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TREATMENT-RELATED RENAL SIDE-EFFECTS IN PEDIATRIC CANCER PATIENTS

by

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To my parents

ABSTRACT

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Treatment-related renal side-effects in pediatric cancer patients

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Background: The long-term side-effects of cancer treatments are of growing importance, since the number of pediatric cancer survivors has considerably increased. Renal side-effects should be noted early to prevent further deterioration. Renal dysfunction may also develop long after cancer treatment. Easy and reliable methods for assessing renal function are needed.

Aims: The aims were to find the mechanisms behind methotrexate-induced renal damage by studying renal tubular cells (LLC-PK_1 cells), and to evaluate the usefulness of laboratory tests in assessing glomerular function in pediatric cancer patients by comparing an isotope clearance method with alternative methods. The aim was also to study the long-term effects of bone marrow transplantation (BMT) and high-dose methotrexate (HD-MTX) treatment in renal function.

Results: Methotrexate induced time-dependent renal tubular cell swelling and cell death. In patients treated with HD-MTX a significant decrease in GFR was noted after a follow-up time of one to ten years. One year after BMT the GFR was reduced, especially in patients treated with total body irradiation (TBI). GFR recovered slightly but remained stable thereafter. In glomerular function assessment the serum cystatin C (cysC) concentration showed a significant association with GFR measured by the isotope method.

Conclusions: Methotrexate induced acute damage in renal tubular cells. In assessing GFR the isotope method still remains the method of choice, but the assay of cystatin C was the most reliable of other alternatives. Long-term follow-up of renal function is needed in BMT patients and patients treated with HD-MTX.

Key words: Childhood cancer, late-effects, glomerular filtration rate, cystatin C, methotrexate, bone marrow transplantation

TIIVISTELMÄ

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Syöpähoitojen vaikutus munuaisten toimintaan lapsilla

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Tausta: Syövän hoitojen aiheuttamien sivuvaikutusten merkitys on viime vuosina korostunut, koska lasten syövän hoitotulokset ovat parantuneet merkittävästi. Munuaissivuvaikutukset tulisi havaita ajoissa, sillä munuaisten toiminta saattaa heikentyä vielä useita vuosia hoitojen päättymisen jälkeen. Munuaisten toiminnan seuraamiseksi tarvitaan helppoja ja luotettavia menetelmiä.

Tavoitteet: Tavoitteena oli selvittää metotreksaatin aiheuttaman munuaisvaurion mekanismeja munuaistubulussolulinjan (LLC-PK_1) avulla. Lisäksi pyrittiin selvittämään laboratoriotutkimusten soveltuvuutta syöpälasten munuaistoiminnan mittaamiseen vertaamalla eri menetelmien tuloksia isotooppimenetelmällä mitattuun glomerulusten suodatusnopeuteen (GFR). Tavoitteena oli myös tutkia korkea-annosmetotreksaattihoidon ja luuydinsiirron aiheuttamia pitkäaikaisia munuaistoiminnan muutoksia.

Tulokset: Metotreksaatti aiheutti munuaissolujen turpoamista ja solukuolemaa, joka lisääntyi ajan kuluessa. Korkea-annosmetotreksaatilla hoidetuilla potilailla glomerulusten toiminta heikkeni merkitsevästi 1-10 vuoden seuranta-ajan kuluessa. Myös luuydinsiirron jälkeen glomerulusten toiminta heikkeni siten, että vuoden kuluttua siirrosta GFR oli alentunut erityisesti potilailla joiden esihoitoon kuului koko kehon sädehoito. Seurannassa GFR hieman parani mutta jäi matalammaksi kuin ennen siirtoa. Glomerulusten toimintaa tutkittaessa kystatiini C:n pitoisuus vastasi hyvin isotooppimenetelmällä saatuja tuloksia.

Päätelmät: Metotreksaatti tuhosi akuutisti munuaistubulussoluja. Glomerulusten toiminnan arvioinnissa isotooppimenetelmä on edelleen luotettavin, mutta myös kystatiini C:n pitoisuutta voidaan käyttää seurantamenetelmänä. Sekä luuydinsiirrolla että korkea-annosmetotreksaatilla hoidettujen potilaiden munuaisten toimintaa tulee seurata vielä vuosia hoitojen jälkeen.

Avainsanat: Lapsuusiän syöpä, myöhäiset sivuvaikutukset, glomerulusten suodatusnopeus, kystatiini C, metotreksaatti, luuydinsiirto

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ABBREVIATIONS

AFP	Alpha-fetoprotein
ALL	Acute lymphoblastic leukemia
ANOVA	Analysis of variance
BMT	Bone marrow transplantation
BSA	Body surface area
CIS	Cisplatin
CNS	Central nervous system
CP	Cyclophosphamide
$CPDG_2$	Carboxypeptidase-G_2
CrCl	Creatinine clearance
^{51}Cr-EDTA	51-chromium labeled ethylenediaminetetra-acetate
CysC	Serum cystatin C
DAMPA	2,4-diamino-N^{10}-methylpteroic acid
DS	Down's syndrome
EFS	Event free survival
ERPF	Effective renal plasma flow
G-CSF	Granulocyte colony-stimulating factor
GFR	Glomerular filtration rate
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
HD-MTX	High-dose methotrexate
HL	Hodgkin's lymphoma
ICC	Intra-class correlation coefficient
IFO	Ifosfamide
MESNA	Sodium-2-mercaptoethane sulphonate
MDS	Myelodysplastic syndrome
MTX	Methotrexate
NHL	Non-Hodgkin lymphoma
NOPHO	Nordic Society of Paediatric Haematology and Oncology
NSAIDs	Non-steroidal anti-inflammatory drugs
7-OH-MTX	7-hydroxy-methotrexate
PBSC	Peripheral blood stem cells
SAA	Severe aplastic anemia
SCT	Stem cell transplantation
SCr	Serum creatinine
SD	Standard deviation
TBI	Total body irradiation
^{99m}Tc-DTPA	99m-technetium labeled diethylene triaminopentoacetic acid
VOD	Veno-occlusive disease

LIST OF ORIGINAL PUBLICATIONS

1. Grönroos M, Chen M, Jahnukainen T, Capitanio A, Aizman RI, Celsi G: Methotrexate induces cell swelling and necrosis in renal tubular cells. Pediatr Blood Cancer 2006;46:624-629

2. Grönroos MH, Jahnukainen T, Irjala K, Härkönen R, Hurme S, Möttönen M, Salmi TT: Comparison of glomerular function tests in children with cancer. Pediatr Nephrol. 2008;23:797-803

3. Grönroos M, Jahnukainen T, Möttönen M, Perkkiö M, Salmi T: Long-term renal function following high-dose methotrexate (HD-MTX) treatment in children. Pediatr Blood Cancer. 2008;51:535-539

4. Grönroos MH, Bolme P, Winiarski J, Berg UB: Long-term renal function following bone marrow transplantation. Bone Marrow Transplant. 2007;39:717-23

1 INTRODUCTION

The long-term side-effects of cancer treatments are of growing importance, since the number of pediatric cancer survivors has considerably increased due to better treatment regimens and better supportive care during recent years (Lie et al., 2005; Marec-Berard and Philip, 2004; Pui and Howard, 2008; Shankar et al., 2008). Forty years ago the median survival for leukemia was only 3-4 months, now the cure rate among pediatric leukemia patients is more than 80% (Pui and Howard, 2008; Zuelzer et al., 1976).

There are numerous reports on the late-effects of childhood cancer. Approximately 60% of cancer survivors have at least one chronic condition, and 30% have a severe, even life-threatening condition 30 years after cancer diagnosis (Oeffinger et al., 2006). The most common chronic conditions among cancer survivors are cardiovascular problems, endocrinological problems, hearing loss, impaired fertility, learning difficulties and second malignant neoplasms (Fallat et al., 2008; Harila-Saari et al., 2007; Madanat et al., 2008; Nandagopal et al., 2008; Shankar et al., 2008). Pediatric cancer survivors also have a ninefold risk to renal failure or dialysis compared to healthy children (Oeffinger et al., 2006). Many drugs are excreted to a large extent via the kidneys, which makes a sufficient renal function essential. Renal side-effects should be taken into account during later therapy in order to prevent further deterioration. Renal function may be impaired in pediatric cancer patients due to disease burden in the kidneys, radiotherapy, removal of one of the kidneys, or nephrotoxic supportive drugs like foscavir, cidofovir, amphotericin B and cyclosporin.

Several chemotherapeutic drugs, such as platinum compounds and ifosfamide, are known to induce permanent renal failure (Ariceta et al., 1997; Doz and Pinkerton, 1994; Goren, 2003; Skinner et al., 1993). High-dose methotrexate (HD-MTX) is known to induce transient reduction in renal function and delayed elimination of methotrexate, but long-term renal changes following HD-MTX treatment have been less intensively studied (Bardi et al., 2004; Condit et al., 1969; Kaya et al., 2007; Krawczuk-Rybak et al., 2005; Widemann Adamson, 2006). The exact mechanisms by which methotrexate induces renal cell damage in acute renal failure are also not yet known.

Acute renal impairment is a major side effect of bone marrow transplantation (BMT), often leading to permanent renal failure (Kersting et al., 2007; Kist-van Holthe et al., 2002; Zager et al., 1989). Chronic renal impairment is also known to develop without any predisposing acute renal damage (Van Why et al., 1991). However, there are only a limited number of reports on the long-term changes in renal function following BMT (Frisk et al., 2002; Hingorani et al., 2007; Kumar et al., 1996; Leblond et al., 1995).

A reliable and accurate measurement of renal function is particularly important in oncology patients in order to detect a developing renal impairment. Direct assessment of glomerular filtration rate (GFR) by inulin or by radioactive traces is considered as a reference method (Piepsz et al., 2001), but they are labor intensive, invasive and not available in all centers (Effersoe et al., 1990). Therefore, there is a need to find simple and reliable methods to assess glomerular function that can be used as screening tools.

2 REVIEW OF THE LITERATURE

2.1 Assessment of renal function

2.1.1 Glomerular filtration rate

Glomerular filtration rate (GFR) is the most useful measurement of kidney function. GFR represents the volume of plasma ultrafiltrate presented to the nephrons per unit of time in the process of urine formation (Schwartz and Furth, 2007). Effective renal plasma flow (ERPF) is the amount of plasma flowing to the parts of the kidney that have a function in the production of constituents of urine. GFR is affected by renal perfusion and the permeability of the glomerular capillaries, and it is measured indirectly through the concept of clearance. Clearance can be defined as the volume of plasma that can be completely cleared from a particular substance by the kidneys in the unit of time (Laterza et al., 2002). GFR is commonly expressed adjusted for body surface area (BSA) as ml/min/1.73 m^2. The GFR is considered normal if it is ≥90 ml/min/1.73 m^2 (Hogg et al., 2003).

2.1.2 Clearance techniques for assessing GFR

Inulin is considered an ideal marker and the gold standard for measuring GFR, because it is neither reabsorbed nor tubularly secreted or metabolized by the kidneys (Holweger et al., 2005; Rahn et al., 1999; Schwartz and Furth, 2007). Inulin clearance requires constant intravenous infusion and continuous collection of urine, which makes it impracticable in children, and thus isotope methods are often used in clinical practice. The clearance of 51-chromium labeled ethylenediaminetetra-acetate (^{51}Cr-EDTA) is virtually identical with the clearance of inulin, and therefore it is a true GFR marker (Blaufox, 1991; Ditzel et al., 1972). 99m-technetium labeled diethylene triaminopentoacetic acid (^{99m}Tc-DTPA) also shows good agreement with inulin clearance which makes it an excellent substance for measuring GFR (Barbour et al., 1976; Blaufox, 1991; Rehling et al., 1984). Major advantages for ^{99m}Tc-DTPA are low radiation dose and the fact that it can be also used for renal imaging (Blaufox, 1991). In addition to these common contrast media iohexol is used in several centers to evaluate the GFR. Iohexol is a contrast medium that is not radioactive; it is eliminated from plasma exclusively by glomerular filtration, and has a close agreement to GFR measured by inulin clearance (Gaspari et al., 1995).

These clearance techniques include either the compartmental analysis method, which requires several blood samples, or a simplified model that utilizes only one blood sample (Blaufox, 1991). The mathematical model for the disappearance curve is an open two-compartment system, where the GFR marker is injected at the first compartment, equilibrates with the second compartment, and is excreted from the first compartment by glomerular filtration (Schwartz and Furth, 2007). Comparable results can also be obtained using the one-compartment model, by which samples are obtained 2-5 h after injection (Brochner-Mortensen, 1972; Schwartz and Furth, 2007).

2.1.3 Serum creatinine

Serum creatinine (SCr) is generally used as an indirect indicator of GFR, based on the assumption that creatinine is primarily eliminated by glomerular filtration, and the production and excretion of creatinine are believed to be constant. Therefore, SCr is inversely correlated with GFR. However, SCr is influenced by several factors such as age, gender and nutrition. Creatinine concentration is also highly dependent on muscle mass because muscle cells contain about 98% of the total body creatinine pool (Perrone et al., 1992). Dietary protein deficiency leads to negative nitrogen balance and loss of muscle mass, thereby decreasing creatinine production. Other muscle-wasting conditions such as chronic glucocorticoid therapy also decrease the amount of creatinine (Perrone et al., 1992). Due to changes in body composition, SCr has age-dependent reference values for children.

The serum concentration of creatinine may remain within the reference range until there is at least 50% loss of renal function (GFR about 60 ml/min/1.73m^2) (Hogg et al., 2003; Levey et al., 2003). It has also been shown that in moderate renal insufficiency tubular secretion of creatinine may account for up to 60 % of total creatinine excretion (Perrone et al., 1992). In addition, some drugs, such as thrimetoprim, may inhibit creatinine secretion (Berglund et al., 1975). Therefore, creatinine turnover is not only affected by changes in GFR but also by changes in tubular function.

2.1.4 Creatinine clearance

The measurement of creatinine clearance (CrCl) using timed (usually 24-hour) urine collection basicly provides greater accuracy than SCr, but it is difficult to perform and is prone to urine collection failure, even in standardized conditions (Hellerstein et al., 2006; Tsubaki et al., 1993). CrCl is affected by the same factors as SCr, namely age, gender, diet and muscle mass. As a result of proximal tubular secretion, in normal individuals CrCl regularly exceeds GFR by 10-40% (Perrone et al., 1992). The use of a 24-hour urine collection for the estimation of GFR has consistently been shown to be no more, and often less, reliable than prediction equations based on serum creatinine (Hogg et al., 2003).

2.1.5 Creatinine-based mathematical formulae

To overcome the problems in SCr and CrCl, several mathematical equations have been developed for estimating GFR from SCr and biometric data. These formulae are based on the hypothesis that creatinine excretion is constant, and the creatinine pool is predicted by age, gender, weight and height.

Schwartz et al. (1976) and Counahan et al. (1976) presented formulas based on serum creatinine and height (**Table 1**). The only difference between these formulas is the constant (k), which is 0.55 in children in the Schwartz formula, and 0.43 in the Counahan-Barratt formula (Counahan et al., 1976; Schwartz et al., 1976). The difference between the constants appears to be the result of different assays used to measure creatinine (Hogg et al., 2003). It has also been recommended that the optimal value for k should be assessed locally (van Rossum et al., 2005). However, Schwartz later revised his formula by changing the constant to 0.7 in males ≥13 years, since the muscle mass of

adolescent boys is greater than the muscle mass in younger boys or girls (Schwartz and Gauthier, 1985). The modified Schwartz formula has been shown to have a good correlation with inulin clearance in children (Pierrat et al., 2003), but others have shown that the formulae are too imprecise for an accurate determination of GFR, since there might be a difference larger that 10 ml/min/1.73 m^2 between estimated GFR and inulin clearance (van Rossum et al., 2005).

Cockcroft and Gault also presented a formula in 1976, but their formula was based on serum creatinine and body weight instead of height (**Table 1**) (Cockcroft et al., 1976). Their formula is found to be useful only in children over 12 years of age (Pierrat et al., 2003), and has limitations in patients with malnourishment, severe obesity and limb amputation, in whom body weight may not accurately reflect muscle mass.

The Modification of Diet in Renal Disease (MDRD) formula uses SCr, demographic characteristics (age, sex, and ethnicity), and other serum measurements (urea nitrogen and albumin concentrations) (Levey et al. 1999), but it is not useful in children at all (Pierrat et al., 2003).

Table 1. *Formulae used in assessment of GFR*

Schwartz formula	$k \times height \times [SCr / 88.4]^{-1}$	*k=0.55 in females and males <13 years*
		k=0.7 in males ≥13 years
Counahan–Barratt formula	$k \times height \times [SCr / 88.4]^{-1}$	*k=0.42*
Cockcroft–Gault formula	k x (140 - age) × weight ×	*k=1 in males*
	88.4 × $[SCr \times 72]^{-1}$	*k=0.85 in females*

2.1.6. Serum cystatin C

Cystatin C (CysC) is a 13-kDa protein that is produced in all nucleated cells at a constant rate (Abrahamson et al., 1990; Laterza et al., 2002). It is freely filtered by the glomerulus, and almost completely reabsorbed and catabolized in the tubules (Jacobsson et al., 1995). The clearance of CysC is virtually identical to that of ^{51}Cr-EDTA (Tenstad et al., 1996). The production rate of CysC is independent of muscle mass, height, age and sex (Bökenkamp et al., 1998; Helin et al., 1998; Keevil et al., 1998; Laterza et al., 2002). Abnormalities in thyroid function have been shown to have an impact on CysC, and also some drugs have been proposed to alter CysC levels (Fricker et al., 2003; Galteau et al., 2001). Corticosteroids have been shown to increase the concentration of CysC (Cimerman et al., 2000; Pöge et al., 2004; Risch et al., 2005; Wasen et al., 2003). In vitro studies showed a dose-dependent increase of up to 80% of CysC secretion by HeLa cells following dexamethasone exposure (Bjarnadottir et al., 1995). This increase in secretion of CysC is due to a promoter-mediated increase in transcription in the CysC gene.

CysC can be determined by two separate methods: particle-enhanced turbidimetric immunoassay (PETIA) and particle-enhanced nephelometric immunoassay (PENIA). The PETIA method generally produces reference values that are 20-30% higher than those of the PENIA method (Laterza et al., 2002). Cystatin C is age independent in children, except for infants under 1 year of age (Bökenkamp et al., 1998; Filler et al., 1997; Finney et al., 2000; Helin et al., 1998; Randers et al., 1999).

Several meta-analyses show that CysC is clearly superior to SCr as a marker of GFR (Dharnidharka et al., 2002; Roos et al., 2007). CysC been has shown to be superior to SCr, especially for detecting impaired GFR (Filler et al., 2002; Kyhse-Andersen et al., 1994; Laterza et al., 2002; Risch et al., 2001; Stabuc et al., 2000; Ylinen et al., 1999). CysC has been shown to be able to detect mild reductions in GFR earlier than SCr. Coll et al. (2000) showed that CysC levels started to increase when GFR was 88 ml/min/1.73 m^2, whereas SCr began to increase when GFR was 75 ml/min/1.73 m^2 (Coll et al., 2000). CysC has also been shown to be superior to SCr in small children (Corrao et al., 2006).

There are only a small number of studies evaluating CysC in pediatric cancer patients. Bardi et al. (2004) showed that CysC measurement can be used to evaluate glomerular function in children with cancer (Bardi et al., 2004). Lankisch et al. 2006 showed that CysC is a suitable marker for monitoring GFR in pediatric cancer patients, especially in young children (<3 years) as CysC had a better diagnostic value than SCr (Lankisch et al., 2006). It has been proposed that in cancer patients the dying nucleated cells might induce an increase in CysC concentration (Laterza et al., 2002), but later studies failed to confirm the CysC-increasing effect of neoplasms (Mojiminiyi et al., 2002; Stabuc et al., 2000).

