patient group showed fine and gross motor dysfunction, as well as suppression and restoration of Achilles and patella reflexes. The most severe walking difficulties occurred in the youngest patients. Among ALL survivors who were not irradiated, disorders in gross motor functioning were most apparent during treatment, while fine motor dysfunctions did not arise until two to three years after the therapy (in 33% of the children). (Vainionpää 1993.) The results of this neurological survey agree with the late-effect study which showed leukaemia survivors to have fine motor problems two years or more after the cessation of treatment for ALL, suggesting that approximately 25% of leukaemia survivors have handwriting problems (Reinders-Messelink et al. 1996).

Gross motor reactions were studied by Wright and colleagues, and they reported musculoskeletal impairment and difficulties in balance, walking and running in ALL survivors compared to age- and gender-matched controls (Wright et al. 1998). In another study, a limited ankle dorsiflexion range of motion (DF-ROM) was reported in ALL survivors. This impairment may restrict gross motor activities, such as walking and climbing stairs. Females and children diagnosed at a younger age were especially at risk. (Wright et al. 1999.) Both studies have included radiated and non-radiated children, revealing no significant differences between these groups. In a study reported by MacLean and colleagues, radiated children performed less well than non-radiated children. However, sub-average motor performance was found in both groups, suggesting peripheral side effects of systemically administered chemotherapies, i.e. vincristine. (MacLean et al. 1995.)

Studies including radiated children have reported gross motor problems after treatment (MacLean et al. 1995, Wright et al. 1998, Wright et al. 1999), but in ALL survivors without radiation therapy, mainly fine motor difficulties have been seen after treatment (Reinders-Messelink et al. 1996, Kaleita et al. 1999). These results suggest a relationship between CRT and gross motor dysfunctions after treatment. Fine motor dysfunction has, however, been reported in both radiated (Whitt et al. 1984) and non-radiated children (Reinders-Messelink et al. 1996, Copeland et al. 1988), suggesting that fine motor problems after therapy are not only related to CRT.

### 2.3.2 Structural changes of CNS

Delayed treatment-related neurological damage is becoming increasingly important now that more and more children survive cancer treatments. After modification of the treatment protocols, severe symptomatic late effects are rare, and most adverse effects are
detected by sensitive imaging methods, such as MRI, SPECT or PET, or by neuropsychological testing (Wilson et al. 1991, Harila-Saari et al. 1997, Kähkönen et al. 1999). MRI has been found more sensitive than CT in detecting treatment-related changes in ALL survivors, except in identifying calcifications, which are better found by CT (Pääkkö et al. 1992). Calcifications, detected by roentgenograms of the skull, were the first reported structural CNS findings in children with ALL (Mueller et al. 1976). Shortly after that, cranial CT was used to detect diffuse subcortical cerebral calcifications (McIntosh et al. 1977).

In long-term survivors of ALL, the incidence of white matter changes seen on MRI has varied between 0 and 53% (Kramer et al. 1988, Bakke et al. 1993). The incidence of leukoencephalopathy during therapy has also been variable (9–68%), and the lesions have often been reversible (Wilson et al. 1991, Pääkkö et al. 2000). In treatment-related leukoencephalopathy, the variations may be due to heterogeneity of the patient group and differences in the treatment protocols. Young children with immature brains may be more susceptible to develop the kind of white matter changes seen on MRI (Pääkkö et al. 2000). White matter disease seems to be more evident when all the three modalities of treatment are used, including intrathecal and intravenous Mtx, and the syndrome is more severe if radiation therapy is used along with chemotherapy (Ochs et al. 1991, Copeland et al. 1996).

Other types of structural brain damage reported in childhood ALL patients are cerebrovascular complications that manifest as haemorrhage and thrombosis (Vazquez et al. 2002) and enlargement of ventricles and/or sulci indicative of cortical atrophy (Hertzberg et al. 1997). Dystrophic calcifications in the basal ganglia and subcortical white matter were relatively common findings at cranial CT in ALL survivors previously treated with CRT and intrathecal Mtx (Vazquez et al. 2002). Nowadays, with less toxic treatment protocols, subtle mineralising microangiopathy can be seen on MRI as a sign of increased putaminal signal intensity on T1-weighted images and decreased signal intensity on T2-weighted images (Shanley 1995). Secondary brain tumors, vasculopathy and cases of acquired vascular malformations have been reported after treatment for ALL (Laitt et al. 1995).

Efforts have been made to find a correlation between neuroimaging findings and cognitive impairment, but only a small or no correlation has been found (Iuvone et al. 2002, Mulhern et al. 1992, Wilson et al. 1991). Modern functional neuro-imaging methods have been used to study the defects related to treatment for childhood ALL. PET with $^{18}$F-fluorodeoxyglucose (FDG) has been used to evaluate cerebral glucose metabolism, and decrease has been seen in thalamus and white matter (Phillips et al. 1991, Kähkönen et al. 1999). No major differences were seen in regional cerebral glucose utilisation or in neurocognitive performance between chemotherapy-treated and irradiated long-term survivors of ALL in a report of 40 cases, but a high leukocyte count at diagnosis was found to inversely correlate with cerebral glucose utilisation (Kähkönen et al. 2000). On the other hand, another study of 11 long-term survivors of ALL showed lower glucose metabolic rates to associate with more neurocognitive deficits (Suhonen-Polvi et al. 1995). Cerebral blood flow abnormalities were seen in ALL survivors in SPECT during treatment, and the disturbance of regional cerebral blood flow was more pronounced in patients with neurological symptoms (Österlundh et al. 1997, Österlundh et al. 1999). Neurocognitive deficits have not been consistently associated with either the
changes in cerebral blood flow detected in SPECT or the decreased glucose utilisation seen in FDG-PET (Kähkönen et al. 1999).

### 2.3.3 Cognitive function

The effects of certain cancer therapies on intellectual and cognitive function were first reported in children who had survived leukaemia. In 1978, Eiser reported that intrathecal Mtx and CRT were associated with neuropsychological dysfunction (Eiser 1978). The full degree of deficits was not evident until three years or more after the diagnosis (Meadows et al. 1981). The role of CRT in intellectual decline has been most marked in children treated at a younger age, i.e. before the age of five years, and at higher cumulative doses (Meadows et al. 1981, Cousens et al. 1988). The decline in IQ has been reported to increase over time in irradiated survivors of ALL (Jankovic et al. 1994). Memory dysfunctions have been the most frequent specific effects in survivors of ALL, but attention deficits, slowness of processing and visuomotor difficulties have also been reported (Peckham et al. 1988, Brouwers & Poplack 1990, Cousens et al. 1991, Hill et al. 1997).

After the untoward role of CRT therapy was reported, the question of chemotherapy alone producing neurocognitive dysfunction emerged. The investigators at St Jude Children’s Hospital randomised 40 children to receive either 18 Gy CRT plus intrathecal Mtx or intrathecal Mtx plus intravenous high-dose Mtx. There were no treatment- or age-related differences in neuropsychological performance, regardless of whether or not they had received CRT. (Mulhern et al. 1988.) This result is in agreement with a later report showing that patients who had received only parenteral Mtx had neuropsychologic deficits of comparable frequency and severity as patients who had received 18 Gy CRT and intrathecal Mtx (Ochs et al. 1991). Furthermore, no differences in cognition were seen between groups given either moderate doses of intravenous and intrathecal Mtx without CRT or intrathecal Mtx plus 18 or 24 Gy CRT. However, each group showed significant deterioration (≥15 points) of IQ values or significant deviation from age norms, indicating adverse effects of Mtx alone as well (Mulhern et al. 1991). Likewise, a randomised study of 54 ALL patients who received intrathecal Mtx and cytarabine showed cognitive deficits of the same magnitude as those observed in patients who received 24 Gy irradiation (Giralt et al. 1992).