2.1.7 Albuminuria

Small amounts of protein may be found in the urine of healthy children. Persistently increased protein secretion is usually a marker of kidney damage. Increased excretion of albumin is a sensitive marker for glomerular disease (Hogg et al., 2003).

Measurement of protein excretion in a 24-hour collection has long been the “gold standard” for the quantitative evaluation of albuminuria. An alternative method is measurement of the ratio of albumin to creatinine in an untimed “spot” urine specimen. A urine albumin to creatinine ratio of less than 30 mg/g in the first morning urine specimen is considered as normal (Hogg et al., 2003). Standard urine dipsticks are also acceptable in the screening of proteinuria (Hogg et al., 2003).

Detecting albuminuria is important since proteins filtered by the glomerulus are responsible for further tubulo-interstitial injury that leads to renal impairment (Ardissino et al., 2004; Remuzzi and Bertani, 1990). Treatment of proteinuria with ACE inhibitors may slow down the rate of progression of the nephropathy (Simonetti et al., 2007).

2.1.8 Assessment of tubular function

Some components of glomerular filtrate are nearly completely reabsorbed in the proximal tubule provided that the plasma concentration is below a certain value, the renal threshold value. Such substances are bicarbonate, amino acids, phosphate and glucose, so that glucosuria, phosphaturia, amino aciduria and alkaline urine pH are signs of proximal tubular dysfunction (Jones and Chesney, 2004). Large proteins are not filtered into urine at the glomeruli, but low molecular weight (LMW) proteins may reach the urinary space. LMW proteins sized 10 to 44 kDa are then reabsorbed in the proximal tubules. Increased amounts of LMW proteins, such as α_1– and β_2–microglobulin, are found in urine in tubular dysfunction.

Approximately 60% of the filtered sodium and 67% of filtered potassium is reabsorbed in the proximal tubule (Berry et al., 1978; Stanton and Koeppen, 1990). The rest of the sodium and potassium reabsorption takes place in the loop of Henle and the distal tubules via Na^+-K^+-Cl^- transporter and NaCl cotransporter, and some of the sodium is also reabsorbed by passive reabsorption (Jones and Chesney, 2004). Magnesium is mainly reabsorbed by the proximal tubule and the thick ascending limb of Henle's loop (65% and 30%, respectively) (Stanton and Koeppen, 1990). Therefore, in proximal tubular damage blood magnesium levels decline due to decreased reabsorption.

Homeostasis is maintained if tubular function is normal, but in pathological conditions the blood electrolyte levels may change. However, in addition to renal function there are also other factors affecting electrolyte homeostasis, such as the intestinal system.

2.2 Assessment of glomerular function in pediatric cancer patients

There is no consensus on methods that should be used in the assessment of renal function of pediatric oncology patients. In most of the studies on pediatric cancer patients renal function has been evaluated by creatinine clearance (Ferrari et al., 2005; Irwin et al., 1996; Suarez et al., 1991; Yetgin et al., 2004) or mathematical formulae based on creatinine (Hempel et al., 2003). In a limited number of studies the GFR has been evaluated by ^{51}Cr-EDTA (Cole et al., 2004; Skinner et al., 1992; Thomas et al., 2000). Cystatin C is a promising new marker, but the data on children with cancer is still sparse (Bárdi et al., 2004 Pediatr Nephrol; Lankish et al., 2006).

2.3 Nephrotoxic treatments in pediatric cancer patients

2.3.1 High-dose methotrexate

Methotrexate (MTX) is a folate agonist that inhibits dihydrofolate reductase, the enzyme responsible for converting folic acid to reduced folate cofactors (Bleyer, 1978). High-dose methotrexate (HD-MTX) is commonly used in pediatric oncology in the treatment of acute lymphatic leukaemia (ALL), non-Hodgkin lymphoma (NHL), osteosarcoma and certain brain tumors (Cairo et al., 2007; Gustafsson et al., 2000; Koch Nogueira et al., 1998; Patte et al., 2007; Pui and Evans, 2006; Timmermann et al., 2006). The dose of MTX in HD-MTX treatments varies between 0.5-33.6 g/m^2 intravenously according to the treatment protocol (Hempel et al., 2003; Nathan et al., 2006; Pui and Howard, 2008; Treon and Chabner, 1996). MTX is also used at low doses in the maintenance therapy of ALL and in the prophylaxis of graft versus host disease (GVHD) after stem cell transplantation, as well as in treatment of rheumatoid arthritis and other chronic inflammatory disorders (Cutolo et al., 2002; Gustafsson et al., 2000; Helliwell and Taylor, 2008; Hoyt et al., 2008; Schröder and Stein, 2003; Vandell and DiPiro, 2002). In low-dose treatment, the MTX dosage is usually in the range of 7-25 mg/m^2/week, given as a single dose orally or by intramuscular injection (Cutolo et al., 2002; Genestier et al., 2000; Schroder and Stein, 2003). MTX is also administered intrathecally in pediatric ALL and AML protocols usually at doses of 6-12 mg (Gustafsson et al., 2000; Lie et al., 2003; Lin et al., 2008).

The most common side-effects of MTX infusions are myelosuppression, gastrointestinal mucositis, nausea and the transient rise of liver transaminases (Aquerreta et al., 2002; Bleyer, 1978; Moe and Holen, 2000; Rask et al., 1998). Inthratecal methotrexate therapy may cause neurotoxicity, especially chemical arachnoiditis, white matter changes, demyelination and motor dysfunction (Cohen, 2004; Gagliano et al., 1976; Inaba et al., 2008; Mott et al., 1972; Pääkkö et al., 2000).

High-dose MTX therapy is known to cause acute renal dysfunction (Perazella, 1999; Widemann et al., 2006). The etiology of MTX-induced renal failure is believed to be mediated by the precipitation of MTX and its metabolites in the renal tubules, or via a direct toxic effect of MTX on the renal tubules (Deray et al., 1989; Fox, 1979; Fuskevåg et al., 2000; Smeland et al., 1996). Since more than 90% of MTX is eliminated by renal clearance, its elimination time is correlated with glomerular filtration rate (GFR) (Bleyer, 1978; Fox, 1979; Skärby et al., 2003). Critical determinants of MTX cytotoxicity are not only drug concentration but also duration of exposure (Widemann et al., 2006). Therefore, acute renal failure is a serious problem, since it may lead to further systemic toxicity due to delayed elimination of MTX. Although it has been suggested that MTX-related impairment in renal function is reversible, there are only a small number of follow-up reports concerning long-term renal function after HD-MTX therapy (Bárdi et al., 2004; Kakihara et al., 2003; Perrone et al., 1992).

Protection of renal function during HD-MTX treatment includes intensive hydration and alkalinization of urine in order to prevent precipitation of MTX in the renal tubules (Bleyer, 1978; Sand Jacobsen, 1981; Skärby et al., 2003). MTX is poorly soluble at acidic pH. An increase in the urine pH from 6.0 to 7.0 results in a five- to eightfold greater solubility of MTX and its metabolites (Widemann et al., 2006). Folinic acid (also called leucovorin, calcium folinate or citrovorum factor) rescue is also routinely administered to patients after MTX infusion. Serum MTX (S-MTX) levels are assessed and used for the dosing of folinic acid (Bleyer, 1978). Folinic acid competes with MTX in the inhibition of dihydrofolate reductase, thus preventing the inhibition of DNA, RNA and protein synthesis by MTX (Bleyer, 1978). The doses of folinic acid needed to rescue malignant cells are higher than used in clinical protocols (Cohen, 2004). Therefore, the protective action of folic acid is believed to be directed to the protection of non-malignant cells. However, there is also evidence of a reduced cure rate in childhood ALL patients when high leukovorin doses are used (Skärby et al. 2006). In cases of severe acute renal toxicity carboxypeptidase G_2 ($CPDG_2$) is administered (Buchen et al., 2005; Snyder, 2007). It is a recombinant form of the bacterial enzyme $CPDG_2$, cloned from Pseudomonas strain RS-16. Carboxypeptidase G_2 rapidly metabolizes circulating MTX to the inactive metabolite DAMPA, and is more efficient than dialysis-based methods in protecting patients from MTX toxicity (Widemann et al., 2006).

2.3.2 Platinum derivatives

Cisplatin (cis-diamminedichloroplatinum, CIS) is a heavy-metal compound used in the treatment of a large number of carcinomas, including osteogenic sarcoma, Ewing's sarcoma, hepatoblastoma and germ cell tumors (Estlin and Veal, 2003; Gobel et al., 2002; Roebuck and Perilongo, 2006). Cisplatin induces acute changes in glomerular and

tubular function (Brillet et al., 1994). Proximal tubular toxicity causes hypomagnesemia and hypocalcemia, and glomerular damage can be noticed by reduced GFR (Skinner et al., 1998; Womer et al., 1985). Cisplatin has been reported to induce long-term reduce in GFR and renal magnesium wasting (Ariceta et al., 1997; Brillet et al., 1994; Goren, 2003).

Carboplatin (CARBO), a structural analogue of CIS, has been proposed to be less nephrotoxic (Sleijfer et al., 1989). It is now a first-line drug for several pediatric tumors, including germ cell tumours, primitive neuroectodermal tumours, low grade gliomas and malignant mesenchymal tumours (Doz and Pinkerton, 1994; Lashford et al., 1996; Mann et al. 2008). Carboplatin may be also associated with renal magnesium wasting and reduced GFR, but the changes are less severe than those caused by cisplatin (English et al., 1999).

2.3.3 Ifosfamide and cyclophosphamide

Ifosfamide (IFO) is an alkylating agent that is used in pediatric treatment protocols of lymphomas, rhabdomyosarcoma, soft tissue sarcomas, Wilms' tumor, bone sarcomas, germ cell tumors and neuroblastoma (Carli et al., 2003; Einhorn, 2003; Fulfaro et al., 2003). Proximal tubular dysfunction is the commonest presentation of IFO-induced nephrotoxicity (Skinner, 2003). It may lead to Fanconi syndrome, including hypophosphatemic rickets and proximal renal tubular acidosis. Ifosfamide may also cause any combination of chronic glomerular, proximal or distal tubular toxicity (Goren et al., 1989; Rossi et al., 1992; Skinner et al., 1993). Concomitant treatment with CIS may further increase the level of IFO-induced impairment of tubular function (Rossi et al., 1994). IFO may also cause hemorrhagic cystitis, which can be prevented by hydration and the use of sodium-2-mercaptoethane sulphonate (MESNA) (Blowey et al., 1995). There is evidence that IFO-induced tubular toxicity may progress months after treatment, with development of glomerular impairment in some children. Ifosfamide is one of the most important cytotoxic drugs likely to cause chronic renal toxicity, and may cause chronic nephrotoxicity in 30-60% of children (Skinner et al., 1993). The management of IFO-induced nephropathy is usually supportive, including prolonged supplementation of phosphate or bicarbonate (Skinner, 2003).

Cyclophosphamide (CP) is an alcylating agent that resembles ifosfamide but is less nephrotoxic (Rossi et al., 1999). The major side-effect of CP is hemorrhagic cystitis, which can be prevented by MESNA that is capable of binding acrolein, the causative agent of CP-induced hemorrhagic cystitis (Luce and Simons, 1988; Ramu et al., 1995). Cyclophosphamide has also been used in conditioning therapy for stem cell transplantation (Gustafsson et al., 2000).

2.3.4 Radiation therapy

Radiotherapy to the kidney may be applied in the context of total body irradiation (TBI) prior to stem cell transplantation (SCT) or occur when the primary tumor is located in or close to the kidneys (Rossi et al., 1999). Doses exceeding 20 Gy result in interstitial nephritis and glomerulosclerosis (Cassady, 1995). Predominant clinical findings are azotemia, anemia, proteinuria and hypertension (Rossi et al., 1999). Late effects may

include hypertension and overall deterioration of renal function (Irwin et al., 1996). The use of iodinated contrast medium can also result in nephropathy (Aspelin et al., 2003). Combined exposure to nephrotoxic anticancer agents and iodinated contrast medium may place patients at higher risk for renal failure (Harned and Mascarenhas, 2007).

2.3.5 Supportive therapy

As a consequence of aggressive therapy, prolonged periods of immune suppression increase the risk of nosocomial infections (Rossi et al., 1999). In the context of severe infections, acute renal failure may become a part of multi-organ failure (Lane et al., 1994). Antibiotic treatment itself rarely induces relevant nephrotoxicity (Rossi et al., 1999). However, some nephrotoxic antibiotics and antiviral agents, especially aminoglycosides, cidofovir and foscarnet, should be used with caution if a patient is treated with nephrotoxic cytostatics or has and impaired renal function (Bárdi et al., 2007; Cesaro et al., 2005).

Amphotericin B has gained increased importance in both prophylaxis and treatment of fungal infections (Rossi et al., 1999). It may induce decreased GFR and distal tubulopathy with renal salt wasting, hypokalemia, hypomagnesemia and loss of urine concentrating ability (Goldman and Koren, 2004; Nath et al., 2007; Rossi et al., 1999). The liposomal form of amphotericin B has a reduced nephrotoxic potential and may, therefore, be more suitable in the treatment of pediatric cancer patients (Cesaro et al., 2006; Dorea et al., 1997). Several new antifungal agents have now almost replaced amphotericin B (Antachopoulos et al. 2007).

Nonsteroidal anti-inflammatory drugs (NSAIDs) interfere with the synthesis of prostaglandins, and therefore they may reduce renal blood flow and GFR (Clive and Stoff, 1984). The reduction in GFR is fortunately usually reversible, but it is of clinical importance during treatment with potentially nephrotoxic drugs, i.e. methotrexate (Maiche et al., 1988; Thyss et al., 1986). However, NSAIDs are not often administered to cancer patients due to the effect of NSAIDs on platelet aggregation. Diuretics are often used in cancer patients, but they also may exhibit some nephrotoxic side effects during nephrotoxic cancer treatment (Maiche et al., 1988; Rossi et al., 1999).

2.3.6 Stem cell transplantation

Stem cell transplantation (SCT) has improved the prognosis of children with a number of hematological and malignant disorders, metabolic diseases and immunodeficiencies (Gluckman et al., 2008; Hasle, 2007; Malatack et al., 2003; Sato et al., 2007). The source of hematopoietic stem cells is either bone marrow (bone marrow transplantation, BMT), stem cells collected from peripheral blood (PBSC), or umbilical cord blood. Bone marrow cells, mainly harvested from the iliac crest, are sometimes been substituted by G-CSF mobilized hematopoietic stem cells harvested from the peripheral vein (Gianni et al., 1989). Patients with solid tumors usually receive an autologous transplant using bone marrow or sometimes peripheral stem cells if they are treated with high dose chemotrapy. Allogeneic SCTs are generally used for patients with hematologic malignancies (Sanders, 1997). In allogeneic SCT the donor can be a relative, a sibling or other family member, or an unrelated donor can be used. The purpose of SCT is to enable a complete eradication of the dysfunctional or malignant cells of the recipient

by very high-dose chemotherapy, bypassing the otherwise dose-limiting bone marrow aplasia with salvaging autologous or allogeneic graft (Gustafsson Jernberg et al., 2003). The transplanted cells will constitute the new bone marrow with normal hematological and immunological function. The immunological reaction against leukemia cells, graft-versus-leukemia (GVL) effect has also been shown to be of importance in allo-SCT (Horowitz et al., 1990). Infections, graft-versus-host disease (GVHD) and relapsed disease are the main sources of morbidity and mortality (Sanders, 1997). There are also several late effects specific for SCT, mainly depending on pre-transplantation conditioning therapy with or without total body irradiation (TBI), chemotherapy or organ manifestations of GVHD (Ranke et al., 2005). TBI is considered to be the principal cause of chronic SCT nephropathy, the clinical onset of radiation-induced nephropathy typically developing between 6 and 12 months after irradiation (Cohen, 2000; Frisk et al., 2002).

It has been reported that 5-28% of pediatric long-term SCT survivors will develop chronic renal dysfunction (Kist-van Holthe et al., 2002; Miralbell et al., 1996; Patzer et al., 2001; Van Why et al., 1991). Patients treated with SCT have especially high risk for renal impairment, since they have often already been treated with nephrotoxic drugs before SCT. Conditioning with cyclophosphamide, carboplatin and / or TBI may further deteriotare the renal function. Factors after the SCT may also reduce the renal function: GVHD, infections and their treatments, and veno-occlusive disease (VOD). Cyclosporine A (CsA) is often used as an immunosuppressant following stem cell transplantation. Its nephrotoxicity presents as a decrease in GFR (Pape et al., 2007). A high clearance rate and fast elimination of CsA in children neccessitates monitoring of blood cyclosporine levels, especially since CsA has numerous drug-drug interactions that can result in toxic levels of cyclosporine (Burckart, 1983).

2.4 Childhood malignancies

2.4.1 Leukemia

Leukemia is the most common cancer during childhood. **Acute lymphoblastic leukemia (ALL)** accounts for 85% of all childhood leukemias (Gustafsson et al., 1998). In the Nordic countries, the incidence of ALL is 4.0 per 100 000 children under 15 years of age (Hjalgrim et al., 2003). The incidence has been very stable for the last 20 years (Svendsen et al., 2007). ALL is slightly more common in males, and the peak incidence is at the age of 2-5 years (Pui and Howard, 2008). Today the cure rate of ALL is >80% in children (Pui and Howard, 2008).

The diagnosis of ALL is based on morphology, and immunophenotyping of leukemic lymphoblasts by flow cytometry is essential to establish the correct diagnosis and define cell lineage (Pui, 1995). ALL is broadly considered to have two lineages, B (85%) and T (15%) (Pui, 1995). The most common findings with therapeutic importance are B-cell, mature B-cell, and T-cell precursor phenotypes (Pui and Evans, 1998). Children with ALL are stratified into risk groups according to cell type, chromosomal changes, age, white blood cell count at diagnosis (tumor burden), central nervous system (CNS) or testis involvement, and the presence of mediastinal mass (Gustafsson et al., 1998; Pui and Howard, 2008). The response to treatment is also a factor in determination of

the risk group (Pui and Howard, 2008). Assessment of the risk of relapse in individual patients ensures that very intensive treatment is given only to high-risk cases, thus sparing children at lower risk from toxic effects.

In Nordic countries, pediatric ALL patients are treated according to leukemia protocols introduced by the Nordic Society of Paediatric Haematology and Oncology (NOPHO), which resembles those used elsewhere (Gustafsson et al., 1998; Pui and Howard, 2008). Briefly, the treatment consists of the remission-induction phase, where the goal is to eradicate leukemic cells. After restoration of normal hematopoiesis, intensification treatment (consolidation) is generally used to eradicate drug-resistant leukemic cells. After consolidation the ALL patients need continuation treatment to prevent relapse. All patients receive chemotherapy for 2.0 – 2.5 years after the diagnosis. In all risk groups there is also an additional CNS-directed treatment. In the highest risk group and often in the case of relapsed disease the most intensive form of treatment is allogeneic hematopoietic stem cell transplantation.

Acute myeloid leukemia (AML) is rare in the pediatric population. The incidence rate of AML in Nordic countries is 0.72 per 100 000 children under 15 years (Hjalgrim et al., 2003). The female/male case ratio for AML is 1.16 (Hjalgrim et al., 2003). 5-year event-free survival (EFS) in pediatric AML patients is 41-50% (Lie et al., 2005; Pui and Howard, 2008).

Children with Down's syndrome (DS) have a significantly increased risk of developing myelodysplastic syndrome (MDS) and AML (Abildgaard et al., 2006). AML in DS patients typically occurs at the age of 1-2 years. AML patients with DS have a significantly higher survival rate than other children, the 5-year EFS beeing around 75% in DS-AML patients (Abildgaard et al., 2006; Creutzig et al., 2005). However, very intensive therapy is more often associated with higher mortality and more treatment-related toxicity in DS-patients (Lange et al., 1998). Therefore, the treatment is often reduced in DS patients (Abildgaard et al., 2006).

NOPHO-protocols are also used in AML patients in the Nordic countries (Lie et al., 2005). The treatment consists of induction and consolidation blocks and lasts only 6-8 months. The treatment is mainly based on cytarabine, 6-thioguanine and doxorubicin. Relapsed patients are usually treated by allogeneic stem cell transplantation, and even after SCT the children may achieve a cure rate of 60-65% (Abrahamsson et al., 2007; Lie et al., 2005).

2.4.2 Lymphoma

The two main types of lymphomas are Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). Lymphomas are the most common pediatric extracranial solid tumors.

The annual incidence of **NHL** in Nordic countries is 0.9 per 100 000 per year in children under age of 15 years (Marky et al., 2004). Median age at time of diagnosis is 8.0 years, and the female/male ratio is 2.2. The four major subtypes of NHL include Burkitt lymphoma (40%), diffuse large B-cell lymphoma (20%), lymphoblastic lymphoma (30%)

and anaplastic large cell lymphoma (10%) (Sandlund et al., 1996). The treatment of NHL is based on the immunophenotypic subgroup and stage. In Nordic countries most pediatric NHL patients are treated by protocols that include alkylating agents and antimetabolites (HD-MTX and ARA-C) (Marky et al., 2004). The treatment lasts about six months. In T-cell tumours, treatment is usually identical or based on that of ALL and lasts about two years. The prognosis of NHL is very good, EFS exceeding 90% in stage I and II patients (Marky et al., 2004).

The incidence of **HL** is 0.4 per 100 000 in the Nordic countries (Gustafsson et al., 1998). Childhood HL typically affects children at 10 to 14 of age and has a male dominance (Spitz et al., 1986). Asymptomatic cervical or supraclavicular lymphadenopathy is the most common presentation of HL in children, also mediastinal adenopathy is common (Hudson Donaldson, 1997). In early childhood, recent primary EBV infection is a risk factor for developing HL. In most treatment protocols the patients are placed into three risk groups according to the following factors: disease stage, age, tumor bulk and adverse features (Hodgson et al., 2007). The treatment regimen is chosen by the risk group and other individual factors (Nachman et al., 2002), and it usually includes a combination of involved-field radiotherapy and chemotherapy. Since there is an increased risk of secondary cancers in HL patients, the exposure to alkylators and radiation is usually limited to as low as possible. Despite reduction of alkylating agents and radiotherapy, the cure rates in children with localized disease are 94-100%, and even in high-risk group the five-year-EFS is up to 86% (Hodgson et al., 2007; Nachman et al., 2002; Schellong et al., 1999).

2.4.3 Solid tumors

Neuroblastoma is the most common extracranial solid tumor in children. It is an embryonal malignancy arising from the sympathetic nervous system. Neuroblastoma occurs more commonly in young children with 50% of them presenting before 2 years of age (Howman-Giles et al., 2007). The most common primary site is the retroperitoneum: the adrenal glands (35%) or paraspinal ganglia (35%) (Howman-Giles et al., 2007). Neuroblastoma may also originate from the posterior mediastinum, pelvis or neck. The NOPHO survey showed that the incidence of neuroblastic tumors is 0.9 per 100 000 children under age of 15 years (Gustafsson et al., 1998). The clinical behavior of neuroblastoma varies markedly, ranging from spontaneous regression to progressive fatal disease. Treatments are based on the subtype classification by the stage of the disease and on biological and biochemical parameters (Howman-Giles et al., 2007). Light and electron microscopy as well as immunohistochemistry are needed in the diagnostic process and stageing of neuroblastoma. The main genetic marker, which is also a significant prognostic factor, is N-MYC. The amplification of N-MYC, ploidy changes, partial deletions of chromosome 1 and 11, and gains of chromosome 17 are all unfavourable prognostic factors (Bown, 2001; Brodeur, 2003; Tonini et al., 1997). Biochemical markers associated with poor prognosis include high serum levels of lactate dehydrogenase, neuron-specific enolase, and ferritin levels (Howman-Giles et al., 2007). Patients younger than 12 months of age have a better prognosis than older patients, while children older than the age of 2 years with stage 4 disease have very poor prognosis (Evans and D'Angio, 2005). Management is tailored to risk and varies widely. Low-risk disease is sometimes treated

by surgery alone, while high-risk disease requires megatherapy with autologous stem cell transplantation. Most patients are treated with multiagent chemotherapy, surgery and sometimes biologic response modifiers, such as cis-retinoic acid (Howman-Giles et al., 2007).

Wilm's tumor is the most frequently occurring renal tumor in children. It is a very treatment-responsive tumor, which is most common between the ages of one and five years, and the peak age is three years (Varan, 2008). The incidence of Wilm's tumor is 0.9 per 100 000 children yearly in the Nordic countries, and it represents 6% of all childhood malignant tumors (Gustafsson et al., 1998; Varan, 2008). The two most common genetic abnormalities in Wilm's tumor are the WT1 and WT2 gene deletions, and the p53 tumor suppressor gene has been found in 75% of patients with anaplastic histology (Bardeesy et al., 1994; Govender et al., 1998; Varan, 2008). Surgery is the cornerstone of treatment of Wilm's tumor when the affected kidney with the tumor is removed. Pre-and postoperative chemotherapies have been used, and sometimes local radiotherapy has been added to the therapy (Green, 2004; Varan, 2008). The prognosis of patients with Wilm's tumor is the most favourable of all solid tumors; the survival is up to 85% (Varan, 2008).

The incidence of **soft-tissue sarcomas** (rhabdomyosarcoma, embryonal sarcoma, soft tissue Ewing's tumor) is 0.9 per 100 000 children yearly in the Nordic countries (Gustafsson et al., 1998). Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma. 65% of the cases are diagnosed in children younger than 6 years of age, and it is slightly more common in males (Paulino and Okcu, 2008). It is highly malignant, and treatment always includes neoadjuvant and adjuvant chemotherapy in addition to surgery and often even radiotherapy is added after resection (Paulino and Okcu, 2008). Approximately 30% of patients with RMS will relapse, and 50% to 98% of these patients will die of progressive disease (Crist et al., 1995; Paulino and Okcu, 2008; Raney et al., 1983).

The yearly incidence of **malignant bone tumors** (osteosarcoma, chondrosarcoma, Ewing's sarcoma) in the Nordic countries is 0.5 per 100 000 children under 15 years of age (Gustafsson et al., 1998). The peak incidence occurs in the second decade of life. In about 75% of cases, patients with osteosarcoma are between 15-25 years of age. Males are more frequently affected than females (ratio 1.5:1) (Picci, 2007). Osteosarcoma arises predominantly in the long bones and is highly malignant. Drugs used in the treatment of osteosarcoma are HD-MTX, cisplatin, adriamycin and ifosfamide. Prognosis for localized osteosarcoma treated with pre- and postoperative chemotherapy associated with surgery varies between 60-70%, and in patients with metastasis at onset it is about 30% (Picci, 2007). Ewing's sarcoma is the second most common bone tumor in children. Surgery is a major tool, and chemotherapy increases survival from less than 5% to 65-70% for patients with localized tumors and to 23-30% for those with metastases (Marec-Berard and Philip, 2004). Ewing's sarcoma is also quite sensitive to radiation therapy (Donaldson, 2004).

Even though **germ-cell tumors** and other gonadal tumors originate from progenitor germ cells, they comprise a very diverse group of neoplastic tumors. The yearly incidence of germ cell tumors is 0.4 per 100 000 children under the age of 15 years

in the Nordic countries (Gustafsson et al., 1998). Germ cell tumors include teratomas, dysgerminomas, seminomas, choriocarcinomas and endodermal sinus tumors (Horton et al., 2007). The site of the primary tumor (extragonadal) and the degree of elevation of AFP are associated with worse survival. Patients with gonadal germ cell tumors have a 95% 5-year survival for early stage disease and at least 85% 5-year survival for advanced stages, whereas 4-year survival for mediastinal germ cell tumor is 70% (Horton et al., 2007). The treatment consists of surgery and chemotherapy.

Hepatoblastoma is the most common liver tumor in young children. The yearly incidence of hepatoblastomas is 0.2 per 100 000 children under the age of 15 years in the Nordic countries (Gustafsson et al., 1998) It is an embryonal tumor, and 90 percent of cases are manifest by the fourth birthday (Meyers, 2007). In older children the most common primary malignant liver tumor is **hepatocellular carcinoma** (HCC). HCC is relatively chemoresistant and therefore carries a poor prognosis with a dismal 15% cure rate (Meyers, 2007). Multifocal tumors are common, and usually complete surgery is not possible. New treatment modalities including metronomic chemotherapy and adjuvant anti-angiogenic therapy have shown some early promising results (Meyers, 2007; Pang and Poon, 2006).

2.4.4 Central nervous system (CNS) tumors

Malignant primary brain tumors are the leading cause of death from solid tumors in children (Buckner et al., 2007). Common presenting symptoms include headache, seizures, and altered mental status. Treatment depends on the histologic diagnosis. The mortality rate from malignant brain tumors remains high despite initial disease control, and also the morbidity among brain tumor survivals is very high. The yearly incidence of brain tumors is 4.2 per 100 000 children under the age of 15 years in the Nordic countries (Gustafsson et al., 1998). Most children are under the age of eight years at the time of diagnosis. Embryonal CNS tumors (medulloblastoma, primitive neuroectodermal tumor (PNET) and ependymoblastoma) comprise the most common group of malignant childhood CNS tumors (Becker, 1999). Due to poor prognosis CNS tumor patients are heavily treated by surgery, radiotherapy and chemotherapy, but still the overall survival is only about 70% (Äärimaa et al., 1997; Gustafsson et al., 1998). Novel therapies such as high-dose chemotherapy with stem cell transplantation, intrathecal chemotherapy, use of radiosensitizers (topotecan and paclitaxel), imatinib mesylate and gene therapy have improved the prognosis slightly (Macdonald, 2003).

3 AIMS OF THE STUDY

The objective of this thesis was to study the effect of chemotherapy on renal function in pediatric cancer patients, and to evaluate the methods used to assess renal function.

The specific study aims were:

1. To study the effects of methotrexate on renal tubular cells (**I**)
2. To compare an isotope clearance method with alternative methods to determine the glomerular filtration rate in order to find possible screening methods to measure glomerular function in pediatric cancer patients (**II**)
3. To evaluate the long-term renal function after HD-MTX treatment in children (**III**)
4. To study the long-term renal function following bone marrow transplantation in children (**IV**)

4 MATERIALS AND METHODS

4.1 Patients

4.1.1 Study II

Thirty-six children were included in the study, 15 males and 21 females. Patients had been treated for a malignant disease in Turku or Oulu University Hospital. The mean age at the diagnosis was 6.9 years (range 0-17.4 years). The mean age of the patients by the time of the study was 11.3 years (SD 5.7, range 2.8 - 23.9 years). The mean follow-up time after the end of cancer treatments was 1.6 years (SD 3.4, range 0-14.1 years). There were 26 ALL patients, four brain tumor patients, two neuroblastoma patients, and one patient of each of the following diagnoses: non-Hodgkin lymphoma, Ewing's sarcoma, hepatocellular carcinoma and germ cell tumor. ALL and lymphoma patients were treated according to NOPHO (Nordic Society of Paediatric Haematology and Oncology) protocols which include cytostatic drugs such as potentially nephrotoxic high-dose methotrexate (HD-MTX). The patients with solid tumours were treated by multiagent chemotherapy including nephrotoxic drugs such as cisplatin, cyclophosphamide or ifosfamide. Most of the ALL patients also received cyclophosphamide in addition to HD-MTX. None of the patients had a history of previous renal disease. One to ten assessment were performed to each patients, the total number of glometular function assessments was 112. Renal function was measured either before cancer treatment (n=9), during cancer treatment (n=39), or after cessation of all chemotherapy (n=64).

4.1.2 Study III

Twenty-three patients with a follow-up of at least 12 months after chemotherapy were selected from the patients of paper II. In addition, five patients treated at Kuopio University Hospital were included in the study. Of these 28 patients, 12 were males and 16 females.The mean age at the diagnosis was 6.9 years (range 1.5-15.3 years) and at the follow-up it was 13.8 years (range 5.8-23.8 years). The mean follow-up time after the end of cancer treatments was 4.7 years (range 1-10 years). Renal function before the initiation of HD-MTX treatment was collected retrospectively from patient charts, and prospectively evaluated once in all these patients after the end of chemotherapy. Twenty-five patients were treated for ALL, and three patients were treated for lymphoma. One lymphoma patient was treated according to modified COMP-DAUNO protocol (Sposto et al. 2001) and all other patients were treated according to NOPHO protocols (Gustafsson et al., 2000). Patients had received either 5 g/m^2 (n=16) or 8 g/m^2 (n=12) of methotrexate (MTX) two to nine times depending on the treatment protocol. The cumulative dose of MTX was 16-45 g/m^2. All patients were hydrated intravenously by a doubled fluid amount, starting 12 hours immediately prior to the HD-MTX infusion, and the heavy hydration was continued until methotrexate concentration in the blood was low enough. Urine pH was monitored and kept alkaline in order to prevent precipitation of MTX and its metabolites in the renal tubules causing tubular obstruction. MTX concentration was measured, and leukovorin rescue was administered according to the protocol. None of the patients had a history of previous renal disease.

4.1.3 Study IV

A total of 187 BMTs were performed among children at Karolinska University Hospital, Huddinge from 1980 through 2000. All patients with GFR measurements after BMT were included in the study. There were 110 male patients (59%) and 77 (41%) females. Age at the time of BMT was 0.04–17.6 (median 8.0) years, and the age at the follow-up was 1.5-23.3 years (mean 11.7 years). The mean follow-up time was 3.8 years (range 0.1-16.0 years). Renal function tests were performed 1–13 times per patient. A total of 50 subjects, 3–22 (median 11) years of age, with no signs of kidney disease served as controls with regard to renal function.The data was retrospectively reviewed from the patient charts. Ninety percent (n=169) of the patients underwent allogeneic BMT, and the other ten percent (n=18) of the patients underwent autologous BMT. The underlying diseases in the allogeneic BMT patients are listed in **Table 2.**

4.1.3.1 Donor histocompatibility and stem cell source

Most patients were given transplants from an HLA A, B, DRB1-compatible sibling (n=104, 61%). Others received syngeneic (n=4, 2%) and matched related (n=4, 2%) or unrelated (n=27, 16%) grafts, while a small number of patients received one antigen mismatched related (n=10, 6%) or unrelated (n=20, 12%) graft. For class I and II antigens, HLA typing was performed serologically, and when they became available around 1990, genomic methods were used for confirmation. Eighteen patients received autologous grafts. Non-manipulated bone marrow was used as the stem cell source with the exception of recipients of mismatched grafts, who received T cell-depleted (TCD) marrow.

Table 2. *Baseline characteristics of the study population that underwent allogeneic transplantation*

Groups	Age at transplantation (years; mean ± 1 SD)	Diagnosis	Number of patients
Group 1: Hematologic malignancies (n=108)	9.0 ± 4.8	ALL	54
		AML	33
		Lymphomas	6
		MDS	8
		CLL	7
Group 2: Aplastic anemias (n=28)	8.5 ± 4.7	Aplastic anemia	19
		Fanconi's anemia	9
Group 3: Non-malignant diseases (n=33)	5.2 ± 3.3*	Immunodeficiencies	10
		Hemoglobinopathies	5
		Inborn errors of metabolism	18

*significantly lower than in group 1 (P=0.002) and in group 2 (P=0.02)
ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, MDS = myelodysplastic syndrome, CLL = chronic lymphoblastic leukemia

4.1.3.2 Conditioning

Most patients with leukemia were prepared by being given cyclophosphamide (CY; 60 mg/kg/day for two days; total dose 120 mg/kg) and busulfan (BU; 1 mg/kg × 4/day for four days; total dose 16 mg/kg) or 10 Gy of single fraction TBI usually delivered as 7-8 cGy/min. Fractionated TBI was introduced in 1996, and after that some patients received

12–14.4 Gy of fractionated TBI instead of single fraction TBI. A total of 119 patients (64%) received TBI. Lung shielding was used in all patients conditioned with TBI. Antithymocyte globulin (ATG) or other anti-T-cell antibodies were given before BMT to patients with unrelated donors. Most patients with aplastic anemia were prepared by being given CY at 50 mg/kg/day for four days (total dose 200 mg/kg) and ATG. Most patients with inborn errors of metabolism received BU at 4 mg/kg/day for four days, followed by four days of CY at 50 mg/kg/day. In patients with hemophagocytic lymphohistiocytosis (HLH), leukemia in incomplete remission or Philadelphia chromosome-positive ALL, etoposide (900 mg/m^2) was added.

4.1.3.3 Post-BMT immunosuppression and supportive care

Cyclosporin A (CsA) was given as GVHD prophylaxis in combination with a short course of iv methotrexate in most children. A small number of children received single therapy with either CsA or methotrexate (MTX). Tapering of the CsA level was started 3-6 after BMT, and the CsA was withdrawn 9-12 months after BMT in most cases. Prolonged treatment was given to patients with SAA, inborn errors of metabolism or chronic GVHD. Patients with ALL, AML M4 or M5 were given intrathecal prophylaxis with MTX or cytarabine. Antimicrobial prophylaxis included trimetoprim–sulfamethoxazole, fluconazole and acyclovir to HSV sero-positive patients. Since 1992, preemptive treatment with ganciclovir or foscarnet has been given for 2–3 weeks to patients with positive CMV-DNA in peripheral blood leukocytes.

4.2 Assessment of renal function

4.2.1 Studies II and III

4.2.1.1 Isotope assessment of glomerular filtration rate (GFR)

Isotope GFR was measured by plasma clearance of ^{51}Cr-EDTA or ^{99m}Tc-DTPA. ^{51}Cr-EDTA was used in all hospitals, in Turku University Hospital some GFR measurements were done by ^{99m}Tc-DTPA. ^{51}Cr-EDTA (0.74 MBq/kg, maximum 3 MBq) or ^{99m}Tc-DTPA (0.43 MBq/kg, maximum 15 MBq) was administered intravenously as a bolus dose and three (at 2, 3 and 4 hours, ^{99m}Tc-DTPA) or five (at 2½, 3, 3½, 4 and 4½ hours, ^{51}Cr-EDTA) blood samples were drawn from the opposite arm after the injection.

4.2.1.2 Laboratory analyses of renal function

Blood samples for serum creatinine (SCr) and serum CysC analysis were drawn immediately before injection of the isotope for iGFR measurement. Serum and urinary creatinine concentrations were measured using the Jaffé reaction (Roche Diagnostics, Mannheim, Germany, and Hitachi 917; Hitachi Ltd., Tokyo, Japan). Serum CysC concentrations were determined using a particle-enhanced nephelometric immunoassay (N Latex Cystatin C, BN II System; Dade Behring, Marburg, Germany). Urine for creatinine clearance was collected 12 hours prior to the injection of isotope. Collection time of 12 hours was chosen instead of 24 hours in order to improve the quality of the collection. GFR was also estimated by mathematical equations based on serum creatinine concentration **(Table 1)**.

Serum albumin was analyzed using a nephelometric immunoassay (BN II system; Dade Behring, Marburg, Germany) and urinary albumin was analyzed using an immunoturbidimetric method (Optima, Microalbuminuria Kit; Thermo Clinical Labsystem, Helsinki, Finland). A urinary albumin- to- creatinine ratio above 2.5 mg/mmol was considered as abnormal.