In contrast, Copeland and colleagues (Copeland et al. 1996) and Butler and colleagues (Butler et al. 1994) reported no important cognitive deficits in children treated with various protocols of chemotherapy only. Anderson and colleagues (Anderson et al. 1994) found that 50 children receiving chemotherapy only performed similarly to controls on intellectual tests at a mean age of 12 years, six years from diagnosis. Also, children who received cranial irradiation plus intrathecal Mtx had significantly poorer performance in neuropsychological tests than patients who received intrathecal Mtx only in a randomised study of 74 young children with ALL (MacLean et al. 1995). These results are in agreement with other studies, which have shown higher IQ scores following chemotherapy alone compared to chemotherapy and radiation (Butler et al. 1994, Hill et al. 1998, von der Weid 2001, Langer et al. 2002). The variety of results may be due to methodological restrictions and differences in study designs, patient characteristics, types of reference group and chemotherapeutic regimens (Butler & Copeland 1993).
Cumulative deficits have been reported in non-verbal and information processing skills for children treated with CRT and chemotherapy, with other deficits remaining relatively stable over time (Anderson et al. 2000). Treatment for childhood ALL with cranial irradiation and chemotherapy at a young age has been clearly associated with poorer academic achievement (Kingma et al. 2000).

Neurophysiological factors are assumed to underlie the cognitive impairment related to therapy for ALL. Auditory event-related potentials (ERPs); P300 and mismatch negativity (MMN) have been studied to find out the impaired attention orientation in asymptomatic cancer survivors. MMN can be used to measure preattentional auditory discrimination, which is suggested to be essential in reactions to auditory changes in the environment. Cancer survivors had prolonged P300 latencies of ERPs as an indication of prolonged short-term memory processing. The MMN parameters did not differ between the study group and the controls. (Lähteenmäki et al. 2001.) Delayed event-related desynchronisation of the background EEG have been detected in cancer survivors. This may indicate prolongation of the cortical information processing time. (Lähteenmäki et al. 1999.) Slowing down of cortical activity secondary to white matter damage may underlie the cognitive decline in children treated with intensive CNS therapies (Heukrodt et al. 1988, Moore et al. 1992).

The etiology of the changes in cognitive function described in the literature is most likely to be multifactorial. Numerous neurocognitive outcome studies have demonstrated that diagnosis at a younger age, i.e. before the age of five years, increases the risk for disabilities (Robison et al. 1984, Copeland et al. 1985, Jannoun & Chessells 1987, Waber et al. 1990, Hill et al. 1997), and that females treated for ALL have a greater risk of cognitive impairment than males (Bleyer et al. 1990, Kato et al. 1993, Christie et al. 1995). Different drugs may have different impacts on the developing brain. More intensive corticosteroid therapy using dexamethasone instead of prednisolone could have negative cognitive consequences (Waber et al. 2000, Kingma et al. 2002). Neurocognitive deficits may progress and become evident years after treatment, and they cannot be evaluated early while the treatment is still going on (Meadows et al. 1981).

Encouraging reports have, however, been published about neuropsychological late effects in contemporary protocols for treating ALL in children (Brown et al. 1999, Espy et al. 2001, Waber et al. 2001). No major differences were seen in school achievement between 20 non-irradiated ALL survivors and their siblings. The same patients had significantly lower test scores than healthy age- and socioeconomically matched controls in only two of the 14 cognitive items measuring intelligence and attention. Two measures (memory and attention) showed a better outcome in this study compared to the patients treated earlier, who had received higher cumulative doses of dexamethasone, vincristine and intrathecal Mt. (Kingma et al. 2002.) Another study revealed significant group differences between patients who had received chemotherapy and healthy control subjects in memory and fine-motor functioning. No significant differences were seen between nonirradiated patients and their healthy siblings in placement in special primary schools for the learning disabled or in the level of secondary education. (Kingma et al. 2001.) These reports provide evidence that the current therapies are less neurotoxic than those used 10–15 years ago, which is most likely due to the limitation of CRT to a selective group of patients.
3 Purpose of the research

The aim of this study was to evaluate treatment-related changes in the nervous system after childhood ALL and to study Mtx-related changes in the CNS in a controlled animal model.

The specific aims and questions were:

1. To evaluate the motor nervous system impairment in long-term survivors of childhood ALL. (I)
2. To evaluate if the detection of perfusion defects is possible by MRI in children after treatment for ALL and if so, whether the findings correlate with the brain perfusion SPECT results? (II)
3. To evaluate functional MRI as a method of observing Mtx-related changes in the central nervous system in an experimental study with swine. (III)
4. To evaluate if intravenously administered Mtx has an effect on brain perfusion in an animal model that can be detected by SPECT. (IV)
5. To evaluate the effect of Mtx on the amount of DAT density in the swine brain. (IV)
4 Subjects, materials and methods

The tables 3 and 4 summarise the subjects, materials and methods used in the papers I–IV. The subjects in the papers I and II were children treated for ALL in the Paediatric Clinic of Oulu University Hospital. The control subjects in paper I were healthy volunteers recruited from among the children of the hospital staff and from a local school.

### Table 3. Subjects and methods in the clinical studies (I, II).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Number</th>
<th>Subjects</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>27</td>
<td>ALL patients</td>
<td>Clinical examination and neurophysiological (MEP) examination</td>
</tr>
<tr>
<td>I</td>
<td>27</td>
<td>controls</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>19/17</td>
<td>ALL patients</td>
<td>Perfusion MRI (19) and perfusion SPECT (17)</td>
</tr>
</tbody>
</table>

### Table 4. Materials and methods used in the experimental studies (III, IV).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Number</th>
<th>Animals</th>
<th>Methotrexate exposure</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>6</td>
<td>Before Mtx exposure</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td>Functional MRI including perfusion, diffusion and BOLD contrast imaging</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>After Mtx exposure</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>Control animals</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>Before Mtx exposure</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td>Perfusion SPECT, [123I]β-CIT SPECT and whole-hemisphere autoradiography with [125I]β-CIT</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>After Mtx exposure</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>Control animals</td>
<td>5 g/m² x 2 or 2 g/m² x 5</td>
<td></td>
</tr>
</tbody>
</table>

### 4.1 Motor pathway lesions (I)

#### 4.1.1 Subjects

Five years after the cessation of therapy for childhood ALL, twenty-seven children were examined clinically by means of MEPs elicited by magnetic stimulation transcranially and at the spinal cord. The initial treatment had been completed between February 1989 and August 1992. The MEP recordings of the patients were carried out between April
1994 and May 1997. The study series consisted of 10 boys and 17 girls, ranging in age from 8 to 24 years (mean age 13.1 years), whose disease was in continuous remission.

The patients were stratified into three risk groups (SR, IR and HR) based on the criteria used in the Nordic countries (Gustafsson et al. 2000). The treatment protocol had been carried out as described in paper I.

The control children were matched to the patients with regard to age (maximum difference 1 year for subjects aged over 10 years and 6 months for those aged under 10 years), gender and height (maximum difference ± 3.0 cm). All had normal developmental data and no neurological signs or symptoms.

### 4.1.2 Motor evoked potentials

Motor responses were recorded using surface Ag/AgCl electrodes with the active leads placed on the motor points of the abductor pollicis brevis muscle in the hand and the tibialis anterior muscle in the leg. The reference electrodes were placed over the first mid-phalange on the hand and over the lateral malleolus on the leg. The responses were recorded with a conventional electromyography device (Viking II, Nicolet, USA).

A Magstim 2000 stimulator was used to deliver the magnetic stimuli (Magstim Company, Limited, UK).

Lower extremities: Cortical stimulation of the tibialis anterior muscles was performed with a double-cone coil placed anteriorly on the vertex at the middle line, using a stimulation intensity equal to 80% of the maximal output. The site of the maximal response was sought. The subjects were asked to activate the muscles at a force estimated aurally and by opposing the movement provoked by active muscle contraction. Root stimulation was performed with a circular coil (dimension of 90 mm) placed over the fifth lumbar vertebra (LV) without muscle activation at a stimulation intensity that was equal to 80% of the maximal output. Both clockwise and anticlockwise currents were used.

Upper extremities: A circular coil (dimension 90 mm) was used for cortical and plexus stimulation of hand responses. For cortical stimulation, the coil windings were positioned over the motor area of the hand, seeking the site of the maximum response. The left hemisphere was stimulated with a clockwise current flow in the coil and the right hemisphere with an anticlockwise current flow. Stimulation intensity was 90% of the maximal output. For plexus stimulation, the round coil was placed on the cervico-shoulder junction with the current flow towards the vertebral column at a stimulation intensity equal to 70% of the maximal output.