Tubular function was evaluated by assay of serum electrolyte, phosphate and magnesium levels and by acid-base balance (pH and bicarbonate). It was also assessed by measuring the urinary concentration(s) of low molecular weight (LMW) proteins (α_1- or β_2-microglobulin). The LMW proteins were analyzed using a nephelometric immunoassay (BN II system; Dade Behring, Marburg, Germany). The concentration was considered abnormal if that of urinary α_1-microglobulin was above 6 mg/L or that of urinary β_2-microglobulin was above 200 µg/L.

4.2.2 Study IV

Renal function was evaluated as glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) determined by the clearances of inulin (Inutest, 25%, Laevosan Gesellschaft, Linz, Austria) and para-aminohippuric acid (PAH, aminohippurate sodium, 20%, MSD, West Point, NJ), respectively. A standard clearance technique was used, with continuous infusion after a prime dose. Water diuresis was induced by oral ingestion of 20 ml water/kg body weight during the first hour and then 5 ml/kg body weight every 30 min. After an equilibration time of one hour, four 30-min urine samples were collected and midway through each collection period a blood sample was drawn. The clearance values presented are the means of the four clearance periods. Investigations in the youngest children (<2 years of age) were performed with a single injection of inulin and PAH, after which blood samples were drawn regularly during the next 180 min and clearance was calculated by means of the two-compartment method. During the last few years, PAH has not been available, which means that sometimes the number of investigations of GFR and ERPF differ.

4.3 In Vitro studies

Cell culture medium and the plastic ware were from Invitrogen Life Technologies (Carlsbad, CA). The MTT assay kit was from Promega (Madison, WI). Methotrexate was purchased from Wyeth-Lederle. Amiloride and carbachol were purchased from Sigma (St. Louis, MO).

4.3.1 Cell culture and treatment

The LLC-PK_1 cell line from porcine proximal tubule (European Collection of Animal Cell Cultures, ECACC) was maintained in M199 medium supplemented with 10% fetal calf serum, penicillin (50 U/ml), and streptomycin (50 µg /ml), in an atmosphere of 5% CO_2 and 95% air at 37°. For cell viability assay and morphological observations the cells were grown in 12-well plates ($1.5x10^{-5}$ cells/well) or 24-well plates ($0.9x10^{-5}$ cells/well) to confluence and then treated with methotrexate alone or in combination with 0.1 µM amiloride or 1 µM carbachol for different periods of time. The methotrexate

concentrations used were 1 μM, 0.1 μM or 0.01μM. The control plates were treated with equivalent volumes of sterile distilled water.

4.3.2 Cell viability

4.3.2.1 Trypan blue exclusion test

Cells were detached from the flasks by trypsinization. An aliquot of the cell suspension was mixed with an equal volume of 0.2% Trypan blue in PBS (phosphate-buffered saline). Cells were counted with the phase contrast microscope using a Neubauer improved counting chamber. Cells with damaged cell membranes were stained blue (necrotic or late apoptotic cells), while cells with intact plasma membrane remain unstained. The experiments were performed in triplicate and repeated two or three times.

4.3.2.2 MTT assay

Mitochondria of viable cells cleave the tetrazolium rings of the pale yellow MTT (3-(4,5dimethylthiazol)-2,5-diphenyl tetrazolium bromide) resulting in formation of a dark blue formazan product. After removal of medium from 24-well plates, the cells were rinsed and incubated with MTT. The MTT formazan precipitate was dissolved and read under a microplate reader (Dynatech, Chantilly, VA) with a test wavelength of 562 nm and a reference wavelength of 660 nm. The experiments were run at least three times in triplicate. Results from different experiments are normalized to an internal standard (i.e. untreated cells) and are expressed as relative units.

4.3.3 Morphology and cell size

The morphology of control cells and cells incubated with methotrexate were studied by phase contrast microscopy and digital pictures were taken. Then, the borders of about 100 random-selected cells in each group were manually outlined and the areas calculated. Cell selection was made by a member of our team who was blinded to the experimental groups. The software used to identify the cells in the pictures and to calculate the areas was written by this member and is currently used for such measurements at the image analysis core facility of the Department of Pathology of the Karolinska University Hospital/ Karolinska Institute in Stockholm. The results are expressed as pixels.

4.3.4 Annexin-V / propidium iodide (PI) staining

Early apoptotic cells were identified by an Annexin V-fluorescein isothiocyanate (FITC) detection kit containing **propidium iodide (PI)** (Annexin Alexa Fluor, Molecular Probes, Eqgene, OR.) Cells on cover slips were washed in Annexin-binding buffer; then 3μl/100μl of Annexin V-FITC and 0.1μg/100μλ of PI were added for 15 min incubation in darkness at room temperature. The cells were examined under fluorescent microscope (Nikon, Japan). Annexin V labels externalized phosphatidylserine which indicates apoptotic cell membrane disruption. Staining with PI indicates necrotic cells with cell membrane damage.

4.3.5 Western blotting

Protein analysis was performed as previously described (Chen et al. 2004). Briefly, cells were lysed for 15 minutes in urea sample buffer (62.5 mM Tris-HCl, pH 6.8; 6 M urea; 10% glycerol; 2% SDS; 0.00125% bromophenol blue; 5% β-mercaptoethanol), sonicated for 15 s and then incubated at 65°C for 15 minutes. Poly (ADP-ribose) polymerase (PARP) was detected with anti-PARP antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

4.4 Statistical analysis

In **Study I**, data were expressed as ± SEM (standard error of mean). Results of MTT assays were expressed relative to the absorbance of an internal standard (pooled controlled cells) to which an arbitrary value of 1 was given. Statistical analyses were performed using ANOVA multiple comparison tests. The critical value of significance was $P<0.05$.

In **Study II**, linear associations between the results of the iGFR method and those of alternative methods to determine glomerular filtration rate (CysC, SCr, CrCl and formulae) were tested separately using general linear mixed models. “Subject” was taken into account as a random factor because there were multiple observations within subjects. The effects of age, height and weight were used as explanatory variables in the analysis, when appropriate. Residuals were checked for justification of the analyses. In the analysis of cystatin C, the distribution of residuals was a little positively skewed because of two outliers. The data was reanalyzed after removing the outliers, and because the results were sufficiently similar in both analyses, the original analysis is reported. The similarity between average values measured by using iGFR and values calculated with the three different formulae was analyzed by using general linear mixed models. The value of GFR (iGFRs and GFRs estimated with formulae) was used as a response variable and “method” was used as an explanatory variable. For analyzing the reliability of the formulae compared with iGFR, intra-class correlation coefficients (ICCs) were calculated for values measured with iGFR and three formulae. Pairwise comparisons of methods were made so that three formulae were compared separately to the iGFR method. Intra-class correlation coefficients reflect relative homogeneity within groups in relation to the total variation. The effect of corticosteroids was analyzed using general linear mixed models to check if the association between iGFR and alternative GFR methods depends on use of corticosteroids. This was done by calculating regression equations for both groups and testing the interaction between the group and GFR method. A p-value less than 0.05 was considered significant. Statistical analyses were performed using SAS System software version 9.1.3, service pack 4 (SAS Institute Inc., NC). The Bland and Altman plotting method was also carried out to explore the agreement between methods of estimating GFR values.

In **Study III**, the effects of follow-up time, age at the time of diagnosis, MTX dose and other forms of medication (vancomycin, gentamycin, and amphotericin B) were analyzed. Associations among continuous variables were studied by using simple regression analyses. Independent samples t-tests were used for comparison of mean values between dichotomous groups. Paired samples t-tests were used to compare values of iGFR before and after treatment. Simple logistic regression analysis was performed

to examine the effects of continuous variables on dichotomous outcome variables. Comparisons between categorial variables were carried out using Fisher's exact test.

In **Study IV**, the patient groups used in statistical analyses are presented in Table 1. The statistical analyses were conducted using Statistica 7.0 software (StatSoft®, Inc., Tulsa OK, USA) and SAS® System 8.2 (SAS Institute Inc., Cary, NC, USA).

In comparisons of patients and controls before BMT and after 1 year the Mann–Whitney U test was used. For comparisons between controls and different groups of diagnoses, Kruskal–Wallis ANOVA by ranks followed by multiple comparisons between groups based on ranks was performed.

For comparisons over time, two-way ANOVA for repeated measures was performed using the procedure MIXED in SAS® System 8.2. Several models were analyzed, taking into account different between-effect factors, diagnoses, types of BMT, cyclophosphamide and total body irradiation. The residuals from the models were positively skewed, especially for the variable ERPF, and therefore these data were logarithmically transformed in order to meet the requirements for adequate ANOVA.

4.5 Ethical considerations

All studies were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Studies II-III were approved by the Ethics Committee of the Southwest Finland Health Care District, and all subjects or their parents gave their written informed consent. Study IV was approved by the local Human Research Ethics Committee at Karolinska University Hospital, Huddinge, Karolinska Institutet, Stockholm, Sweden.

5. RESULTS

5.1 Study I: Morphological changes induced by methotrexate

Trypan blue exclusion tests revealed that incubation of LLC-PK_1 cells with 0.1 μM MTX decreased the number of viable cells by 18% after 4 hours in comparison with control cells (**Figure. 1**). The difference was significant compared to control cells ($p<0.05$). The number of viable cells further decreased when the cells were incubated with 1 μM MTX, but the difference between treated goups was not significant.The decrease in cell viability was confirmed by MTT assay.

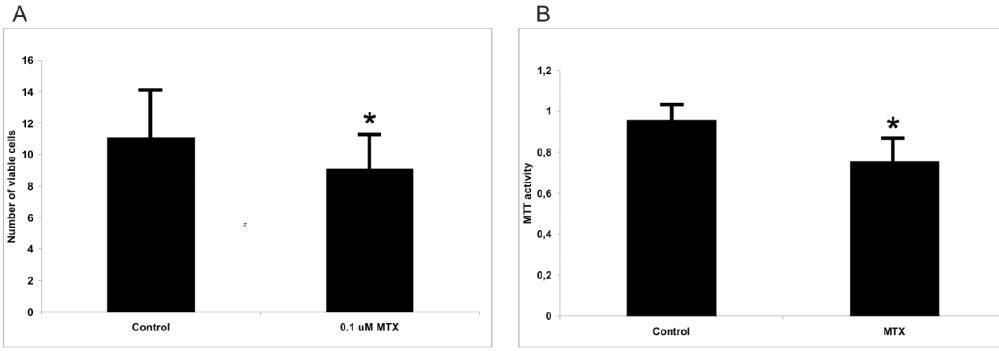


Figure 1. *Cell viability after 4 hours incubation. A) Trypan blue exclusion test B) MTT assay. (In: Grönroos et al. Pediatr Blood Cancer 2006;46:624-629)*

To evaluate if the lower number of viable cells was the result of suppression of cell growth or an increased rate of cell death, the number of dead cells was also studied. We found that the proportion of trypan blue (TB) -positive cells (dead cells) compared with the total number of cells per group was increased by 38% after incubation with 0.1 μM MTX (**Figure 2**).

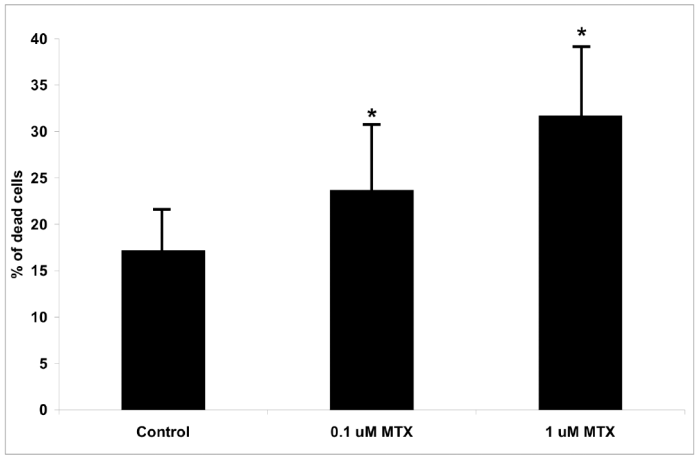


Figure 2. *Cell death after 4 hours' incubation*

Cell death induced by MTX was time-dependent (**Table 3**). After four hours' incubation with 1 μM MTX the number of living cells was still relatively high (82% vs. controls), but after 96 hours' incubation with MTX the number of viable cells was only 10% of the number of viable control cells. The difference in the number of viable cells was significant after 12 hours.

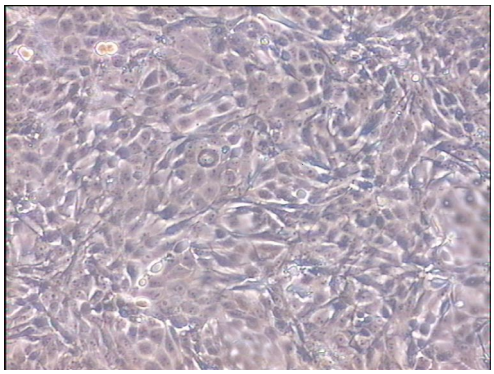
Table 3. *Number of viable cells*

	Control	1 μM Methotrexate
4 hours	11.1 ± 0.8	9.1 ± 0.7
12 hours	18.5 ± 1.1	8.4 ± 1.8*
72 hours	30.5 ± 6.9	5.3 ± 0.4*
96 hours	41.8 ± 6.4	4.1 ± 0.5*

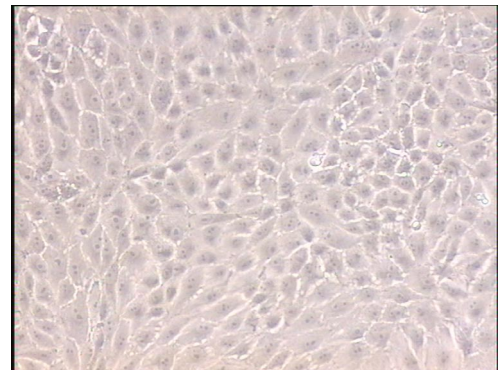
Values are mean ± SEM, n=5 in each group.
**P<0.05 versus controls*

To study if MTX induces necrosis or apoptosis, cells were checked by means of phase contrast microscopy. No signs of apoptotic nuclear shrinkage were observed. The absence of apoptotic cell death was confirmed by biochemical tests. PI/Annexin V labelling revealed only nuclear staining with PI, which indicates necrotic cell death (data not shown). Western blotting did not show cleavage of PARP (data not show).

Morphological studies revealed that cell volume increased significantly after exposure to MTX (**Figure 3**). Maximal cell swelling was found after 12 hours' incubation with MTX. Exposure to both 0.01 μM and 1 μM MTX increased cell volume 1.5-fold vs. controls (**Figure 4**).

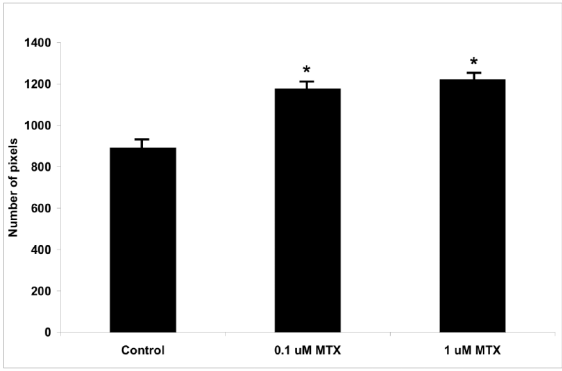


***A:** Control cells*



***B**: Cells incubated with 1 μM MTX*

Figure 3. *Phase contrast microscopic images of LLC-PK$_1$ cells after incubation for 12 hours. (In: Grönroos et al. Pediatr Blood Cancer 2006;46:624-629)*



Values are mean ± SEM. n (control) = 149, n (0.01 μM) = 173, n (1 μM) = 136 and n (100 μM) = 78. p<0.05 (control compared to all of the concentrations).

Figure 4. *Cell size expressed as pixels after incubation for 12 hours. (In: Grönroos et al. Pediatr Blood Cancer 2006;46:624-629)*

To see if the cell swelling is mediated via ion channel activation, the cells were incubated with MTX in the presence of amiloride, a Na^+/H^+ antiporter inhibitor, or carbachol, a potassium effux stimulator. Incubation with 10 μM amiloride completely inhibited MTX-induced cell death, and also incubation with 1 μM carbachol had a protective effect (**Figure 5**).

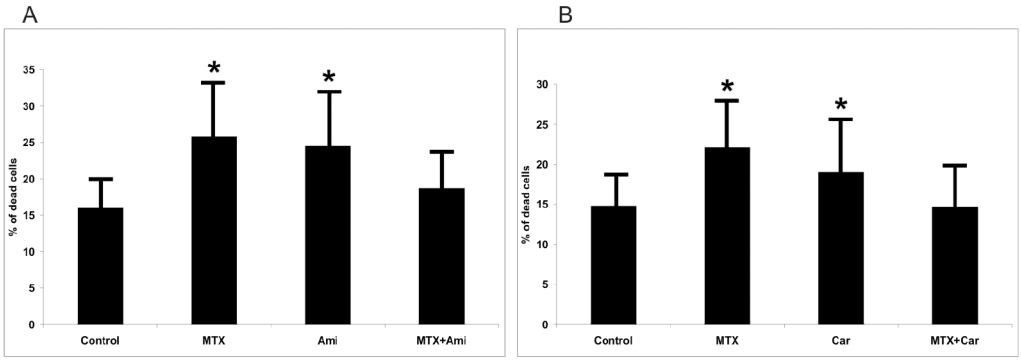


Figure 5. *Incubation for 4 hours with methotrexate and amiloride or carbachol*

$LLC\text{-}PK_1$ cells were incubated with 0.1 μM MTX in the presence or absence of 10 μM amiloride and 1 μM carbachol. Percentages of dead cells compared with all cells were checked by the TB exclusion test. Values are mean ± SEM, n=5 in each group. *P<0.05 versus controls.

5.2 Study II: Evaluation of glomerular function tests

A total of 112 renal function tests were performed on 36 children. The mean iGFR was 113.4 mL/min/1.73 m^2 (SD 30.2, range 51.7–201.2 mL/min/1.73 m^2). The distribution of iGFR results is presented in **Table 4.** Mean SCr and CysC concentrations as well as

GFR estimates based on CrCl and GFR formulae are shown in **Table 5.** All results are presented in **Figure 6**. The associations between iGFR and other parameters are shown as scatter plots in **Figure 7**.

Table 4. *iGFR results*

iGFR	n	%
Normal (> 90 mL/min/1.73 m^2)	89	79
Moderately reduced (60–90 mL/min/1.73 m^2)	21	19
Clearly reduced (≤ 60 mL/min/1.73 m^2)	2	2

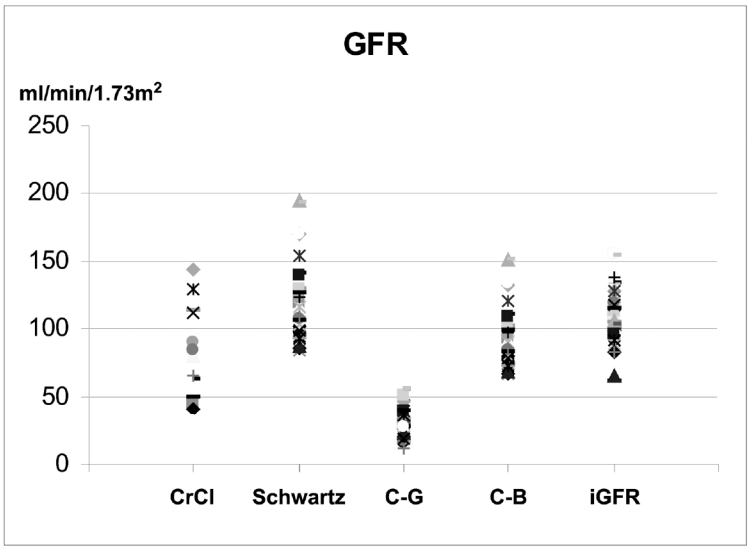


Figure 6. *Glomerular filtration rate (GFR) measured by different methods (CrCl = creatinine clearance, Schwartz = Schwartz formula, C-G = Cockroft–Gault formula, C-B = Counahan–Barratt formula, iGFR = isotope GFR). (In: Grönroos et al. Pediatr Nephrol. 2008;23:797-803)*

Table 5. *Glomerular functions*

	Mean	Range	SD
iGFR	113.4 (ml/min/1.73m^2)	51.7 – 201.2 (ml/min/1.73m^2)	30.2
Schwartz (k=0.55)	116.4 (ml/min/1.73m^2)	83.5 – 194.3 (ml/min/1.73m^2)	23.2
Schwartz (k=0.7 in boys ≥ 13 years)	122.3 (ml/min/1.73m^2)	83.5 – 194.3 (ml/min/1.73m^2)	26.4
Counahan-Barratt	91.0 (ml/min/1.73m^2)	65.3 – 152.0 (ml/min/1.73m^2)	18.1
Cockroft-Gault	93.9 (ml/min)	32.4 – 202.1 (ml/min)	38.4
Creatinine clearance	76.3 (ml/min/1.73m^2)	23.4 – 148.8 (ml/min/1.73m^2)	26.9
Creatinine	61.0 µmol/l	33.9 – 92.0 µmol/l	14.4
Cystatin C	0.77 mg/l	0.49 – 1.16 mg/l	0.14

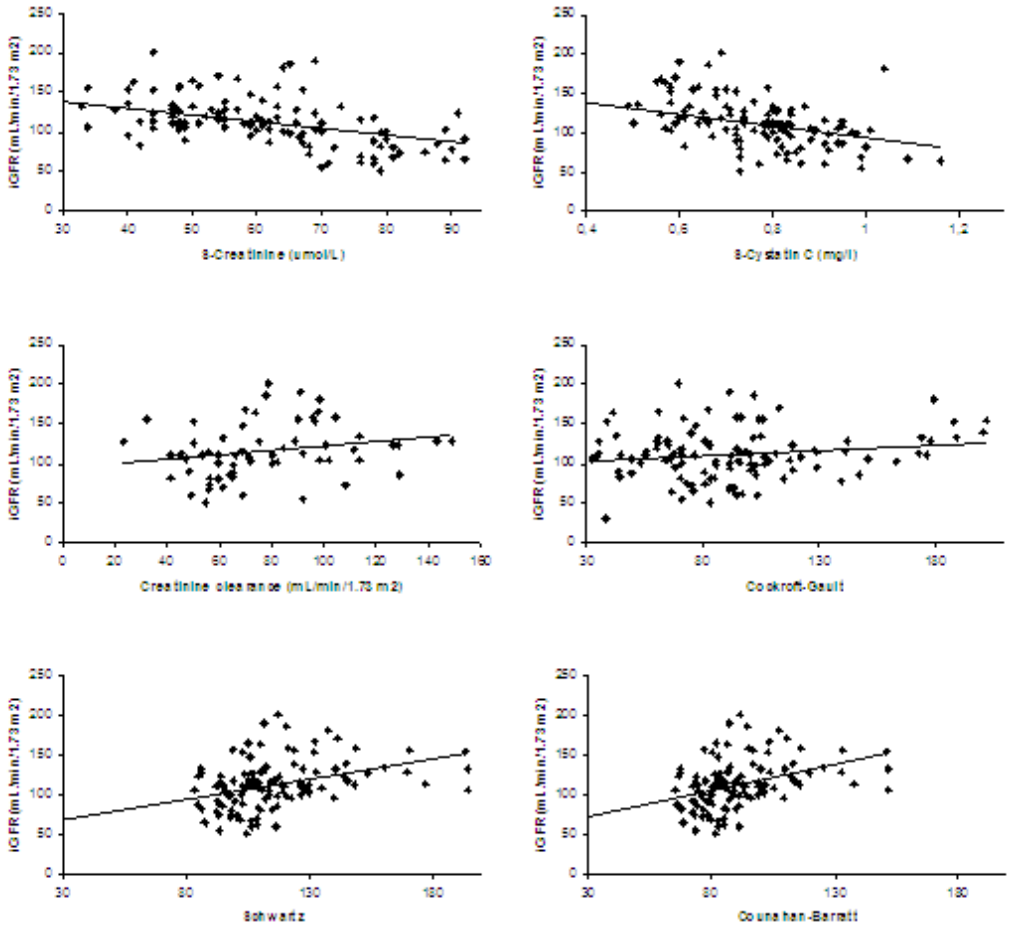


Figure 7. *All methods compared to isotope GFR. (In: Grönroos et al. Pediatr Nephrol. 2008;23:797-803)*

5.2.1 Cystatin C, Serum Creatinine and Creatinine Clearance

Significant associations were found between iGFR and serum CysC (p<0.001), and between iGFR and SCr (p<0 .001). Multivariate analysis was performed for the explanatory variables CysC, SCr, height, weight and age. Height, weight and age were removed from the model because of non significant effects, and the final model included only serum CysC (p=0.006) and SCr (p=0.0005). According to the results of the final model, CysC and SCr were associated with iGFR. Based on univariate analysis, the formula for association of CysC with iGFR is iGFR = 179.04 – 85.27*CysC. Intra-class correlation (ICC) was calculated for values of iGFR and values estimated with the formula for CysC. ICC was only 0.3062 which reflects quite poor reliability. Based on multivariate analysis, the formula for association of CysC and SCr with iGFR is iGFR = 203.33 – 58.38*CysC – 0.73*SCr. ICC for the iGFR method and this formula was 0.4011, which is better than the formula based on CysC alone.

In order to study if corticosteroids have an effect of CysC concentrations, the association between CysC and iGFR was studied. In 24 evaluations (21%) the patient had received corticosteroids prior to the assessment of glomerular function. The association between

serum CysC and iGFR was not statistically significantly different in patients who had received corticosteroids (mean CysC 0.78 mg/L) before GFR measurements and those who had not (mean CysC 0.76 mg/L).

There was no linear relationship between iGFR and CrCl (p=0.7) (**Figure 7**).

5.2.2 Mathematical formulae

There was a linear relationship between iGFR and results of the Counahan–Barratt formula (p=0.004) and between iGFR and Schwartz formula results (p=0.004). In contrast, there was no association between iGFR and Cockroft–Gault formula results (p=0.33). These associations were not influenced by the age, height or weight of the patient. In addition to linear relationships, intra-class correlation was calculated for iGFR and GFR estimates calculated by the formulae. This analysis revealed poor association between iGFR and the results of all three formulae, i.e. the deviations between the GFR values were significantly different. Similarity of the average values of the different formulae, and iGFR was also analyzed by means of linear mixed models, where GFR was the response variable and method was the explanatory variable. Analyses were carried out separately for the three formulae. According to these analyses there was no statistically significant difference between the average values of iGFR and the Schwartz formula. The difference between iGFR and Schwartz formula data was nonsignificant when a k value of 0.55 was used for all patients including pubertal boys (p=0.30). When a k value of 0.7 for pubertal boys was used, the difference between iGFR and Schwartz formula data became significant (p=0.006).

5.3 Study III: Long-term changes in renal function after high-dose methotrexate treatment

5.3.1 Renal function before and during HD-MTX treatment

Baseline renal function of 28 patients treated with HD-MTX is presented in **Table 6**. All of the values were within age-dependent reference limits. None of the patients had significant acute renal failure before or during HD-MTX treatment. In five patients, iGFR was abnormally low at least once during the cancer treatment, and in one of these patients the elimination of methotrexate was delayed.

Table 6.

	Before HD-MTX treatment (mean)	Range	At the follow-up (mean)	Range
Age, years	7.1	1.5 – 15.4	13.8	5.8 – 23.8
Follow-up time, years	-	-	4.7	1.0 – 10.0
Isotope GFR (ml/min/1.73m^2)	136.7 (n=17)	87.0 – 237.0	113.9 (n=28)	75.7–185.6
Schwartz formula GFR (ml/min/1.73m^2)	109.4 (n=11)	79.5 – 152.3	-	-
Serum creatinine	57	34 - 95	64	34 - 92
Proteinuria (n)	not known	not known	9 (32%)	

5.3.2 Renal function at follow-up

5.3.2.1 Glomerular filtration rate

None of the patients had acute renal failure at follow-up 1-10years after chemotherapy. The glomerular filtration rate results at the end of follow-up are presented in **Table 7**. The iGFR declined significantly with increasing follow-up time (p=0.02, estimate of change in one day: -0.29, confidence interval: -0.53 ; -0.04) (**Figure 8**).

Table 7. *iGFR results at the end of follow-up*

iGFR	n	%
Normal (>90 ml/min/1.73 m^2)	25	89%
Moderately reduced (60–90 mL/min/1.73 m^2)	3	11
Clearly reduced (≤ 60 mL/min/1.73 m^2)	0	0

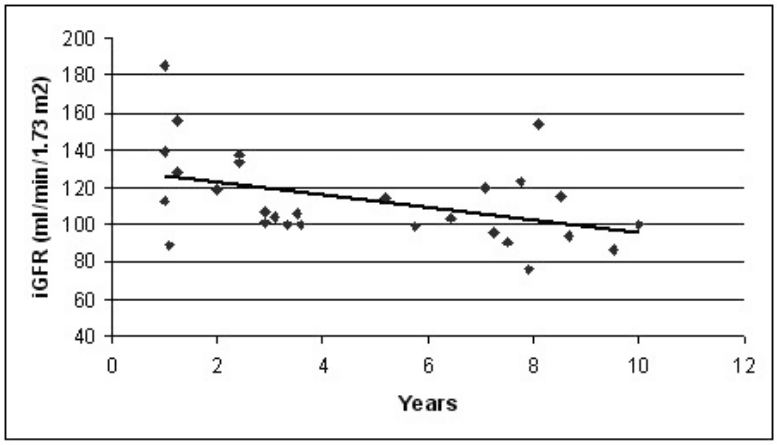


Figure 8. *iGFR and follow-up time. (In: Grönroos et al. Pediatr Blood Cancer. 2008;51:535-539)*

In 17 patients, iGFR measurement was carried out both before treatment and at follow-up. In these patients the mean iGFR dropped from 136.7 mL/min/1.73m^2 (before treatment) to 118.8 mL/min/1.73m^2 (at follow-up), although the difference was not statistically significant (**Figure 9**).

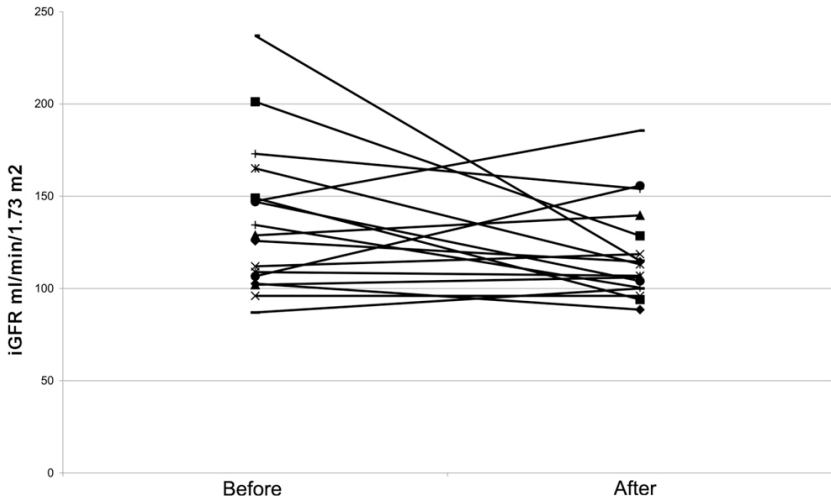


Figure 9. *iGFR before HD-MTX treatment and at the end of follow-up. (In: Grönroos et al. Pediatr Blood Cancer. 2008;51:535-539)*

The age of the patients at the time of diagnosis did not have a significant effect on the change of iGFR. The single dose of MTX (5 g/m^2 or 8 g/m^2), the cumulative MTX dose (**Figure 10**), or the use of amphotericin B, vancomycin or gentamycin did not have an influence on iGFR (data not shown). The five patients who had had reduced glomerular function at some point during cancer treatment had slightly lower iGFRs after the follow-up compared with the other patients (mean 105.1 *vs.* 115.8 mL/min/1.73m^2), but the difference was not statistically significant.

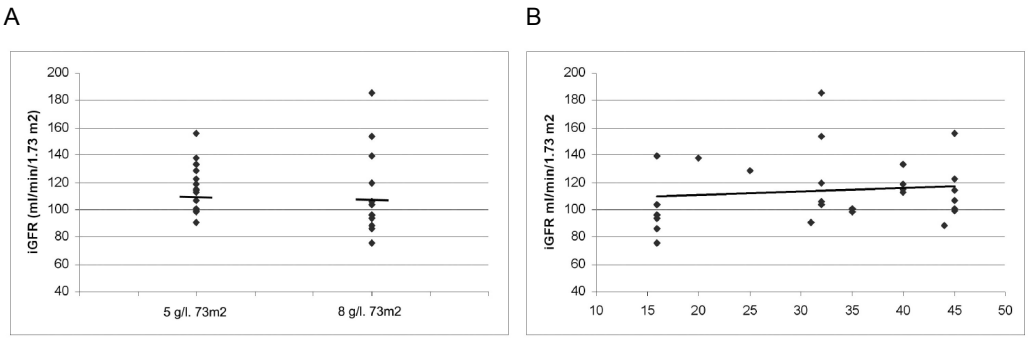


Figure 10. *Influence of single (A) or cumulative (B) MTX dose on iGFR. (In: Grönroos et al. Pediatr Blood Cancer. 2008;51:535-539)*

5.3.2.2 Proteinuria

Significant albuminuria was found in eight patients (29%) and low molecular weight (LMW) proteinuria in four patients (14%) at follow-up. Young age at the time of diagnosis and long follow-up time tended to correlate to albuminuria and LMW proteinuria (**Figure**

11). However, these findings were not statistically significant. The single dose of HD-MTX (5 or 8 g/m^2) and the cumulative MTX dose did not affect the incidence of albuminuria or LMW proteinuria. Albuminuria was more frequently diagnosed in patients who had received gentamycin during chemotherapy (**Table 8**). Albuminuria also tended to be more frequent in patients treated with vancomycin or amphotericin B (**Table 8**).

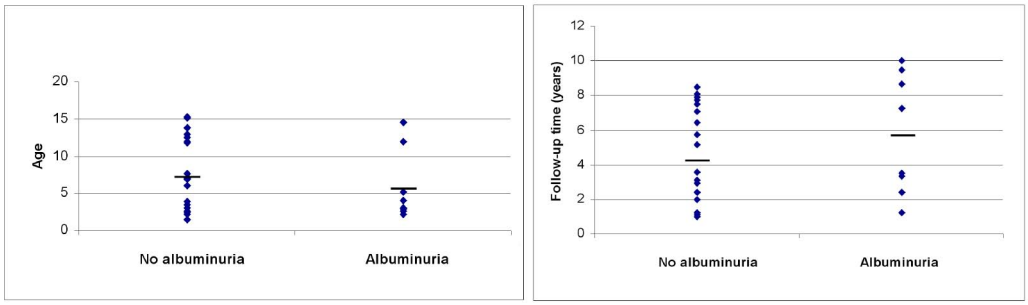


Figure 11. *The effect of age at diagnosis and length of follow-up time on albuminuria. (In: Grönroos et al. Pediatr Blood Cancer. 2008;51:535-539)*

Table 8. *Factors affecting the incidence of proteinuria at follow-up (In: Grönroos et al. Pediatr Blood Cancer. 2008;51:535-539)*

		Albuminuria	p	OR	CI	LMW proteinuria *	p	OR	CI
HD-MTX dose (g/m^2)	5	4/16 (25%)	0.29	1.50	0.29-0.78	1/15 (7%)	0.29	4.67	0.42-52.12
	8	4/12 (33%)				3/12 (25%)			
Renal problems during treatment	yes	2/5 (40%)	0.61	1.89	0.25-14.19	1/4 (25%)	0.50	2.22	0.17-28.98
	no	6/23 (26%)				3/23 (13%)			
Amphotericin B	yes	5/9 (56%)	0.07	6.67	1.10-40.43	2/9 (22%)	0.58	2.29	0.27-19.66
	no	3/19 (16%)				2/18 (11%)			
Vancomycin	yes	3/8 (38%)	0.65	1.80	0.31-10.39	1/8 (13%)	1.00	0.76	0.07-8.66
	no	5/20 (25%)				3/19 (16%)			
Gentamycin	yes	4/6 (67%)	0.04	9.00	1.20-67.42	2/6 (33%)	0.20	4.75	0.50-44.48
	no	4/22 (18%)				2/21 (10%)			

5.3.2.3 Tubular function

None of the patients had glucosuria at follow-up. Serum potassium, sodium, magnesium and bicarbonate concentrations were normal in all patients. One patient had a reduced serum phosphate concentration.

5.3.2.4 Blood pressure

In ten patients (36%) blood pressure was above the 50th percentile of height-based reference values, and two patients (7%) had blood pressure above the 95th percentile at follow-up. Both of them were put on angiotensin-converting enzyme (ACE) inhibitors, with good responses.

5.4 Study IV: Long-term renal function following bone marrow transplantation (BMT)

Among the 187 transplanted patients, 415 clearance tests were performed. Most patients (169; 90%) received allogeneic transplants and 18 patients (10%) received autologous transplants. By the time of the last clearance test, 124 patients (66%) were alive and 63 (34%) had died. During follow-up 63 patients died, and the cause of death was transplantation-related in 28 cases (44%) and due to disease progression or relapse in 35 (56%) of the cases. None of the patients received a renal transplant; two patients were treated with dialysis because of multi-organ failure in the initial post-transplant period.

5.4.1 Renal function before allogeneic BMT

Before transplantation the 67 patients with hematological malignancies had lower GFRs than the controls (108±33 *vs.* 116±11 ml/min per 1.73 m^2, *p*=0.02, **Figure 12**), but the mean GFR in this group was still within the normal range, as was the mean ERPF (590 ml/min per 1.73 m^2). Mean GFRs in the two other groups (aplastic anemia and nonmalignant diseases) were 114±38 and 130±50 ml/min per 1.73 m^2, respectively; neither was there any difference in mean ERPF between these groups (574 and 587 ml/min per 1.73 m^2) and the controls (611 ml/min per 1.73 m^2).

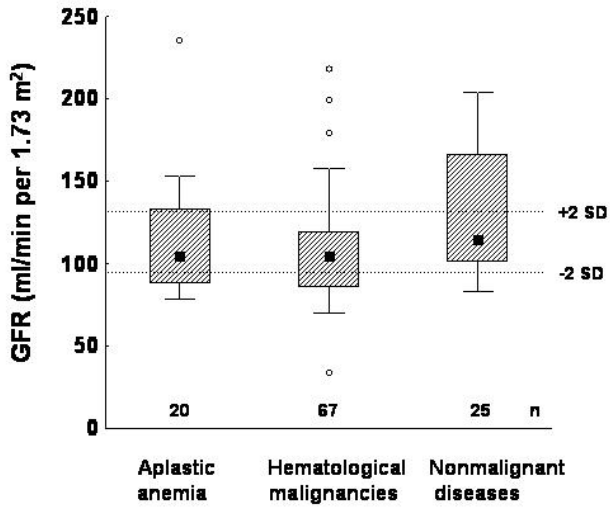


Figure 12. *GFR values before BMT. Box plots with median values, 25th and 75th percentiles, and 10th and 90th percentiles. The dotted lines indicate ± 2SD of GFR of control subjects. (In: Grönroos et al. Bone Marrow Transplant. 2007;39:717-23)*

5.4.2 Changes in renal function one year after allogeneic BMT

In 59 patients GFR and ERPF were evaluated both before and one year after BMT. Both GFR and ERPF were significantly reduced compared with the situation before BMT

(p<0.0001). When analyzing the changes in the different diagnosis groups, GFR was decreased significantly in all groups. However, ERPF decreased only in the hematological malignancy group (p<0.01) (**Figure 13a and b**).