At least eight consecutive stimuli were given cortically in the examination of both the upper and the lower extremities. The shortest latency and the highest amplitude (peak to peak) were used for further analysis.
4.1.3 Neurological examination

Neurologic and clinical examinations were performed five years after the cessation of treatment. The clinical examination was performed according to Touwen (Touwen 1979).

4.2 Perfusion after treatment for childhood ALL (II)

4.2.1 Subjects

Nineteen children or young adults, consisting of 9 female and 10 male patients, underwent perfusion MRI and 17 (7 females and 10 males) patients were examined by brain perfusion SPECT after treatment for ALL. The initial diagnoses had been made between April 1987 and January 1998. Nine of the subjects were examined at the end of treatment and ten underwent the examination 4–8 years after the end of treatment. The mean age at diagnosis was 6.0 years, range 2.1–14.8 years. Nine of the children had been under five years of age at the time of diagnosis. The patients had been treated according to the Nordic protocol, with the exception of four patients in the IR group and one patient in the HR group, who received their treatment according to the Berlin-Frankfurt-Munster (BFM) 83 protocol (Gustafsson et al. 2000).

4.2.2 Perfusion MRI

MRI was performed using a 1.5-T scanner (Signa Echospeed, General Electric, Milwaukee, WI, USA). T1-weighted sagittal (repetition time (TR) 400 msec, echotime (TE) 9 msec, field of view (FOV) 23 cm, matrix 256 x 224, two number of excitations (NEX), slice 5 mm, gap 2 mm) and T2- and intermediate-weighted (TR 3500, TE 14/98, FOV 23 x 17, matrix 256 x 224, two NEX, slice 5 mm, gap 1 mm) axial images were obtained before the perfusion series. A contrast-enhanced T1-weighted coronal series was performed after perfusion (TR 600, TE 14, FOV 23 x 17, matrix 256 x 224, two NEX, slice 6 mm, gap 1 mm). Perfusion MRI was carried out using a Spin Echo-Echo Planar Imaging (SE-EPI) technique, TR 1500, TE 80, 128 x 128 matrix, 24–40 cm FOV. The sequence allowed the operator to obtain 10 slices with a 10 mm slice thickness and a 1 mm gap 46 times (= 460 images) during the 70 s acquisition with a time resolution of 1.5 s/image. A contrast agent bolus gadopentate dimeglumine (Magnevist®, Schering AG, Berlin, Germany) 0.2 mmol/kg body weight was injected into the antecubital vein at 3–5 ml/s, starting 10 s after the initiation of the scan and using a MR-compatible power injector (Spectris®, MR Injection System, Medral Europe B.V., Maastricht, Netherlands). Relative cerebral blood volume (CBV) and relative cerebral blood flow (CBF) maps were calculated on a pixel-by-pixel basis. Relative CBV was calculated numerically by integrating the area under the time-intensity curve for each pixel. The mean transit time (MTT) for each pixel was approximated by evaluating the width of the curve. Relative
CBF was then obtained from the relation: relative CBF = relative CBV/MTT. The resulting images were displayed as relative CBV and relative CBF maps.

These maps were analysed visually for perfusion abnormalities by a radiologist experienced in MR analyses. This procedure was first done without knowledge of the SPECT scans, after which the images were compared to the SPECT images. Relative CBV maps were also used to obtain the relative intensities of the interesting areas by drawing the regions of interest (ROI) in ten areas at the level of the lateral ventricles: gray and white matter in the right and left frontotemporal and parieto-occipital areas as well as the thalami. Each area was measured twice, and the mean was calculated. The mean overall values for gray and white matter as well as the thalami were also calculated. The relations of thalamus to white matter, frontotemporal gray to white matter, parieto-occipital gray to white matter and whole gray to white matter were then calculated in different patient groups.

4.2.3 Brain perfusion SPECT

Brain perfusion SPECT was carried out on seventeen patients using Technetium-99m-ethyl cysteinate dimer (ECD) (Neurolite®, Bristol-Myers Squibb Medical Imaging Inc., USA) as a tracer according to weight (Piepsz et al. 1990). The patients were lying supine in a darkened, quiet room with their eyes covered by patches. SPECT was performed 15–60 minutes after the intravenous injection of the tracer. The images were acquired with a double-head rotating camera equipped with a fan beam collimator (ADAC Vertex, ADAC Laboratories, Milpitas, CA, USA). Sixty-four angular projections in a 128x128 matrix were acquired over a 360° orbit at a radius of about 14 cm. The filtered backprojection algorithm used a Butterworth filter with a cutoff frequency of 0.22 and an order of 5.0. The images were reconstructed in three orthogonal planes, including transverse, coronal and sagittal images. The SPECT images were analysed visually for regions of asymmetric perfusion by an experienced specialist in nuclear medicine. The interval between MRI and SPECT was 0–3 days in 14 cases and 1.5, 3 and 6 months in three cases, respectively.

4.3 Functional MRI, brain perfusion SPECT, [123I]β-CIT SPECT and autoradiography after Mtx administration to swine (III, IV)

4.3.1 Animal model

The experiments were done using the swine model we have designed to study the neurological side effects of Mtx therapy.

Fifteen two- to three-month-old pigs of native stock of both genders were assigned to three groups: to receive Mtx (Trexan® 25 mg/ml, Orion, Espoo, Finland) intravenously
either twice 5 g/m² (five animals) at 14-day intervals or five times 2 g/m² (five animals) every three to four days within one month, while the remaining five animals served as a control group. The intervals between the administration of Mtx and the rates of dosage were chosen to resemble the protocol used in children. The animals weighed 14–15 kg at the beginning of the study and 19.5–27 kg at the end of the one-month follow-up in the treatment group. The weights in the control group were 14–15 kg at the beginning of the study and 24–29 kg at the end of the study.

All animals received care in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes 85/90 and Directive 86/609/ETY. The pigs were kept in animal rooms with a temperature of 21 ± 1°C and ambient humidity of 55 ± 10%. The automatic light and dark cycle of the room was 12 hours of light and 12 hours of dark. The animals were fed twice a day, and tap water was available ad libitum. Before the experiment, the pigs were allowed to adapt to the environmental conditions for one week. The protocol of these experiments had been approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia was induced in the first three animals with medetomidin 200 mikrog/kg intramuscularly (Domitor®, Orion Pharma, Espoo, Finland) and ketamine hydrochloride 12 mg/kg intramuscularly (Ketalar® 10 mg/ml, Pfizer inc., New York, NY, USA), while the rest of the animals received midazolam 1.2 mg/kg intramuscularly (Dormicum®, F. Hoffman La Roche Ltd., Basel, Switzerland) and ketamine hydrochloride 12 mg/kg intramuscularly. The change in the medication was made because of the lack of a BOLD response with the former combination in functional MRI. The pigs were intubated to ensure breathing and placed on their right or left side, and their auricular vein was cannulated for intravenous administration of propofol (Diprivan®, 20 mg/ml, AstraZeneca, London, UK), which was used during the imaging sessions and Mtx infusions. The animals’ temperature was not monitored, but blankets were used to maintain stable body temperature. Heart rate and oxygen saturation were monitored using a pulse oximeter. A specialised nurse from the animal laboratory monitored the clinical conditions of the animal throughout the period of anesthesia and the experimental procedures.

A central catheter (Porth-A-Cath®, Deltec, Philadelphia, PA, USA) was surgically implanted before the Mtx infusions. During the operation, anesthesia was maintained with isoflurane (Forene®, Abbot Laboratories, Chigago, IL, USA) with the same premedication as described before. Cephalosporin 20 mg/kg (Lifurox®, Eli Lilly and Company, Indianapolis, IN, USA) was given intravenously at the time of operation to prevent infections.