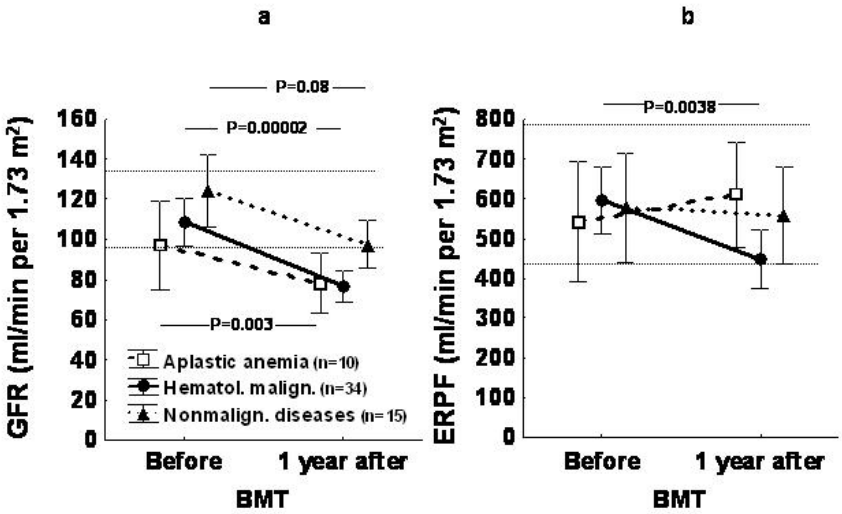


Figure 13. *GFR (a) and ERPF (b) both before and one year after BMT. Mean values with 95% confidence intervals are given. (In: Grönroos et al. Bone Marrow Transplant. 2007;39:717-23)*

5.4.3 Changes in renal function over time after allogeneic BMT

Both GFR and ERPF were significantly reduced 1 year after BMT (**Figure 14a and b**). Before BMT the mean GFR in 112 patients was 114±39 ml/min per 1.73 m^2 and the mean ERPF (in 105 patients) was 586±222 ml/min per 1.73 m^2. One year after BMT the mean GFR (in 91 patients) was 85±26 ml/min/1.73 m^2 and the mean ERPF (in 87 patients) was 508±189 ml/min/1.73 m^2. The mean GFR one year after BMT also differed from that in the controls (p<0.001). One year after transplantation there were 37 patients (41%) with renal impairment, defined as GFR < 80 ml/min/1.73 m^2.

Three years after transplantation a slight but statistically significant recovery after the initial drop was noticed in GFR (p=0.04), after which it remained stable (**Figure 14a**). Renal impairment was noted in 31% of the patients three years after BMT, in 11% of the patients seven years after BMT and in 23% of the patients 10 years after BMT.

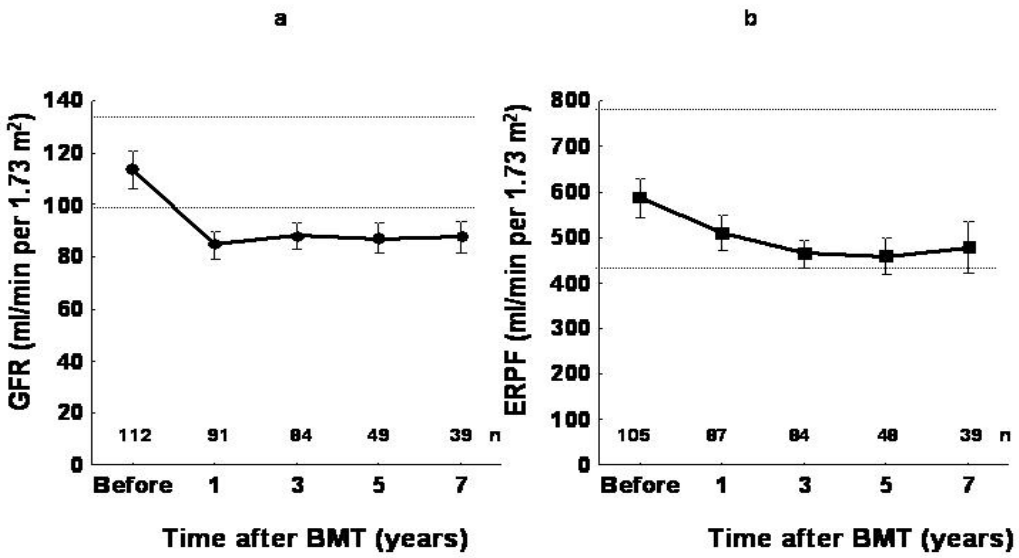


Figure 14. *Changes in GFR (**a**) and ERPF (**b**) over time. Number of patients at each time point is presented above the x-axis. The outlines presented are values of control subjects ± 2SD. Mean values with 95% confidence intervals are given. (In: Grönroos et al. Bone Marrow Transplant. 2007;39:717-23)*

5.4.4 Comparison of renal function between diagnosis groups after allogeneic BMT

There was a difference in GFR but not in ERPF between patients with hematological malignancies and those with nonmalignant diseases at all time points. In patients with hematological malignancies GFR was significantly lower than in those with nonmalignant diseases (p=0.01).

5.4.5 Influence of total body irradiation (TBI) on renal function

The majority (169) of patients were treated with allogeneic BMT. Of these, TBI status was available in 168 cases. One hundred and fifteen patients (68%) received TBI (TBI+ group) and 53 patients (32%) did not (TBI- group). There was no significant difference in GFR or ERPF between the TBI- and TBI+ groups before BMT. After BMT, both GFR and ERPF were reduced in both groups compared with the values beforehand (p<0.0001). In the TBI+ group the fall in GFR and ERPF was more profound than in the TBI- group at all time points (p=0.02) (**Figure 15a and b**).

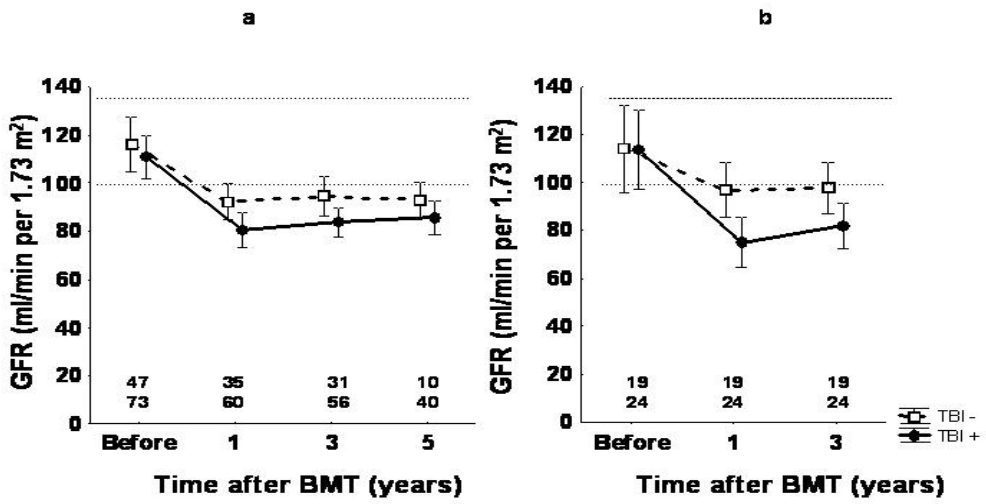


Figure 15. ***a***. *All patients with or without TBI. Number of patients at each time point is presented above the x-axis. Mean values with 95% confidence intervals are given.* ***b***. *Changes in GFR in those 19 TBI- and 24 TBI+ patients repeatedly investigated at all time points. Mean values with 95% confidence intervals are given. (In: Grönroos et al. Bone Marrow Transplant. 2007;39:717-23)*

5.4.6 Influence of donor type on renal function

No difference in GFR or ERPF was found between donor types (matched *vs.* mismatched) at any time point.

5.4.7 Influence of acute graft-versus-host disease (GVHD) on renal function

The patients were divided into two groups according to the grade of acute GVHD: group 1: GVHD grade 0–I (*n*=135), group 2: grade II–IV GVHD (*n*=22). In a third group the grade was not known (*n*=30). No difference in GFR or ERPF between any of these groups was found at any time point (*p*=0.74).

5.4.8 Type of BMT

No differences in GFR or ERPF were noticed between autologous and allogeneic transplantation patients, either before or after BMT.

5.4.9 Cyclophosphamide

129 patients (69%) received cyclophosphamide in their conditioning regimen and 58 patients (31%) did not. These patients included both autologous and allogeneic BMT patients. No significant difference in either GFR or ERPF was noticed before BMT or during follow-up between patients with or without cyclophosphamide.

6 DISCUSSION

6.1 Study population and controls

In studies II and III the patients were enrolled into the study either soon after diagnosis before any nephrotoxic medications were given, or at a follow-up visit. Subjects were collected from three centers. All eligible patients were asked to participate, and only a small number of families refused. All study patients went through the whole study protocol. Patients served as their own controls. Information of renal function and of the nephrotoxic medications given during the whole period were collected at the time of the last follow-up visit. In some patients the glomerular function was not assessed by the isotope clearance method before cancer treatment, and in these cases the glomerular function was evaluated by serum creatinine if it was obtainable.

In study IV all of the data were collected from medical records from 1980 to 2000 by exactly same methods. Therefore, all of the data could not be collected, i.e. the use of nephrotoxic antibiotics, or information on tubular function was not obtainable. We did not carry out any analysis concerning cyclosporin A (CsA), which is known to be nephrotoxic (Bobadilla and Gamba, 2007), since almost all patients were treated with CsA, which was withdrawn at 9–12 months. Almost all of the analyses in study IV are based on allogenous BMT patients, since due to the heterogeneic population and small number of patients the autologous BMT group coud not be further analyzed.

6.2 Glomerular filtration rate measurements and laboratory analyses of renal function

In study IV GFR was assessed by the inulin clearance, which is considered to be the golden standard (Rahn et al., 1999). However, in studies II and III it was not possible to assess inulin clearance, since the method in question is very expensive, cumbersome and not established in the study centers. Therefore GFR was measured either by by plasma clearance of ^{51}Cr-EDTA or ^{99m}Tc-DTPA. The latter method was only used in Turku University Hospital on 14 patients. It has been shown that the clearance of ^{51}Cr-EDTA is virtually identical with the clearance of inulin, and clearance of ^{99m}TcDTPA also shows good agreement with inulin clearance (Barbour et al., 1976; Blaufox, 1991; Ditzel et al., 1972). The GFR measured by the isotope method, iGFR, was then considered as a standard against which other methods were compared. There may be pitfalls in iGFR due to the distribution volume. To overcome this problem, in this study 3-5 blood samples were obtained after the injection of tracer in order to have a reliable disappearance curve. The tracer was injected through an i.v. cannula put to the basilica vein, and the blood samples were obtained from the central venous catheter (CVC) in order to avoid the accumulation of the tracer in the CVC. To our knowledge, there were no abnormal distribution volumes in the iGFR measurements of the study patients.

The clinically used cut-offs for renal impairment varies between countries and centers. In study IV the cut-off for renal impairment was considered as <80 ml/min/1.73m^2, whereas in study III the GFR was was considered normal if it was ≥ 115 mL/min/1.73m^2, reduced

as 90–114 mL/min/1.73m^2, and more clearly reduced as ≤ 89 mL/min/1.73m^2. However, since the National Kidney Foundation (Kidney Disease Outcomes Quality Initiative: K/DOQI) recommends that GFR <60 ml/min/1.73m^2 is reduced, GFR 60-90 ml/min/1.73m^2 is mildly reduced and GFR >90 ml/min/1.73m^2 is normal (Levey et al., 2003), we used these cut-offs in study II where other methods were compared to iGFR.

Timed urine collection would have been the method of choice in detecting proteinuria, but since collection of urine is cumbersome and not reliable in pediatric patients, the LMW proteins and albumin were measured from one early morning spot urine sample. The urinary concentration of albumin was then corrected by the urinary concentration of creatinine, so that a urinary albumin- to- creatinine ratio above 2.5 mg/mmol was considered as abnormal. However, the problem of sampling errors still remains, since even though the participants were instructed to obtain midstream urine sample, it is possible that a few patients failed to do so. The urine collection problems may also have affected the results of CrCl, although we tried to minimize the collection problems by collecting the urine for 12 hours instead of 24 hours. However, this in turn may weaken the reliability of our results.

The mathematical equations were generated from creatinine values measured by methods based on the Jaffé chromogen reaction (Schwartz et al., 2007). In our study, the serum and urinary creatinine were measured using the Jaffé reaction, which makes formulae useful in this setting. However, in modern autoanalyzers serum creatinine is determined enzymatically, which gives 10-20% lower values than those measured by the Jaffé method, so that the k-values should probably be smaller (Filler et al., 2002). The k-value should also be calibrated in the individual laboratory depending on the local creatinine assay, since differences among clinical laboratories in creatinine calibration can account for errors in GFR estimates as high as 20% (Hogg et al., 2003). Unfortunately, this was not possible, and therefore standard k-values were used.

6.3 Changes in renal tubular cells induced by methotrexate

This study presents evidence that methotrexate possesses a direct toxic effect on renal tubular cells, which may lead to permanent tubular damage. It was previously shown that methotrexate induces permanent renal tubular cell damage, but the mechanisms behind cell damage were not known (el-Badawi et al., 1996; Fuskevåg et al., 2000). Metabolites of methotrexate, such as 17-OH-methotrexate, are known to be nephrotoxic, and it has been suggested that most of the methotrexate-induced renal damage is due to the metabolites (Fuskevåg et al., 2000; Smeland et al., 1996). In this study we aimed to study pure metothexate, and demonstrated that methotrexate is also toxic to renal tubular cells. According to our results, methotrexate-induced renal cell damage is due to disturbances in cell volume regulation. Therefore, we did not analyze the effect of leukovorin, which competes with methotrexate in the inhibition of dihydrofolate reductase, thus preventing the inhibition of DNA, RNA and protein synthesis by methotrexate (Bleyer, 1978).

In HD-MTX treated patients the renal tubular cells are heavily exposed to methotrexate since methotrexate is mainly eliminated through renal clearance (Bleyer, 1978). If the patient is not hydrated well enough and if the urine is not kept alkaline, methotrexate

easily precipitates in the renal tubules (Fox, 1979; Fuskevåg et al., 2000), thus increasing the intratubular methotrexate concentration. Methotrexate induced time-dependent cell death in renal tubular cells, suggesting that prolonged exposure to methotrexate further increases the tubular damage. Tubular obstruction may also secondarily affect the glomerular filtration rate.

The methotrexate concentrations used in this study were selected by clinical data: in high-dose methotrexate treatment the peak level in plasma is around 100 μM, after 24 hours the concentration is around 1 μM and after 48 hours it is around 0.1 μM (Aquerreta et al., 2002). In this study we showed that methotrexate is toxic to renal tubular cells even at a low dose. Even though the difference was not statistically significant, exposure to a higher methotrexate concentration induced more cell death that exposure to a lower concentration. This finding indicates that the risk for renal damage increases if the elimination of methotrexate is prolonged and the blood concentration of methotrexate remains high.

Disturbances in the regulation of cell volume may play a key role in methotrexate-induced tubular cell damage, since it has been proven to be essential to a variety of physiological processes, such as cell proliferation and cell fate. Methotrexate induces apoptosis in cancer cells (Genestier et al., 2000; Taub and Ge, 2005), but we did not find any evidence of apoptotic cell shrinking in renal tubular cells. On the contrary, we found remarkable cell swelling. This finding is supported by El-Badawi et al. (1996), who showed in an animal model that methotrexate induces cell swelling and damage in distal convoluted tubule in guinea-pigs. In the present *in vitro* study, we demonstrated that methotrexate-induced cell death could be prevented by amiloride, an inhibitor of Na^+/H^+ antiporter, indicating that methotrexate induces renal tubular cell swelling and cell death by activating the Na^+/H^+ –antiporter, leading to increased sodium influx and proton efflux. However, clinical studies describe only minor or no signs of tubular toxicity and inflammatory response (Hempel et al., 2003). *In vivo* the volume regulatory mechanisms are probably able to prevent massive death after cellular swelling induced by methotrexate. This is supported by our finding where Carbachol, an M-cholinergic agonist that activates K^+-channels, protected tubular cells from methotrexate-induced death. K^+ channel activation is a regulatory volume decrease mechanism, so it is likely that *in vivo* M-cholinergic agonists stimulate regulatory volume decrease mechanisms, thus preventing cells from swelling. However, this is only speculative and needs further studies to confirm it.

6.4 Long-term changes in renal function after HD-MTX treatment

We have shown that methotrexate induced acute damage in renal tubular cells. There is also clinical evidence of acute renal dysfunction following high-dose MTX therapy (Perazella, 1999; Widemann et al., 2006). Progressive kidney damage after acute leukemia has been reported (Hovi et al., 1989; Kaya et al., 2007), but long-term renal changes following HD-MTX treatment have been less intensively studied (Bardi et al., 2004). This is the first prospective study describing long-term changes in glomerular and tubular function in pediatric oncology patients in which the glomerular filtration rate has been evaluated by using isotope methods. Previous studies found no evidence of reduced

GFR after several years of follow-up, whereas in our study population surprisingly many patients had either a slightly or clearly reduced iGFR at the end of the follow-up period (Bardi et al., 2004; Koch Nogueira et al., 1998). However, in previous studies, GFR was evaluated by laboratory analyses, which may fail to detect renal impairment as shown in study II. Another finding suggesting glomerular or tubular damage was albuminuria that was found in 29% of patients at the end of follow-up. Similar results have been shown in previous studies (Bardi et al., 2004). In our study, young age at the time of diagnosis and a long follow-up time tended to be risk factors for albuminuria. However, these factors were not statistically significant, probably as a result of the small number of patients.

There is some evidence of mild tubular damage following HD-MTX treatment (Koch Nogueira et al., 1998). The only finding indicating tubular damage in our study was LMW proteinuria in 14% of the patients. This finding is supported by the results of a study by Yetgin et al. (2004), where they found abnormal β_2-microglobulin excretion in 6% of cases of pediatric leukemia after a median follow-up period of 35 months. However, they also found abnormal tubular phosphorus reabsorption in 16.4% of patients, whereas in our material only one patient had a slightly reduced serum phosphorus level.

The majority of our patients were exposed to different combinations of nephrotoxic antibiotics (amphotericin B, vancomycin and / or gentamycin), which makes the interpretation of our results difficult. The influence of additional use of nephrotoxic antibiotics and HD-MTX was analyzed. We did not find any significant changes in tubular function or glomerular filtration rate in these patients compared with those who did not receive other nephrotoxic drugs. However, albuminuria was noted significantly more often in patients treated with gentamycin. This indicates that the glomerular filtration barrier may be damaged as a result of being exposed to several nephrotoxic agents at the same time.

Secondary hypertension is frequently seen in patients with renal damage. Blood pressure was increased in 7% of our patients, and as many as 36% had slightly increased blood pressure. Only one of our patients had a BMI ≥ 25 kg/m^2, suggesting that obesity and metabolic syndrome were not a cause of elevated blood pressure. The incidence of renal hypertension following HD-MTX treatment has also been shown to be similar in other studies (Chow et al., 2007; Yetgin et al., 2004). A weakness of our study was that we used single blood pressure measurements instead of ambulatory blood pressure monitoring, which gives more detailed information (Hölttä et al., 2001). Follow-up of blood pressure is important, since elevated blood pressure is an indicator of renal problems as well as being an indicator of metabolic syndrome, which is increasing among pediatric cancer survivors. If antihypertensive medication is indicated, the presence of proteinuria should be taken into consideration when choosing appropriate medication.