The Mtx infusion lasted for six hours. A calcium folinate bolus (Antrex® 3 mg/ml, Orion Pharma, Espoo, Finland) 15 mg/m² was given intravenously to block the systemic toxicity of MTX. The first dose of calcium folinate was administered 22 hours after the end of Mtx infusion, and this dose was repeated at six and 12 hours’ intervals until the Mtx concentration was below 0.08 umol/l. None of the animals needed calcium folinate rescue of more than three doses, and the last dose was given 42 hours after the end of Mtx infusion. During the Mtx infusion, the animals received glucose at a dose of 3000ml/m²/day for 6 to 7 hours (Glucosteril 50 mg/ml®, Baxter Healthcare Corporation, Deerfield, AL, USA) with potassium 10 mmol/l (Kaliumklorid Braun 150 mg/ml®, B. Braun
Melsungen AG, Melsungen, Germany) and sodium bicarbonate 50 mmol/l (Natriumbicarbonate Braun 75 mg/ml®, B. Braun Melsungen AG, Melsungen, Germany). Samples of cerebrospinal fluid were obtained at the end of the MTX infusion by lumbar puncture.

The brain perfusion SPECT and SPECT with $^{[123I]}\beta$-CIT were carried out before the Mtx exposure and after the one-month follow-up. Six of the animals also underwent MRI, including functional MRI. The MRI images were used to analyse the SPECT images. The immediate Mtx-related changes in the pigs were manifested as susceptibility to infections, weakness and loss of appetite.

After the one-month follow-up period and the last imaging session, the animals were electively killed and the entire brains were immediately harvested, weighed and stored at $-80\,^\circ\text{C}$. The density of DATs was analysed by whole-hemisphere autoradiography using $^{[125I]}\beta$-CIT as the radioligand.

The procedures in the control group were otherwise identical except that the control animals did not have a surgically implanted central catheter and hence did not have the anesthesia required for that and for the Mtx infusions.

**4.3.2 Functional MRI**

MRI was performed with a GE (General Electric, Milwaukee, WI, USA) Signa EchoSpeed 1.5-T scanner with a standard head coil. The animal’s head was propped firmly with foam pads onto the headrest. Anatomic images were obtained using the routine head protocol T2 Proton Density (PD) sequence, i.e. two-dimensional fast spin-echo (FSE) with flow compensation (flip angle 90°, TE 15/105 msec, TR 3500 msec, slice thickness/spacing 3/0.5 mm, FOV 20 x 15 cm, matrix 256 x 224, two NEX, 12–14 axial slices). No anatomical changes (signal changes, expansion) were detected by the experienced neuroradiologist who analysed the anatomical T2- and PD-weighted images of all groups.

**4.3.2.1 Perfusion MRI**

The SE-EPI sequence (flip angle 90°, TE 80 msec, TR 1500 msec, slice thickness/spacing 10/2 mm, FOV 23 x 23 cm, matrix 128 x 64, one NEX, four axial slices) was used with the bolus (0.4 ml/kg, 5 ml/sec) of gadopentetate dimeglumine (Magnevist®, Schering AG, Berlin, Germany) as a contrast medium for perfusion imaging. The intravenous bolus was administered with a power injector (Spectris® MR Injection System, Medral Europe B. V., Maastricht, Netherlands) over 10 seconds into a superficial vein either in an ear or in a foreleg (out of the total imaging duration of 70 seconds). A 0.9% sodium chloride bolus of 20–50 ml was given immediately after the contrast medium bolus.
4.3.2.2 Diffusion MRI

A diffusion-weighted two-dimensional axial SE-EPI (flip angle 90°, TE 71.6 msec, TR 5000 msec, slice thickness/spacing 3/0.5 mm, FOV 20 x 15 cm, matrix 128 x 128, one NEX, 10 axial slices) sequence was used for diffusion imaging. To accomplish diffusion weighting, a gradient pulse (duration $\delta = 32$ msec, interval $\Delta = 38.1$ msec, maximum $G_{\text{max}} = 22$ mT/m, ramp time = 184 µsec) was added to both sides of the 180° refocusing pulse (a Stejskal-Tanner sequence) in the z-direction (along $B_0$). The imaging protocol included a sagittal localiser scan and sets of diffusion-weighted axial images consisting of seven diffusion weighting factors (b-values) of 0, 31.25, 80.0, 180.0, 245.0, 361.25 and 500.0 seconds/mm².

4.3.2.3 BOLD contrast MRI

The series were taken with an interleaved gradient recalled echo single-shot EPI sequence (flip angle 90°, TE 60 msec, TR 3000 msec, slice thickness/spacing 10/2 mm, FOV 23 x 23 cm, matrix 64 x 64, four axial slices). The stimulation pattern (off/on/off: 60 seconds/30 seconds/60 seconds) was realised by somatosensory stimulation with electrical pulses (3 Hz, 10–15 MA, 0.3 msec) with a needle electrode inserted under the skin near the left or right foreleg. The stimulation was tested to reach motor activation, i.e. movement of the minor digits before the imaging. Modified protective ear pads were used to diminish auditory activation due to gradient noise. In the post-Mtx group, BOLD imaging was repeated twice.

4.3.3 Brain perfusion SPECT and $[^{123}\text{I}]\beta$-CIT SPECT

SPECT images were acquired using a dual-head rotating camera (ADAC Vertex, ADAC Laboratories, Milpitas, CA, USA) equipped with high-resolution fan beam collimators. The images were acquired stepwise over 360° (32 projections per head, 128x128 matrix, zoom 1.85, pixel size 2.54 mm), at 100 s per projection in the striatum studies, resulting in 1 – 2 millicounts (Mcnts) per acquisition. For the perfusion studies, the corresponding figures were 45 s and, on an average, 4 Mcnts. The tomographic radius varied within 12–14 cm.

Reconstruction was performed in a HERMES (Nuclear Diagnostic, Stockholm, Sweden) workstation with the OS-EM algorithm. Transversal slices were filtered with a Butterworth filter (0.9 cycles/cm, order 6). Five sagittal slices were summed up for data analysis. The ROIs over the striatum and the cerebellum were drawn manually. The cerebellum was used as reference for nondisplaceable activity due to its very low DAT density. The appearance of the striatum showed marked interindividual variation. Only the maximum pixel count was accepted from the striatum ROI for quantitation, while mean counts were used for the cerebellum.
Brain SPECT was performed using ECD (Neurolite®, Bristol-Myers Squibb Medical Imaging Inc., Billerica, MA, USA) as a tracer, administered according to the swine’s body weight. SPECT was performed 5–30 minutes after the intravenous injection of tracer. The imaging session lasted for approximately 30 minutes. The perfusion images were interpreted visually. The imaging system used for clinical studies in humans did not achieve sufficient resolution to allow semiquantitative analysis of the swine brain. The brain SPECT was classified as abnormal if a clearly visible hemispheric change in tracer uptake was observed in at least two consecutive slices by two analysts. Image analysis was performed in a double-blind manner.

Striatal DAT was evaluated before and after the Mtx infusions in all groups, using SPECT with $[123I] \beta$-CIT. The time of the maximum binding of this tracer to DAT was evaluated by taking images of the first five animals for up to 14 hours post-injection. The remaining animals were studied using this information, i.e. the images for evaluation were taken at 6–7 hours post-injection.

### 4.3.4 In vitro whole-hemisphere autoradiography

Autoradiography was performed with a modification of the published methods (Hall et al. 1998). The frozen porcine brains were embedded in carboxymethylcellulose (CMC) by pouring CMC semiliquid gel (+4°C) over and around the whole brain placed in a 8x18 cm metal frame and frozen with liquid nitrogen. The block was kept in a heavy-duty cryomicrotome cabinet (LKB PMV model 2250, LKB, Stockholm, Sweden) until the next day, to reach the cutting temperature (–15°C). Sagittal 100 µm cryosections were cut with a cryotome by pressing the non-sticking side of the cryotape (Leica type 810, Leica Microsystems, Nussloch, Germany) against the block and guiding the section onto a sturdy plastic sheet attached to the knife. The sections were transferred onto precooled (–15°C) gelatinised glass plates with the aid of a plastic sheet and attached to glass by allowing the section to quickly thaw on the plate at room temperature before transferring it to –20°C. The sections were allowed to freeze-dry and were stored at –20°C until labelling.

For in vitro receptor autoradiography, sections were chosen from two levels to include the brain regions known to contain DAT in other species (nucleus accumbens, nucleus caudatus and putamen). Anatomic structures were defined with the help of an anatomic atlas (Felix et al. 1999). The sections were incubated in 20 pM $[125I] \beta$-CIT (MAP Medical Technologies Ltd., Helsinki, Finland) in 50 mM Tris-HCl buffer pH 7.4 with 100 mM NaCl for one hour. Washing was performed in cold buffer (3 x 10 min) followed by a brief dip into ice-cold distilled water. The sections were dried with a gentle stream of warm air for 10 min and left in a fume cupboard at room temperature for two hours. Nonspecific binding was determined using incubation medium that contained 10 µM unlabelled $\beta$-CIT in addition to the radioligand. The plates were placed in Kodak X-ray cassettes with fine screens and pressed against X-ray film (Kodak X-Omat AR, Eastman Kodak Company, Rochester, NY, USA), exposed at –20°C and developed in an automatic X-ray processor. Each cassette had its own $^{125}$I autoradiographic microscale strip (RPA 523L, Amersham Pharmacia Biotech, Buckinghamshire, UK) to permit quantitation.
Quantitative image analysis of the autoradiograms was performed by computerised densitometry using a Mikrotek Scanmaker E6 connected to an Osborne PC Pentium II. The software packages included Adobe Photoshop 5.02 (Mountain View, CA, USA) and Scion Image for Windows, version 3b (Frederik, MD, USA). The resulting pixel values of the binding data were mathematically transformed by an exponential calibration equation into relative radioactivity values by using the $^{125}$I-calibrating scales.

### 4.3.5 Statistical analysis

The organisation and analysis of the data using the SPSS for Windows (Statistical Package for Social Sciences, version 9.0) is described in the papers I, II, and IV. In paper I, the differences in the mean latencies and amplitudes between the patients and their age, sex- and height-matched controls were assessed with the paired T-test. Natural logarithmic transformations were used to analyse the amplitudes. Two-tailed probability values of less than 0.05 were regarded as significant.

In paper II, Student’s t-test was used to assess the differences of the means in variables with normal distributions, while Mann-Whitney’s test was used with skewed distributions.

In paper III, the figures were drawn by using Microcal Origin version 3.78 and Student’s t-test was used to examine the amount of BOLD response.

As far as the $[^{123}]$β-CIT SPECT results were concerned in paper IV, the differences between the groups were analysed using the Mann-Whitney U-test, while Wilcoxon’s matched pairs test was used to compare the values before and after the Mtx treatment. The statistical analysis of the autoradiography results was performed by using the Mann-Whitney U-test to compare the values of the control and treated animals.
5 Results

5.1 MEP (I)

5.1.1 MEP latencies

The latencies of the MEPs in the hands and legs elicited by stimulation of the cortex were significantly prolonged in the children treated for ALL compared to the control group (Table 5.). Latency is the period between the moment of stimulation and the beginning of the response. The latency delay from the cortex to the thenar (abductor pollicis brevis muscle) was 2.2 ms (p < 0.001) on the right side and 2.0 ms (p < 0.001) on the left side, and that from the cortex to the leg (tibialis anterior muscle) 1.4 ms (p = 0.004) on the right side and 1.3 ms (p = 0.004) on the left side. The latency from the LV spinal level to the leg was also prolonged, the delay being 1.0 ms (p = 0.005) on the right side and 0.8 ms (p = 0.005) on the left. When the latencies from the LV level to the popliteal fossa and from the popliteal fossa to the leg were compared separately between the patients and the controls, a difference was found in the proximal segment: the delay from the LV to the popliteal fossa was 0.8 ms (p = 0.015) on the right side and 0.7 ms (p = 0.004) on the left, whereas that from the popliteal fossa to the leg was 0.1 ms (p = 0.371) on the right side and 0.1 ms (p = 0.342) on the left. There were no significant differences in the MEP latencies from the brachial plexus to the distal hands, the delay in the patients being 0.3 ms (p = 0.328) on the right side and 0.01 ms (p = 0.982) on the left side.

Central motor latencies were calculated by subtracting the MEP latency obtained in the leg (tibialis anterior muscle) by stimulation at the LV spinal level from the latency obtained by stimulation over the cortex. The calculated latency between the cortex and the LV spinal level was not significantly longer in the patients treated for ALL than in their healthy controls, although that between the cortex and the brachial plexus was significantly longer in the patients, by 1.9 ms (p = 0.002) on the right side and 1.9 ms (p = 0.012) on the left. The irradiated patients did not have significantly prolonged MEP latencies compared to the non-irradiated ones, nor was there any significant correlation between the total individual doses of vincristine and Mtx per square metre and the MEP latency prolongation in the lower or upper limbs on either side.
Table 5. Mean latencies (ms) of MEPs in patients with ALL and their age-, gender- and height-matched controls and mean differences.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No</th>
<th>Mean latencies (SD) of patients (ms)</th>
<th>Mean latencies (SD) of controls (ms)</th>
<th>Mean difference (ms) between patients and controls</th>
<th>95% CI for the mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latencies of the entire motor pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right cortex – left hand (APB)</td>
<td>27</td>
<td>19.1 (2.5)</td>
<td>16.8 (2.0)</td>
<td>2.2</td>
<td>1.1–3.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left cortex – right hand (APB)</td>
<td>27</td>
<td>19.1 (2.1)</td>
<td>17.1 (1.7)</td>
<td>2.0</td>
<td>1.1–2.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Right cortex – left leg (TA)</td>
<td>27</td>
<td>24.7 (2.1)</td>
<td>23.3 (2.5)</td>
<td>1.4</td>
<td>0.5–2.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Left cortex – right leg (TA)</td>
<td>27</td>
<td>24.7 (2.1)</td>
<td>23.4 (2.5)</td>
<td>1.3</td>
<td>0.5–2.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Latencies in the peripheral nerve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right brachial plexus – hand (APB)</td>
<td>27</td>
<td>10.4 (1.1)</td>
<td>10.1 (1.1)</td>
<td>0.3</td>
<td>−0.3–0.8</td>
<td>0.328</td>
</tr>
<tr>
<td>Left brachial plexus – hand (APB)</td>
<td>27</td>
<td>10.2 (3.2)</td>
<td>10.2 (1.0)</td>
<td>0.0</td>
<td>−1.3–1.4</td>
<td>0.982</td>
</tr>
<tr>
<td>Right LV – leg (TA)</td>
<td>25</td>
<td>11.5 (1.7)</td>
<td>10.5 (1.5)</td>
<td>1.0</td>
<td>0.3–1.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Left LV – leg (TA)</td>
<td>26</td>
<td>11.2 (1.6)</td>
<td>10.4 (1.4)</td>
<td>0.8</td>
<td>0.3–1.3</td>
<td>0.005</td>
</tr>
</tbody>
</table>

APB; abductor pollicis brevis muscle, TA; tibialis anterior muscle, LV; fifth lumbar vertebrae ms; milliseconds

5.1.2 MEP amplitudes

The MEP amplitudes of the legs after LV spinal stimulation tended to be lower in the ALL patients than in the control group, but all the other MEP amplitudes elicited by cortical or brachial plexus stimulation were equal in the patients and the controls.

5.1.3 Clinical neurological findings

Depressed deep tendon reflexes were detected in two IR patients in the neurological evaluation, while fine motor difficulties were seen in 9/26 patients (35%) and dysdiadochokinesia was evident in 7/26 patients (27%). Seven of the patients had gross motor difficulties (27%), whereas fourteen did not suffer from neurological symptoms of any kind 14/26 (51%). One patient had problems with clumsiness due to extreme obesity. Four of the 26 patients (15%) had difficulty jumping. Two of the 26 patients (8%) were unable to walk on their heels and toes, and three (12%) showed instability in standing on one leg or walking along a straight line. One girl (2.1 years at diagnosis) with motor clumsiness and delayed speech was considered to have these problems independent of the therapy. The neurological signs of the patients are presented as recorded during the treatment (Vainionpää 1993), at the end of the treatment (Vainionpää 1993) and five years following the cessation of treatment in Table 6.
Table 6. Neurologic findings in ALL patients.