6.5 The assessment of glomerular function in pediatric cancer patients

6.5.1 Serum creatinine

The concentration of SCr is probably the most widely used indirect indicator of GFR. However, the serum concentration of creatinine may remain within the age-dependent reference range until there is at least 50% loss of renal function (GFR about 60 mL/

min/1.73 m^2) (Hogg et al., 2003). In the present study the serum concentration of creatinine had relatively good correlation with iGFR, except in patients with clearly reduced renal function. Perrone et al (1992) reported that there was an enhanced tubular secretion of creatinine in patients with reduced glomerular function, which may explain why in our patients with reduced iGFR the SCr did not increase as expected. Falsely low serum creatinine values may also be found due to the fact that pediatric cancer patients often have reduced muscle mass and their creatinine turnover is variable due to protein-calorie malnutrition (Perrone et al., 1992). In the present study, height and weight of the patients did not have any effect on results, indicating that muscle wasting was not important. However, the estimation of muscle mass based only on body weight is difficult and would require methods such as bioimpedance analysis which was not performed in the present study. Although creatinine concentration alone is not totally reliable, the finding of an elevated SCr concentration is an almost certain indication that GFR is reduced (Levey et al., 1988; Perrone et al., 1992).

6.5.2 Creatinine clearance

Endogenous CrCl has been widely used in the assessment of kidney function. However, creatinine clearance requires timed urine collection, which is relatively unreliable in a pediatric population without catheterization (Narayanan and Appleton, 1980). Creatinine clearance depends not only on glomerular filtration but also on tubular secretion. Therefore, in patients with reduced glomerular function CrCl may overestimate GFR by as much as 10–40% compared with GFR assessed by inulin clearance (Perrone et al., 1992). In cancer patients, both glomerular and tubular function may be affected, either reversibly or irreversibly. According to recent statements, the measurement of creatinine clearance using timed urine collections should not be used routinely (Hogg et al., 2003; Levey et al., 2003). Our results support this statement, since we did not find any association between CrCl and iGFR. These findings do not encourage the use of CrCl in pediatric cancer patients.

6.5.3 Mathematical equations

The recognized problems concerning SCr and CrCl have led to the concept of estimating GFR from the serum creatinine concentration by way of several mathematical formulae. We found that these formulae overestimated GFR considerably. However, the mathematical estimates of GFR based on SCr and patient's height (formulae of Schwartz and Counahan-Barratt) had better correlation to iGFR than SCr alone. In the original Schwartz formula the constant was 0.55 in all patients, but in the revised formula a constant of 0.7 was used in adolescent boys (Schwartz et al., 1976; Schwartz and Gauthier, 1985). In our study, use of the formula with a constant of 0.55 correlated better with iGFR, probably because of the low muscle mass of pediatric cancer patients. We did not find any association between iGFR data and results of the Cockroft–Gault formula, which is based on the age and weight of the patient. This is in agreement with the results of Filler et al. (2005), who also found that Cockroft-Gault formula showed poor agreement with GFR. However, there is some evidence that the reliability of the Cockroft–Gault formula is age-dependent, being more accurate in children over 12 years of age (Pierrat et al., 2003). However, due to the small number of patients we were

unable to differentiate the group of children over 12 years of age in whom the Cockroft-Gault formula might have been useful.

6.5.4 Cystatin C

The concentration of serum cystatin C is independent of muscle mass, age, sex and inflammatory conditions (Abrahamson et al., 1990; Bökenkamp et al., 1998; Filler et al., 2005; Filler et al., 2005; Grubb, 1992; Laterza et al., 2002). Therefore, it has been suggested to be more reliable than the concentration of SCr in the estimation of renal function in pediatric patients. Our results show that CysC is slightly better correlated with iGFR than serum creatinine, which is in unison with several other trials (Corrao et al., 2006; Kyhse-Andersen et al., 1994; Laterza et al., 2002; Risch et al., 2001; Stabuc et al., 2000; Ylinen et al., 1999).

In this study, we determined CysC by particle-enhanced nephelometric immunoassay (PENIA), which produces a slightly lower result than particle-enhanced turbidimetric immunoassay (PETIA) method that has been used in most pediatric studies (Laterza et al., 2002). In our population, CysC varied from 0.49 to 1.16 mg/l, which is similar to other studies (Corrao et al., 2006; Filler et al., 1997; Finney et al., 2000; Randers et al., 1999).

Corticosteroids, which are frequently used in cancer patients, have been suggested to increase serum CysC concentrations (Risch et al., 2001; Wasen et al., 2003). In our study, no difference in serum CysC concentrations in patients with glucocorticoids within one month and without glucocorticoids was found. It has also been observed that serum CysC levels may be increased as a result of non-Hodgkin's lymphoma and certain other malignancies, whereas cellular proliferation in patients with proliferative hematological disorders has been shown not to increase serum CysC concentrations (Finney et al., 2001; Kos et al., 1998; Mojiminiyi et al., 2002; Mulaomerovic et al., 2007). Since there might be a confounding effect of CysC production by some tumor cells, more studies are needed to evaluate the effects of malignant cells on CysC concentrations in order to definitely estimate the usefulness of CysC in pediatric cancer patients.

6.6 Long-term renal function following bone marrow transplantation in children

We found significant decrease in GFR and ERPF when comparing the results pre-BMT and one year after BMT. These findings are consistent with previous reports of permanent impairment of glomerular function (Berg and Bolme, 1989; Kist-van Holthe et al., 1998; Patzer et al., 2001). The reduction remained constant during 10 years of follow-up, except that between one and three years after transplantation a slight but statistically significant recovery after the initial fall was noticed in GFR, after which it remained stable. This slight recovery may be explained by the fact that almost all patients were treated with Cyclosporin A, which was withdrawn at 9–12 months in most cases. Patients with a malignant hematological disease as their underlying diagnosis had even lower GFRs, probably because they had been treated more heavily before BMT.

Subclinical renal dysfunction following BMT has been noted to be common; 56% of patients have been reported to have a >20% decrease in GFR during long-term follow-

up after BMT (Leblond et al., 1995). We found similar results, since in our patients 54% had a >20% decrease in GFR seven years after BMT. It has been reported that currently 5–28% of long-term BMT survivors will develop chronic renal dysfunction (Borg et al., 2002; Cohen, 2000; Kist-van Holthe et al., 2002; Lawton et al., 1997; Miralbell et al., 1996; Patzer et al., 2001; Van Why et al., 1991). This is in accordance with our results, since we found that 11% of the patients had a GFR < 80 ml/min/1.73 m^2 seven years after BMT.

There are several risk factors as regards the development of chronic renal impairment following BMT. First, renal function may already be compromised before BMT, since most patients with malignant disease will have been exposed to potentially nephrotoxic chemotherapy, antibiotics or antifungal agents (Lawton et al., 1997; Lönnerholm et al., 1991; Miralbell et al., 2004; Patzer et al., 2001; Van Why et al., 1991). This might be an explanation for the reduced GFR found in our patients with hematological malignancies. However, renal impairment may develop after BMT even if renal function had been within normal limits prior to the event (Kletzel et al., 2005). Secondly, BMT is associated with several factors that may be harmful as regards renal function.

To evaluate if conditioning chemotherapy is involved in the deterioration of renal function, we analyzed the effect of total body irradiation in the condition regimen. We discovered that TBI was a strong risk factor as regards the development of chronic renal impairment following BMT; the fall in GFR and ERPF was more profound in patients conditioned with TBI than those without TBI at all time points. There are several reports supporting our finding (Leblond et al., 1995; Lönnerholm et al., 1991; Miralbell et al., 1996; Van Why et al., 1991). We also evaluated the effect of cyclophosphamide in the conditioning regimen, and found no correlation between the use of cyclophosphamide and the development of chronic renal impairment.

Renal dysfunction after allogeneic BMT has been shown to be strongly related to the presence of acute or chronic GVHD (Lawton et al., 1997). Patients with severe GVHD have shown to have renal tubulitis and peritubular capillaritis, and nephrotic syndrome resulting from membranous glomerulonephritis has also been described (Brukamp et al., 2006; Heras et al., 2007; Kusumi et al., 2008). We found no difference in our patients in the development of chronic renal impairment between cases of no or very mild acute GVHD versus those with clinically important acute GVHD. This finding also is similar in previous studies (Borg et al., 2002; Leblond et al., 1995). However, in our study we only had a small number of patients with severe GVHD, probably due to the large number of sibling transplantations.

Based on the results of this thesis, renal function should be carefully followed after pediatric cancer treatment. Glomerular function should be assessed before and one year after BMT by a reliable isotope method. The renal function of high-risk patients, such as patients transplanted for hematological malignancies after heavy previous treatments and those conditioned with TBI, should be monitored for several years after BMT if GFR is reduced. The renal function of patients treated with HD-MTX should also be followed-up for several years after treatment. GFR should be measured at least once by an isotope method, and if the iGFR is normal, the follow-up may be continued by measuring cystatin C and creatinine consentrations from the blood. Blood pressure and urinary albumin- to- creatinine ratio should be measured yearly.

7 CONCLUSIONS

On the basis of the study results obtained, the following conclusions were made:

1. Methotrexate has a direct toxic effect on renal tubular cells (LLC-PK_1 cells). Since more cell death occurred as the exposure time of tubular cells to methotrexate increased, tubular obstruction in patients treated with HD-MTX may further increase the risk of renal damage. Therefore, alkalinization of the urine and excessive fluid intake still remain essential in HD-MTX treatment. Na^+/H^+ antiporter and possibly other volume regulators may be involved in methotrexate-induced renal failure, but this needs to be further studied.

2. Direct assessment of the glomerular filtration rate by radioactive traces remains the most reliable method that can not be replaced by any laboratory analyses. Cystatin C is a promising screening tool that can be used in the follow-up. Creatinine clearance is not a reliable method in assessing glomerular function in pediatric cancer patients.

3. Glomerular function may deteriorate years after HD-MTX treatment even though there have been no renal problems before or during cancer treatments. Long-term follow-up of glomerular function and blood pressure is needed after HD-MTX treatment. Albuminuria should also be screened, particularly in patients who have also been treated with nephrotoxic antibiotics.

4. The glomerular function of pediatric bone marrow transplantation patients should be measured before transplant and one year after BMT. If the GFR is significantly reduced, the assessment should be repeated regularly. The patients at highest risk for long-term renal impairment are those transplanted because of a hematological malignancy, and especially those conditioned with total body irradiation.

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9 REFERENCES

Abildgaard L, Ellebaek E, Gustafsson G, Abrahamsson J, Hovi L, Jonmundsson G, Zeller B, & Hasle H (2006) Optimal treatment intensity in children with down syndrome and myeloid leukaemia: Data from 56 children treated on NOPHO-AML protocols and a review of the literature. Ann Hematol 85: 275-280.

Abrahamsson J, Clausen N, Gustafsson G, Hovi L, Jonmundsson G, Zeller B, Forestier E, Heldrup J, Hasle H, & Nordic Society for Paediatric Haematology and Oncology (NOPHO) (2007) Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol 136: 229-236.

Abrahamson M, Olafsson I, Palsdottir A, Ulvsback M, Lundwall A, Jensson O, & Grubb A (1990) Structure and expression of the human cystatin C gene. Biochem J 268: 287-294.

Antachopoulos C, Walsh TJ, & Roilides E (2007) Fungal infections in primary immunodeficiencies. Eur J Pediatr 166:1099-117.

Aquerreta I, Aldaz A, Giraldez J, & Sierrasesumaga L (2002) Pharmacodynamics of high-dose methotrexate in pediatric patients. Ann Pharmacother 36: 1344-1350.

Ardissino G, Testa S, Dacco V, Vigano S, Taioli E, Claris-Appiani A, Procaccio M, Avolio L, Ciofani A, Dello Strologo L, Montini G, & Ital Kid Project (2004) Proteinuria as a predictor of disease progression in children with hypodysplastic nephropathy. Data from the ital kid project. Pediatr Nephrol 19: 172-177.

Ariceta G, Rodriguez-Soriano J, Vallo A, & Navajas A (1997) Acute and chronic effects of cisplatin therapy on renal magnesium homeostasis. Med Pediatr Oncol 28: 35-40.

Aspelin P, Aubry P, Fransson SG, Strasser R, Willenbrock R, Berg KJ, & Nephrotoxicity in High-Risk Patients Study of Iso-Osmolar and Low-Osmolar Non-Ionic Contrast Media Study Investigators. (2003) Nephrotoxic effects in high-risk patients undergoing angiography. N Engl J Med 348: 491-499.

Barbour GL, Crumb CK, Boyd CM, Reeves RD, Rastogi SP, & Patterson RM (1976) Comparison of inulin, iothalamate, and 99mTc-DTPA for measurement of glomerular filtration rate. J Nucl Med 17: 317-320.

Bardeesy N, Falkoff D, Petruzzi MJ, Nowak N, Zabel B, Adam M, Aguiar MC, Grundy P, Shows T, & Pelletier J (1994) Anaplastic wilms' tumour, a subtype displaying poor prognosis, harbours p53 gene mutations. Nat Genet 7: 91-97.

Bárdi E, Bobok I, Olah AV, Olah E, Kappelmayer J, & Kiss C (2004) Cystatin C is a suitable marker of glomerular function in children with cancer. Pediatr Nephrol 19: 1145-1147.

Bárdi E, Bobok I, V Olah A, Kappelmayer J, & Kiss C (2007) Anthracycline antibiotics induce acute renal tubular toxicity in children with cancer. Pathol Oncol Res 13: 249-253.

Bárdi E, Olah AV, Bartyik K, Endreffy E, Jenei C, Kappelmayer J, & Kiss C (2004) Late effects on renal glomerular and tubular function in childhood cancer survivors. Pediatr Blood Cancer 43: 668-673.

Becker LE (1999) Pathology of pediatric brain tumors. Neuroimaging Clin N Am 9: 671-690.

Berg U, & Bolme P (1989) Renal function in children following bone marrow transplantation. Transplant Proc 21: 3092-3094.

Berglund F, Killander J, & Pompeius R (1975) Effect of trimethoprim-sulfamethoxazole on the renal excretion of creatinine in man. J Urol 114: 802-808.

Berry CA, Warnock DG, & Rector FC,Jr (1978) Ion selectivity and proximal salt reabsorption. Am J Physiol 235: F234-45.

Bjarnadottir M, Grubb A, & Olafsson I (1995) Promoter-mediated, dexamethasone-induced increase in cystatin C production by HeLa cells. Scand J Clin Lab Invest 55: 617-623.

Blaufox MD (1991) Procedures of choice in renal nuclear medicine. J Nucl Med 32: 1301-1309.

Bleyer WA (1978) The clinical pharmacology of methotrexate: New applications of an old drug. Cancer 41: 36-51.

Blowey DL, Ben-David S, & Koren G (1995) Interactions of drugs with the developing kidney. Pediatr Clin North Am 42: 1415-1431.

Bobadilla NA, & Gamba G (2007) New insights into the pathophysiology of cyclosporine nephrotoxicity: A role of aldosterone. Am J Physiol Renal Physiol 293: F2-9.

Borg M, Hughes T, Horvath N, Rice M, & Thomas AC (2002) Renal toxicity after total body irradiation. Int J Radiat Oncol Biol Phys 54: 1165-1173.

Bown N (2001) Neuroblastoma tumour genetics: Clinical and biological aspects. J Clin Pathol 54: 897-910.

Brillet G, Deray G, Jacquiaud C, Mignot L, Bunker D, Meillet D, Raymond F, & Jacobs C (1994) Long-term renal effect of cisplatin in man. Am J Nephrol 14: 81-84.

Brochner-Mortensen J (1972) A simple method for the determination of glomerular filtration rate. Scand J Clin Lab Invest 30: 271-274.

Brodeur GM (2003) Neuroblastoma: Biological insights into a clinical enigma. Nat Rev Cancer 3: 203-216.

Brukamp K, Doyle AM, Bloom RD, Bunin N, Tomaszewski JE, & Cizman B (2006) Nephrotic syndrome after hematopoietic cell treansplantaiton: do glomerular lesions represent renal graft-versus-host disease? Clin J Am Soc Nephrol 1:685-694.

Buchen S, Ngampolo D, Melton RG, Hasan C, Zoubek A, Henze G, Bode U, & Fleischhack G (2005) Carboxypeptidase G2 rescue in patients with methotrexate intoxication and renal failure. Br J Cancer 92: 480-487.

Buckner JC, Brown PD, O'Neill BP, Meyer FB, Wetmore CJ, & Uhm JH (2007) Central nervous system tumors. Mayo Clin Proc 82: 1271-1286.

Burckart GJ (1983) Monitoring cyclosporine therapy. Clin Pharm 2: 568.

Bökenkamp A, Domanetzki M, Zinck R, Schumann G, & Brodehl J (1998) Reference values for cystatin C serum concentrations in children. Pediatr Nephrol 12: 125-129.

Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, & Brodehl J (1998) Cystatin C--a new marker of glomerular filtration rate in children independent of age and height. Pediatrics 101: 875-881.

Cairo MS, Raetz E, Lim MS, Davenport V, & Perkins SL (2005) Childhood and adolescent non-hodgkin lymphoma: New insights in biology and critical challenges for the future. Pediatr Blood Cancer 45: 753-769.

Cairo MS, Gerrard M, Sposto R, Auperin A, Pinkerton CR, Michon J, Weston C, Perkins SL, Raphael M, McCarthy K, Patte C, & FAB LMB96 International Study Committee (2007) Results of a randomized international study of high-risk central nervous system B non-hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. Blood 109: 2736-2743.

Carli M, Passone E, Perilongo G, & Bisogno G (2003) Ifosfamide in pediatric solid tumors. Oncology 65 Suppl 2: 99-104.

Cassady JR (1995) Clinical radiation nephropathy. Int J Radiat Oncol Biol Phys 31: 1249-1256.

Cesaro S, Xhou X, Manzardo C, Buonfrate D, Cusinato R, Tridello G, Mengoli C, Palu G, & Messina C (2005) Cidofovir for cytomegalovirus reactivation in pediatric patients after hematopoietic stem cell transplantation. J Clin Virol 34:129-32.

Cesaro S, Zignol M, Burlina AB, Tridello G, Visintin G, & Messina C (2006) Assessment of nephrotoxicity of high-cumulative dose of liposomal amphotericin B in a pediatric patient who underwent allogeneic bone marrow transplantation. Pediatr Transplant 10: 255-258.

Chow EJ, Pihoker C, Hunt K, Wilkinson K, & Friedman DL (2007) Obesity and hypertension among children after treatment for acute lymphoblastic leukemia. Cancer 110: 2313-2320.

Cimerman N, Brguljan PM, Krasovec M, Suskovic S, & Kos J (2000) Serum cystatin C, a potent inhibitor of cysteine proteinases, is elevated in asthmatic patients. Clin Chim Acta 300: 83-95.

Clive DM, & Stoff JS (1984) Renal syndromes associated with nonsteroidal antiinflammatory drugs. N Engl J Med 310: 563-572.

Cockcroft DW, & Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16: 31-41.

Cohen EP (2000) Radiation nephropathy after bone marrow transplantation. Kidney Int 58: 903-918.

Cohen IJ (2004) Defining the appropriate dosage of folinic acid after high-dose methotrexate for childhood acute lymphatic leukemia that will prevent neurotoxicity without rescuing malignant cells in the central nervous system. J Pediatr Hematol Oncol 26: 156-163.