<table>
<thead>
<tr>
<th>Neurological sign</th>
<th>After induction a</th>
<th>At the end of treatment a</th>
<th>Five years after cessation of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed Achilles or patella reflexes</td>
<td>34/39 (87%)</td>
<td>8/33 (24%)</td>
<td>2/26 (8%)</td>
</tr>
<tr>
<td>Fine motor difficulties</td>
<td>0/39 (0%)</td>
<td>6/33 (18%)</td>
<td>9/26 (35%)</td>
</tr>
<tr>
<td>Gross motor difficulties</td>
<td>9/39 (23%)</td>
<td>10/33 (30%)</td>
<td>7/26 (27%)</td>
</tr>
</tbody>
</table>

a These were patients whom Dr. Vainionpää had followed until the end of treatment (Vainionpää 1993)

5.2 Perfusion MRI and brain perfusion SPECT after treatment for childhood ALL (II)

5.2.1 Perfusion MRI and brain perfusion SPECT

We did not detect any significant differences by MRI perfusion in the relative CBV ratios between gray matter and white matter or thalamus and white matter in any of the patient groups. Of the different treatment groups, the SR group had the highest thalamus-to-white matter ratio, but the difference was not statistically significant.

At the time of perfusion MRI, all patients had normal white matter, while one of them had had transient white matter changes earlier during the therapy. Two patients had small areas of hemorrhage (in the form of hemosiderin) on their MR images at the time of perfusion. The perfusion values of these three patients did not differ from those of the patients with normal MRI.

Abnormalities on SPECT were observed in five out of 17 children examined (29%), while no such defects were seen on relative CBF or relative CBV maps in perfusion MRI. The perfusion defects on SPECT were small and located in the left basal, frontal or temporal areas. In two of these patients, the defects were observed at the end of the treatment, while three of them were studied 4–8 years after the treatment. Two patients belonged to the SR group, two to the IR group and one to the HR group. Four of the patients were under four years old at the time of diagnosis, and none of them had received cranial irradiation.

5.2.2 Neurological evaluation

In the neurological evaluation on admission, one patient with neuroleukaemia had had dizziness and headache. Two patients out of 19 had had motor clumsiness before the initiation of therapy. Eight of the patients did not suffer from neurological symptoms of any kind at the end of the treatment. Among the five patients with abnormalities in SPECT, neurological difficulties were seen in three patients, and two of them had motor clumsiness and dysdiadochokinesia at the time of SPECT imaging, but there were no focal signs corresponding to the small defects seen on SPECT (Table 7.).
Table 7. Neurological findings in ALL patients.

<table>
<thead>
<tr>
<th>Neurological sign</th>
<th>After induction</th>
<th>At the end of treatment</th>
<th>Four to eight years after the cessation of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed Achilles or patella reflexes</td>
<td>16/19</td>
<td>6/19</td>
<td>1/10</td>
</tr>
<tr>
<td>Fine motor difficulties</td>
<td>1/19</td>
<td>3/19</td>
<td>1/10</td>
</tr>
<tr>
<td>Gross motor difficulties</td>
<td>5/19</td>
<td>6/19</td>
<td>1/10</td>
</tr>
</tbody>
</table>

5.3 Functional MRI of Mtx-exposed swine brain

5.3.1 Perfusion MRI

Perfusion imaging and analysis were successfully applied to three control/four pre-Mtx/four post-Mtx pigs. No changes were detected in the relative values of the perfusion measures of relative CBF and MTT between the control, pre-Mtx and Mtx-exposed groups.

5.3.2 Diffusion MRI

In diffusion imaging, apparent diffusion coefficient (ADC<sub>z</sub>) was successfully determined for the cortex in 3 control/5 pre-Mtx/4 post-Mtx pigs and for the deep structures in 3/5/2, respectively. The data of two pigs for ADC<sub>z</sub> in the deep structures were rejected because of the low signal-to-noise ratio of voxel intensity in the function of b-values, S(b<sub>k</sub>). No changes were seen in ADC<sub>z</sub> between the control, pre-Mtx and Mtx-exposed groups.

5.3.3 BOLD contrast MRI

The sum curves of the voxel intensity time course from one control and one pre-Mtx pig and two subjects of the post-Mtx group were selected after center of mass analysis (max 0.8 mm during activation).

A positive response (of ≈ 2–4%) was present in the control and pre-Mtx subjects. On an average, 33 voxels fulfilled the criteria (p < 0.01) out of the average of altogether 92 voxels of the ROI (i.e., 33/92 ROI voxels, ≈ 36%). The post-Mtx group either did not show a positive response or showed a reduced positive response of ≈ 1% (respectively, average 25/123 ROI voxels, ≈ 20%).

The negative response was absent in the pre-Mtx subject. In the control animal, no clear negative response was seen (transient spikes) during stimulation. A negative response of approximately −2% to −3% was seen in the BOLD responses of the post-Mtx group; respectively, an average of 57/126 (≈ 45%) ROI voxels.
The voxels with no response accounted for 32/74 ROI voxels in the control subject and 66/109 ROI voxels in the pre-Mtx subject. Respectively, an average of 51/126 ROI voxels with no response was found in the post-Mtx group.

5.4 Brain perfusion SPECT, $^{123}$I$\beta$-CIT SPECT and autoradiography after Mtx administration to swine (IV)

5.4.1 Brain perfusion SPECT

All the control animals had normal brain perfusion SPECT scans in the first evaluation at the beginning of the one-month surveillance period. Three of the control animals (60%) had abnormal SPECT scans in the second imaging one month later. One of the eight animals in the Mtx group had an abnormal SPECT scan before the Mtx treatment, and five animals had abnormal SPECT scans (63%) after the treatment.

Table 8. Brain perfusion SPECT results in methotrexate-exposed swine and control animals in preliminary imaging and after one-month follow-up.

<table>
<thead>
<tr>
<th>Timing of imaging</th>
<th>Normal result</th>
<th>Perfusion defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial imaging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>5/5 (100%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Treatment group</td>
<td>7/8 (88%)</td>
<td>1/8 (13%)</td>
</tr>
<tr>
<td>After one-month follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>2/5 (40%)</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>Treatment group</td>
<td>3/8 (38%)</td>
<td>5/8 (63%)</td>
</tr>
</tbody>
</table>

5.4.2 $^{123}$I$\beta$-CIT SPECT

Maximum binding of $^{123}$I$\beta$-CIT to DAT in porcine was detected at 6–7 hours post-injection. The variation of the binding change ratio was wider in the control group than in the treatment group, with three out of five animals showing equally high or higher ratios compared to the Mtx-treated animals. The mean $^{123}$I$\beta$-CIT DAT binding change ratio in the treatment group was 1.06, range 0.91 to 1.30. The mean binding change ratio for the animals with five Mtx infusions was 1.13 and that for the group with two infusions 0.99. The mean binding change ratio in the control group was 1.30, range 0.93 to 1.77. These figures imply more abundant binding in the control group than in the treatment group, but the total striatal $^{123}$I$\beta$-CIT DAT uptake in the treatment group did not differ significantly from that in the control group (p = 0.22).
5.4.3 *In vitro* whole-hemisphere autoradiography

$[^{125}\text{I}]\beta$-CIT showed dense labelling in the nucleus caudatus, putamen and nucleus accumbens, indicating high densities of DAT in these structures. The whole-hemisphere images also showed, to variable extents, labelling of the frontal cortex and the colliculus inferior area. The median DAT density in the nucleus caudatus (level 1) was 182.25 Bq/mg in the Mtx group and 191.89 Bq/mg in the controls. The median density in the putamen (level 1) was 197.18 Bq/mg in the treatment group and 265.32 Bq/mg in the control group. The minimum and maximum values in the area of n. caudatus were 147.23 Bq/mg and 271.17 Bq/mg in the treatment group and 113.10 Bq/mg and 308.20 Bq/mg in the controls. The minimum and maximum values in the area of the putamen were 86.53 Bq/mg and 325.93 Bq/mg in the treatment group and 173.11 Bq/mg and 330.68 Bq/mg in the control group, respectively. The binding to DAT was also evaluated at another level (nucleus caudatus and nucleus accumbens), but the results did not yield any further information. The values in the treatment group did not differ significantly from those in the control group. The cerebellum was devoid of specific $[^{125}\text{I}]\beta$-CIT binding in all sections.
6 Discussion

6.1 Motor system impairment after treatment for ALL

In the survivors of childhood ALL, decreased motor nerve conduction in the peripheral nerves was still present five years after the treatment, and one third of the cases had clinical neurological findings. The most impressive slowing of conduction was located in the proximal nerve tracts in the legs, when the motor impairment within the CNS seen in MEP recordings at the end of the treatment for ALL (Harila-Saari et al. 2001) was no longer visible at this stage. Magnetic stimulation of the brain, spinal roots and peripheral nerves provides a non-invasive means of evaluating the motor pathway and the motor cortex, and it can detect both clinical and sub-clinical injuries (Maegaki et al. 1994, Di Lazzaro et al. 1999).

Significantly prolonged latencies within the entire motor pathway were reported in a previous investigation of MEPs at the end of ALL therapy in children. The same study also showed significantly decreased MEP amplitudes in the peripheral motor nerves, indicating both demyelination and loss of descending motor fibres or loss of muscle fibres (Harila-Saari et al. 2001). The present results show that these MEP disturbances are partially reversible. Conduction velocity continued to be significantly lower in the proximal parts of the peripheral nerves five years after the end of therapy, especially in the lower extremities. As the long motor CNS tracts from the cortex to the LV vertebral level showed equal conduction in the patients and the controls, we presume that the latency prolongation between the cortex and the brachial plexus in the patients was also located below the CNS, i.e. in the nerve root and/or the proximal brachial plexus.

The present report points to long-lasting sequelae in the motor pathway, which seem to concentrate primarily in the proximal nerve tracts at least in the legs, indicating vincristine-induced neuropathy, as described widely in previous reports (Casey et al. 1973, Reinders-Messink et al. 1996). The effects of the other neurotoxic drugs used, or even paraneoplastic sequelae, cannot be excluded, however. Our results also support the reversibility of motor injury within the CNS some years after therapy, since the motor impairment within the CNS observed in MEP recordings at the end of the treatment for ALL was no longer detected later.
Children treated for ALL often develop impaired motor function during the treatment, which is manifested as motor clumsiness, fine motor difficulties and depressed or lost deep tendon reflexes. In our study, gross and fine motor difficulties developed during the treatment and persisted for five years following the cessation of treatment. One third of our patients had fine or gross motor difficulties or dysdiadochokinesia, which is relevant to the study of problems with handwriting and fine motor skills two years after treatment for childhood ALL (Reinders-Messelink et al. 1996). A minority of the patients continued to have depressed deep tendon reflexes. Most of the detrimental neurotoxic side-effects are thought to disappear gradually after the cessation of treatment. In our study, the fine motor performance declined over time, but the lack of controls warrants further speculation on the matter. The neurological findings obtained in a clinical examination may be caused by vincristine neuropathy, because the central pathways were not affected. Our comparisons of non-irradiated patients with patients who had received radiotherapy suggested that cranial irradiation did not play a role in the motor disturbances. Diadochokinesis and fine motor skills may be impaired because of peripheral neuropathy, and cerebellar motor dysfunction may be associated with CNS therapy that involves high-dose systemic chemotherapy.

The clinical disturbances found in our children with ALL were not associated with the MEP latency prolongation. This may be explained by the fact that MEP is only a measure of the descending motor tract and involves considerable physiological variance (Ellaway et al. 1998), whereas walking and other motor actions are complicated, dynamic motor processes at multiple levels of the neuronal system. So far, however, there is no more precise neurophysiological method, by which we could examine both the central and the peripheral motor tracts.

This study focused on the motor impairment following the treatment of childhood ALL. However, the impairment of neuropsychologic functioning after childhood ALL, which has been described in several studies to occur as long as six years after the therapy, possibly causes an even more significant negative impact on the quality of the life of these patients (Espy et al. 2001).

6.2 Brain perfusion after treatment for childhood ALL

In the current study, small perfusion defects were detected in brain perfusion SPECT in 29% of the patients (5/17). In our former study, such defects were observed in 44% of the patients examined at the end of the treatment. In this study, two of the patients with perfusion defects were examined at the end of the treatment, while three were examined 4-8 years after the cessation of treatment. Perfusion defects were observed in 75% of the patients during therapy by Vera and colleagues, most of them having AML (Vera et al. 1999). In these studies, the neurotoxic agents contributing to the perfusion defects are considered to be Mtx and cytarabine.

It is not really known how the perfusion defects change over the time and whether they are permanent or not. In their study, Vera and colleagues observed abnormal SPECT scans, though with some improvement, in three patients out of six during their 3- to 42-month follow-up. These patients had received cytarabine. Hence, according to this study,
the prognosis of perfusion defects related to treatment may be favourable. (Vera et al. 1999). This is in agreement with the studies that showed white matter changes in MRI, which may be transient and disappear during follow-up (Wilson et al. 1991, Pääkkö et al. 2000).

There has not been any correlation between the white matter changes in MRI and the perfusion defects in SPECT in the former studies (Harila-Saari et al. 1997, Vera et al. 1999). Our results are similar. The two methods measure brain perfusion in different ways. In MRI, perfusion is studied during the first pass of an intravascular contrast agent, which remains in the vessels when the blood-brain barrier is intact and the total time of measurement during the bolus is of the order of seconds. In SPECT, on the other hand, the estimation of perfusion is based on a lipophilic substrate that passes through the blood-brain barrier and accumulates in the parenchyma, depending on its vascular supply. The vascular changes caused by neurotoxic drugs probably lead to endothelial damage in small vessels without total obstruction (Quinn et al. 1997). This type of injury may not be visible in first-pass perfusion MRI, whereas SPECT detects even small local blood flow disturbances. Perfusion MRI is able to detect defects in patients with stroke and shows a good correlation with SPECT (Karonen et al. 1999). Total obstruction of larger vessels often underlies the ischemia in these patients, which is more susceptible to study with perfusion MRI.

We did not observe any significant differences in the perfusion ratios between the different treatment groups. If the transient white matter changes observed on conventional MRI in SR patients (Pääkkö et al. 2000) were the result of vascular insufficiency, no such insufficiency was detectable by perfusion MRI after the treatment. Nor did the different patient groups have any significant differences in their perfusion ratios with respect to age at diagnosis, time from the end of treatment, brain radiation or SPECT or MRI findings.

In conclusion, SPECT may show regional brain perfusion defects in children with leukaemia that are not detectable by perfusion MRI. All patients had visually normal relative CBV and relative CBF maps on perfusion MRI. We were not able to confirm the perfusion defects visible by SPECT on perfusion MRI. It thus seems that SPECT is better suited to detecting perfusion defects caused by leukaemia treatment, but the clinical significance as well as prognosis of these perfusion defects is still not known.

6.3 Functional MRI in the Mtx exposed swine brain

Functional MRI was performed to evaluate perfusion, diffusion and BOLD imaging after Mtx exposure of swine brain. A positive BOLD response changed into an attenuated positive or negative BOLD response after Mtx exposure. Neither perfusion nor diffusion images showed changes after Mtx exposure.

Functional MRI is a novel method to study CNS changes before the defects are seen in anatomic MRI. Exposure to Mtx may cause local or diffuse white matter changes, which may be transient (Pääkkö et al. 2000). Functional changes in brain perfusion have been studied earlier with SPECT and PET (Harila-Saari et al. 1997, Kähkönen et al. 1999). In this study, we wanted to evaluate the possible changes in functional MRI after Mtx exposure with no other chemotherapy.
Earlier studies with SPECT in children have shown perfusion defects in the cortical areas and in the basal ganglia (Harila-Saari et al. 1997, Österlundh et al. 1997, Österlundh et al. 1999). Mtx-associated damage of endothelium in cerebral blood vessels might cause brain perfusion defects. A brain perfusion study in rats with autoradiography indicated reduced CBF in all brain regions after Mtx infusion (Mizusawa et al. 1988). We selected ROIs preferring areas known to be susceptible to Mtx-induced changes in regional CBF. The basal ganglia area was not segmented separately due to the limited image resolution. No clear changes related to Mtx exposure in the relative values of perfusion were seen in the swine brain. Arterial input function could not be used because the resolution in perfusion imaging did not meet the requirements for its estimation. We cannot, however, exclude the possibility of simultaneous changes in perfusion because, if the changes in perfusion in the different anatomical areas are parallel, the relative values remain the same.

Mtx-associated changes in myelin metabolism have been hypothesised to cause accumulation of interstitial fluid in splitting myelin and vacuoles (Asato et al. 1992). This change in myelin integrity, which causes an increase in the extracellular water fraction, was assumed to cause increased ADC\textsubscript{z}. On the other hand, the reduction of cerebral glucose metabolism after Mtx exposure may interfere with brain water diffusion, as the ion exchange pumps maintaining fluid and ion homeostasis require energy (Komatsu et al. 1990, Phillips et al. 1991). The distortion of ion balance may lead to cell swelling, i.e., the extracellular water content is diminished in favour of intracellular water. Hence, a decrease in ADC\textsubscript{z} may follow, as seen in ischemic brain damage (Thornton et al. 1998). In the current study, ADC\textsubscript{z} did not change. This could mean that no change in myelin metabolism took place after the Mtx exposure in our model. The anatomical images did not reveal white matter changes either, which supports the explanation. It is, however, also possible that minor white matter changes were undetectable in anatomical MRI because there was no way to compare the pre- and the post-Mtx images directly. If the changes in regional CBF or ADC\textsubscript{z} after Mtx exposure are transient, correct timing of the experiment may be important.

The flow-metabolism coupling, i.e., the local increase in blood flow to meet the demands of glucose and oxidative metabolism during neural activation, was expected to be disturbed after Mtx exposure. The regulation of cerebrovascular resistance may not work properly due to Mtx-related endothelial deterioration or to the synthesis of vasoactive neurotransmitters. Defects in flow-metabolism coupling during activation can be visualised in BOLD contrast MRI. Our results suggest that defects of this kind are possible, but the data are insufficient to warrant conclusions. The hypothesis is that Mtx-related changes in the brain MRI may be detected as reduced or negative BOLD responses to somatosensory activation, indicating changes in flow-metabolism coupling. The MR perfusion imaging with contrast agent is hampered by a lack of absolute quantification. Determination of ADC (in one direction) did not display Mtx-related changes in the swine brain.
6.4 Mtx-related changes in brain perfusion SPECT and DAT density in animal model

In this study, brain perfusion defects were revealed by SPECT after the chosen follow-up time both in the Mtx group and in the control group. In vivo \({\text{[^{123}I]}}\)\(\beta\)-CIT SPECT did not show the specific DAT binding ratio to be any different in the Mtx group from the control group. Perhaps the power of the setup could not prove the hypothesis of the deleterious effect of Mtx on dopaminergic neurons.

In our previous clinical study (Harila-Saari et al. 1997), the patients with normal SPECT results had received high-dose Mtx less frequently, while those with abnormal SPECT had received low doses at shorter intervals, the total dose of Mtx being the same in both groups. Land and colleagues reported a treatment trial in which frequent administration of intravenous Mtx was found to involve a risk of increased neurotoxicity. (Land et al. 1994). Österlundh and colleagues saw nonhomogenous cerebral hypoperfusion in all patients during induction treatment (Österlundh et al. 1999). The long-term impact of these findings remains open. In children with leukaemia, other chemotherapeutic agents (corticosteroids, cytarabine) and radiation therapy, which are used in the treatment of leukaemia, also play an important role in neurotoxicity, not to mention the leukaemia itself, especially neuroleukaemia.

Dopamine and its major metabolites localise primarily in the basal ganglia (Mefford et al. 1982). The affinity of the \(\text{[^{123}I]}\)\(\beta\)-CIT tracer to serotonin transporter is lower than its binding to DAT, and the maximum value is reached at 2-4 hours post-injection in the human brain. The striatal \(\text{[^{123}I]}\)\(\beta\)-CIT activity, which is primarily due to binding to DATs, increases slowly, reaching its peak value at about 20 hours post-injection (Brucke et al. 1993). In our present study, we found the maximum binding of this tracer to DAT to occur at 6-7 hours post-injection. There was a tendency towards lower binding in the Mtx group compared to the controls. The variation, however, was wide and overlapping in both groups.

Basal ganglia play an important role in motor and memory functions, in which children after treatment for ALL have been reported to have deficits (Ochs et al. 1991, Vainionpää 1993). The concentration of dopamine is high in basal ganglia (Mefford et al. 1982). Impairment of bipterin metabolism, which leads to decreased availability of monoamine neurotransmitters, has been suggested to explain Mtx neurotoxicity (Millot et al. 1992). In the present swine study, although the in vivo images showed higher binding in the striatal area in most individuals in the control group than in the Mtx group, the results were inconclusive. A similar tendency regarding DAT density was also seen in in vitro autoradiography. Thus, our results further do not contradict the idea that Mtx may cause changes in dopamine metabolism.

The limitations of this study made the interpretation of the results difficult. Propofol, which was used as an anaesthetic regimen during the imaging sessions and Mtx infusions, has been reported not to affect cerebral blood flow, metabolism and cerebral autoregulation in anaesthetised pigs in general, but it may abolish autoregulation in individual animals even in the absence of major pathological changes (Lagerkranser et al. 1997). A recently reported study by PET in humans showed global reduction of regional CBF related to intravenous propofol infusion (Kaisti et al. 2002). The choice of the
anaesthetic regimen was based on the studies reported earlier, but propofol might have affected the brain perfusion SPECT in both groups. Also, individual features might explain the presence of perfusion defects in general. The present results showed that Mtx is not the only factor causing perfusion defects in brain SPECT.
7 Conclusions

Average life expectancy after childhood leukaemia has improved during the past few decades, making late effects an important issue in pediatric oncology. The quality of life after childhood ALL may depend on CNS late effects, which may manifest as neuropsychological and motor dysfunction.

In the evaluation of MEPs five years after the cessation of therapy for ALL, decreased motor nerve conduction was still present in the peripheral nerves, and clinical neurological findings could be obtained in one third of the cases. Gross and fine motor difficulties developed during treatment and were still seen five years after the cessation of treatment. On the other hand, the motor impairment within the CNS seen in MEP recordings at the end of the treatment for ALL was no longer detectable at this stage. The current study focused on motor impairment after childhood ALL, but the impairment in neuropsychological function probably constitutes a more significant threat to the quality of life and career prospects of these patients.

In our study, a novel method of functional MRI was used to evaluate treatment-related changes. Perfusion MRI is able to detect defects in patients with stroke and appears to correlate with SPECT. In the present study, long-term survivors of ALL had defects visible in brain perfusion SPECT, which could not be detected in perfusion MRI. The vascular changes caused by neurotoxic drugs are probably the result of endothelial damage in small vessels without total obstruction, and such injuries may not be visualisable by perfusion MRI.

In our series, functional MRI was unable to detect perfusion or diffusion changes after Mtx exposure in an animal model designed to study possible Mtx-related changes in detail, with all other chemotherapy excluded. Mtx-related changes in the brain may be detected as a reduced or negative BOLD response to somatosensory activation in BOLD contrast MRI, indicating changes in flow-metabolism coupling. The results obtained by the BOLD MRI technique were inconclusive.

Brain perfusion SPECT has shown defects in brain perfusion in patients with childhood ALL. The clinical importance of perfusion defects seen in SPECT is not known, however. To evaluate this finding, the animals were examined by brain perfusion SPECT in order find out the role of Mtx in the perfusion defects. Perfusion defects were seen in both the Mtx-exposed and the control animals. Thus, individual features of the animals and the anaesthesia might explain the findings even without Mtx exposure.
In vivo $^{[123]}$I-$\beta$-CIT SPECT and in vitro $^{[125]}$I-$\beta$-CIT receptor autoradiography were carried out to evaluate the effect of Mtx on the amount of DATs in the swine brain. We hoped to be able to explain the possible mechanisms related to Mtx neurotoxicity. Both in vivo $^{[123]}$I-$\beta$-CIT SPECT and in vitro $^{[125]}$I-$\beta$-CIT receptor autoradiography suggested a lowered DAT density compared to the control group, but the limitations of this study made the interpretation of the results difficult.

By now, we know that some of the late effects show reversibility over time and some emerge during follow-up. Long-term follow-up will be needed to tell the whole story. Thanks to the improved treatments for childhood ALL, there are more and more people living with a background of cured childhood haematologic malignancy. The duty of clinicians in pediatric oncology is to strive to further minimise the late effects and to ensure the best possible quality of life after childhood cancer treatment. This goal necessitates continuous research in the area.
References