Cole M, Price L, Parry A, Keir MJ, Pearson ADJ, Boddy AV, & Veal GJ (2004) Estimation of glomerular filtration rate in paediatric cancer patients using ^{51}CR-EDTA population pharmacinetics. Br J Cancer 90:60-64.

Coll E, Botey A, Alvarez L, Poch E, Quinto L, Saurina A, Vera M, Piera C, & Darnell A (2000) Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. Am J Kidney Dis 36: 29-34.

Condit PT, Chanes RE, & Joel W (1969) Renal toxicity of methotrexate. Cancer 23: 126-131.

Corrao AM, Lisi G, Di Pasqua G, Guizzardi M, Marino N, Ballone E, & Chiesa PL (2006) Serum cystatin C as a reliable marker of changes in glomerular filtration rate in children with urinary tract malformations. J Urol 175: 303-309.

Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, & Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child 51: 875-878.

Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, & Zimmermann M (2005) AML patients with down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia 19: 1355-1360.

Crist W, Gehan EA, Ragab AH, Dickman PS, Donaldson SS, Fryer C, Hammond D, Hays DM, Herrmann J, & Heyn R (1995) The third intergroup rhabdomyosarcoma study. J Clin Oncol 13: 610-630.

Cutolo M, Seriolo B, Pizzorni C, Craviotto C, & Sulli A (2002) Methotrexate in psoriatic arthritis. Clin Exp Rheumatol 20: S76-80.

Deray G, Khayat D, Cacoub P, Bourbouze R, Musset L, Baumelou A, Jacquillat C, & Jacobs C (1989)

The effects of diltiazem on methotrexate-induced nephrotoxicity. Eur J Clin Pharmacol 37: 337-340.

Dharnidharka VR, Kwon C, & Stevens G (2002) Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. Am J Kidney Dis 40: 221-226.

Ditzel J, Vestergaard P, & Brinklov M (1972) Glomerular filtration rate determined by 51 Cr-EDTA-complex. A practical method based upon the plasma disappearance curve determined from four plasma samples. Scand J Urol Nephrol 6: 166-170.

Donaldson SS (2004) Ewing sarcoma: Radiation dose and target volume. Pediatr Blood Cancer 42: 471-476.

Dorea EL, Yu L, De Castro I, Campos SB, Ori M, Vaccari EM, Lacaz Cda S, & Seguro AC (1997) Nephrotoxicity of amphotericin B is attenuated by solubilizing with lipid emulsion. J Am Soc Nephrol 8: 1415-1422.

Doz F, & Pinkerton R (1994) What is the place of carboplatin in paediatric oncology? Eur J Cancer 30A: 194-201.

Effersoe H, Rosenkilde P, Groth S, Jensen LI, & Golman K (1990) Measurement of renal function with iohexol. A comparison of iohexol, 99mTc-DTPA, and 51Cr-EDTA clearance. Invest Radiol 25: 778-782.

Einhorn LH (2003) Ifosfamide in germ cell tumors. Oncology 65 Suppl 2: 73-75.

el-Badawi MG, Abdalla MA, Bahakim HM, & Fadel RA (1996) Nephrotoxicity of low-dose methotrexate in guinea pigs: An ultrastructural study. Nephron 73: 462-466.

English MW, Skinner R, Pearson AD, Price L, Wyllie R, & Craft AW (1999) Dose-related nephrotoxicity of carboplatin in children. Br J Cancer 81: 336-341.

Estlin EJ, & Veal GJ (2003) Clinical and cellular pharmacology in relation to solid tumours of childhood. Cancer Treat Rev 29: 253-273.

Evans AE, & D'Angio GJ (2005) Age at diagnosis and prognosis in children with neuroblastoma. J Clin Oncol 23: 6443-6444.

Fallat ME, Hutter J, American Academy of Pediatrics Committee on Bioethics, American Academy of Pediatrics Section on Hematology/Oncology, & American Academy of Pediatrics Section on Surgery (2008) Preservation of fertility in pediatric and adolescent patients with cancer. Pediatrics 121: e1461-9.

Ferrari S, Pieretti F, Verri E, Tolentinis L, Cesari M, Versari M, Zolezzi C, Lamanna G, & Bacci G (2005) Prospective evaluation of renal function in pediatric and adult patients treated with high-dose ifosfamide, cisplatin and high-dose methotrexate. Anti-Cancer Drugs 16:733-738.

Filler G, Foster J, Acker A, Lepage N, Akbari A, & Ehrich JH (2005) The cockcroft-gault formula should not be used in children. Kidney Int 67: 2321-2324.

Filler G, Priem F, Lepage N, Sinha P, Vollmer I, Clark H, Keely E, Matzinger M, Akbari A, Althaus H, & Jung K (2002) Beta-trace protein, cystatin C, beta(2)-microglobulin, and creatinine compared for detecting impaired glomerular filtration rates in children. Clin Chem 48: 729-736.

Filler G, Witt I, Priem F, Ehrich JH, & Jung K (1997) Are cystatin C and beta 2-microglobulin better markers than serum creatinine for prediction of a normal glomerular filtration rate in pediatric subjects? Clin Chem 43: 1077-1078.

Finney H, Newman DJ, Thakkar H, Fell JM, & Price CP (2000) Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child 82: 71-75.

Finney H, Williams AH, & Price CP (2001) Serum cystatin C in patients with myeloma. Clin Chim Acta 309: 1-6.

Fox RM (1979) Methotrexate nephrotoxicity. Clin Exp Pharmacol Physiol Suppl 5: 43-45.

Fricker M, Wiesli P, Brandle M, Schwegler B, & Schmid C (2003) Impact of thyroid dysfunction on serum cystatin C. Kidney Int 63: 1944-1947.

Frisk P, Bratteby LE, Carlson K, & Lönnerholm G (2002) Renal function after autologous bone marrow transplantation in children: A long-term prospective study. Bone Marrow Transplant 29: 129-136.

Fulfaro F, Valerio MR, Badalamenti G, Gebbia N, & Russo A (2003) Antiblastic drug combinations with ifosfamide: An update. Oncology 65 Suppl 2: 21-30.

Fuskevåg OM, Kristiansen C, Lindal S, & Aarbakke J (2000) Leucovorin and maximum tolerated dose toxicity of methotrexate in rats. Pediatr Hematol Oncol 17: 651-658.

Fuskevåg OM, Kristiansen C, Olsen R, Aarbakke J, & Lindal S (2000) Microvascular perturbations in rats receiving the maximum tolerated dose of methotrexate or its major metabolite 7-hydroxymethotrexate. Ultrastruct Pathol 24: 325-332.

Gagliano RG, & Costanzi JJ (1976) Paraplegia following intrathecal methotrexate: Report of a case and review of the literature. Cancer 37: 1663-1668.

Galteau MM, Guyon M, Gueguen R, & Siest G (2001) Determination of serum cystatin C: Biological variation and reference values. Clin Chem Lab Med 39: 850-857.

Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, Guerini E, Daina E, & Remuzzi G (1995) Plasma clearance of nonradioactive

iohexol as a measure of glomerular filtration rate. J Am Soc Nephrol 6: 257-263.

Genestier L, Paillot R, Quemeneur L, Izeradjene K, & Revillard JP (2000) Mechanisms of action of methotrexate. Immunopharmacology 47: 247-257.

Gianni AM, Siena S, Bregni M, Tarella C, Stern AC, Pileri A, & Bonadonna G (1989) Granulocyte-macrophage colony-stimulating factor to harvest circulating haemopoietic stem cells for autotransplantation. Lancet 2: 580-585.

Gluckman E, & Wagner JE (2008) Hematopoietic stem cell transplantation in childhood inherited bone marrow failure syndrome. Bone Marrow Transplant 41: 127-132.

Goldman RD, & Koren G (2004) Amphotericin B nephrotoxicity in children. J Pediatr Hematol Oncol 26: 421-426.

Goren MP, Pratt CB, & Viar MJ (1989) Tubular nephrotoxicity during long-term ifosfamide and mesna therapy. Cancer Chemother Pharmacol 25: 70-72.

Goren MP (2003) Cisplatin nephrotoxicity affects magnesium and calcium metabolism. Med Pediatr Oncol 41: 186-189.

Govender D, Harilal P, Hadley GP, & Chetty R (1998) P53 protein expression in nephroblastomas: A predictor of poor prognosis. Br J Cancer 77: 314-318.

Green DM (2004) The treatment of stages I-IV favorable histology wilms' tumor. J Clin Oncol 22: 1366-1372.

Grubb A (1992) Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 38 Suppl 1: S20-7.

Gustafsson G, Kreuger A, Clausen N, Garwicz S, Kristinsson J, Lie SO, Moe PJ, Perkkiö M, Yssing M, & Saarinen-Pihkala UM (1998) Intensified treatment of acute childhood lymphoblastic leukaemia has improved prognosis, especially in non-high-risk patients: The Nordic experience of 2648 patients diagnosed between 1981 and 1996. Nordic society of paediatric haematology and oncology (NOPHO). Acta Paediatr 87: 1151-1161.

Gustafsson G, Langmark F, Pihkala U, deVerdier B, & Lilleaas I (1998) Childhood cancer in the nordic countries. Report on epidemiologic and therapeutic results, from registries and working groups. NOPHO Annual Meeting 11-122.

Gustafsson G, Schmiegelow K, Forestier E, Clausen N, Glomstein A, Jonmundsson G, Mellander L, Mäkipernaa A, Nygaard R, & Saarinen-Pihkala UM (2000) Improving outcome through two decades in childhood ALL in the nordic countries: The impact of high-dose methotrexate in the reduction of CNS irradiation. Nordic society of pediatric haematology and oncology (NOPHO). Leukemia 14: 2267-2275.

Gustafsson Jernberg A, Remberger M, Ringden O, & Winiarski J (2003) Graft-versus-leukaemia effect in children: Chronic GVHD has a significant impact on relapse and survival. Bone Marrow Transplant 31: 175-181.

Göbel U, Calaminus G, Schneider DT, Schmidt P, Haas RJ, & MAKEI and MAHO Study Groups of the German Society of Pediatric Oncology and Hematology, and the SIOP CNS GCT Study Group (2002) Management of germ cell tumors in children: Approaches to cure. Onkologie 25: 14-22.

Harila-Saari AH, Lähteenmäki PM, Pukkala E, Kyyrönen P, Lanning M, & Sankila R (2007) Scholastic achievements of childhood leukemia patients: A nationwide, register-based study. J Clin Oncol 25: 3518-3524.

Harned TM, & Mascarenhas L (2007) Severe methotrexate toxicity precipitated by intravenous radiographic contrast. J Pediatr Hematol Oncol 29: 496-499.

Hasle H (2007) Myelodysplastic and myeloproliferative disorders in children. Curr Opin Pediatr 19: 1-8.

Helin I, Axenram M, & Grubb A (1998) Serum cystatin C as a determinant of glomerular filtration rate in children. Clin Nephrol 49: 221-225.

Hellerstein S, Berenbom M, Erwin P, Wilson N, & DiMaggio S (2006) Timed-urine collections for renal clearance studies. Pediatr Nephrol 21:96-101.

Helliwell PS, & Taylor WJ; CASPAR Study Group (2008) Treatment of psoriatic arthritis and rheumatoid arthritis with disease modifying drugs -- comparison of drugs and adverse reactions. J Rheumatol 35:472-476.

Hempel L, Misselwitz J, Fleck C, Kentouche K, Leder C, Appenroth D, Rost M, & Zintl F (2003) Influence of high-dose methotrexate therapy (HD-MTX) on glomerular and tubular kidney function. Med Pediatr Oncol 40: 348-354.

Heras M, Saiz A, Sánchez R, Fernandez-Reyes MJ, Mampaso F, Queizán JA, Molina A, Vásquez L, & Alvarez-Ude F (2007) Nephrotic syndrome resulting from focal segmental glomerulosclerosis in a peripheral blood stem cell transplant patient. J Nephrol 20:495-498.

Hingorani S, Guthrie KA, Schoch G, Weiss NS, & McDonald GB (2007) Chronic kidney disease in long-term survivors of hematopoietic cell transplant. Bone Marrow Transplant 39: 223-229.

Hjalgrim LL, Rostgaard K, Schmiegelow K, Söderhäll S, Kolmannskog S, Vettenranta K, Kristinsson J, Clausen N, Melbye M, Hjalgrim H, & Gustafsson G (2003) Age- and sex-specific incidence of childhood leukemia by immunophenotype in the nordic countries. J Natl Cancer Inst 95: 1539-1544.

Hodgson DC, Hudson MM, & Constine LS (2007) Pediatric Hodgkin lymphoma: Maximizing efficacy

and minimizing toxicity. Semin Radiat Oncol 17: 230-242.

Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, Balk E, Lau J, Levin A, Kausz AT, Eknoyan G, Levey AS, & National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (2003) National kidney foundation's kidney disease outcomes quality initiative clinical practice guidelines for chronic kidney disease in children and adolescents: Evaluation, classification, and stratification. Pediatrics 111: 1416-1421.

Holweger K, Bokemeyer C, & Lipp HP (2005) Accurate measurement of individual glomerular filtration rate in cancer patients: An ongoing challenge. J Cancer Res Clin Oncol 131: 559-567.

Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, Rimm AA, Ringden O, Rozman C, & Speck B (1990) Graft-versus-leukemia reactions after bone marrow transplantation. Blood 75: 555-562.

Horton Z, Schlatter M, & Schultz S (2007) Pediatric germ cell tumors. Surg Oncol 16: 205-213.

Hovi L, Koskimies O, Holmberg C, Rajantie J, Rautonen J, & Siimes MA (1989) Risk of progressive kidney damage after acute leukemia. Acta Paediatr Scand 78: 608-614.

Howman-Giles R, Shaw PJ, Uren RF, & Chung DK (2007) Neuroblastoma and other neuroendocrine tumors. Semin Nucl Med 37: 286-302.

Hoyt R, Ritchie DS, Roberts AW, Macgregor L, Curtis DJ, Szer J, & Grigg AP (2008) Cyclosporin, methotrexate and prednisolone for graft-versus-host disease prophylaxis in allogeneic peripheral blood progenitor cell transplants. Bone Marrow Transplant 41:651-658.

Hudson MM, & Donaldson SS (1997) Hodgkin's disease. Pediatr Clin North Am 44: 891-906.

Hölttä T, Happonen JM, Rönnholm K, Fyhrquist F, & Holmberg C (2001) Hypertension, cardiac state, and the role of volume overload during peritoneal dialysis. Pediatr Nephrol 16: 324-331.

Inaba H, Khan RB, Laningham FH, Crews KR, Pui CH, & Daw NC (2008) Clinical and radiological characteristics of methotrexate-induced acute encephalopathy in pediatric patients with cancer. Ann Oncol 19: 178-184.

Irwin C, Fyles A, Wong CS, Cheung CM, & Zhu Y (1996) Late renal function following whole abdominal irradiation. Radiother Oncol 38: 257-261.

Jacobsson B, Lignelid H, & Bergerheim US (1995) Transthyretin and cystatin C are catabolized in proximal tubular epithelial cells and the proteins are not useful as markers for renal cell carcinomas. Histopathology 26: 559-564.

Jones DP, & Chesney RW (2004) Tubular function. In: E. D. Avner, W. E. Harmon and P. Niaudet, eds. Pediatric Nephrology, 5th ed. edn. Lippincott Williams & Wilkins, Philadelphia, USA, pp 45.

Kakihara T, Imai C, Hotta H, Ikarashi Y, Tanaka A, & Uchiyama M (2003) Impaired tubular excretory function as a late renal side effect of chemotherapy in children. J Pediatr Hematol Oncol 25: 209-214.

Kaya Z, Gursel T, Bakkaloglu SA, Kocak U, Atasever T, & Oktar SO (2007) Evaluation of renal function in turkish children receiving BFM-95 therapy for acute lymphoblastic leukemia. Pediatr Hematol Oncol 24: 257-267.

Kist-van Holthe JE, Goedvolk CA, Brand R, van Weel MH, Bredius RG, van Oostayen JA, Vossen JM, & van der Heijden BJ (2002) Prospective study of renal insufficiency after bone marrow transplantation. Pediatr Nephrol 17: 1032-1037.

Keevil BG, Kilpatrick ES, Nichols SP, & Maylor PW (1998) Biological variation of cystatin C: Implications for the assessment of glomerular filtration rate. Clin Chem 44: 1535-1539.

Kersting S, Koomans HA, Hene RJ, & Verdonck LF (2007) Acute renal failure after allogeneic myeloablative stem cell transplantation: Retrospective analysis of incidence, risk factors and survival. Bone Marrow Transplant 39: 359-365.

Kist-van Holthe JE, van Zwet JM, Brand R, van Weel MH, Vossen JM, & van der Heijden AJ (1998) Bone marrow transplantation in children: Consequences for renal function shortly after and 1 year post-BMT. Bone Marrow Transplant 22: 559-564.

Kletzel M, Pirich L, Haut P, & Cohn RA (2005) Comparison of tc-99 measurement of glomerular filtration rate vs. calculated creatinine clearance to assess renal function pretransplant in pediatric patients undergoing hematopoietic stem cell transplantation. Pediatr Transplant 9: 584-588.

Koch Nogueira PC, Hadj-Aissa A, Schell M, Dubourg L, Brunat-Mentigny M, & Cochat P (1998) Long-term nephrotoxicity of cisplatin, ifosfamide, and methotrexate in osteosarcoma. Pediatr Nephrol 12: 572-575.

Kos J, Stabuc B, Cimerman N, & Brunner N (1998) Serum cystatin C, a new marker of glomerular filtration rate, is increased during malignant progression. Clin Chem 44: 2556-2557.

Krawczuk-Rybak M, Kuzmicz M, & Wysocka J (2005) Renal function during and after treatment for acute lymphoblastic leukemia in children. Pediatr Nephrol 20: 782-785.

Kumar M, Kedar A, & Neiberger RE (1996) Kidney function in long-term pediatric survivors of acute lymphoblastic leukemia following allogeneic bone marrow transplantation. Pediatr Hematol Oncol 13: 375-379.

Kusumi E, Kami M, Hara S, Hoshino J, Yamaguchi Y, Murashige N, Kishi Y, Shibagi Y, Shibata T, Matsumura T, Yuji K, Masuoka K, Wake A, Miyakoshi S, & Taniguchi S (2008) postmortem

examination of the kidney in allogeneic stem cell transplantation recipients: possible involvement of graft-versus-host disease. Int J Hematol 87:225-30.

Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, & Grubb A (1994) Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 40: 1921-1926.

Lane PH, Mauer SM, Blazar BR, Ramsay NK, & Kashtan CE (1994) Outcome of dialysis for acute renal failure in pediatric bone marrow transplant patients. Bone Marrow Transplant 13: 613-617.

Lange BJ, Kobrinsky N, Barnard DR, Arthur DC, Buckley JD, Howells WB, Gold S, Sanders J, Neudorf S, Smith FO, & Woods WG (1998) Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with down syndrome: Children's cancer group studies 2861 and 2891. Blood 91: 608-615.

Lankisch P, Wessalowski R, Maisonneuve P, Haghgu M, Hermsen D, & Kramm CM (2006) Serum cystatin C is a suitable marker for routine monitoring of renal function in pediatric cancer patients, especially of very young age. Pediatr Blood Cancer 46: 767-772.

Lashford LS, Campbell RH, Gattamaneni HR, Robinson K, Walker D, & Bailey C (1996) An intensive multiagent chemotherapy regimen for brain tumours occurring in very young children. Arch Dis Child 74: 219-223.

Laterza OF, Price CP, & Scott MG (2002) Cystatin C: An improved estimator of glomerular filtration rate? Clin Chem 48: 699-707.

Lawton CA, Cohen EP, Murray KJ, Derus SW, Casper JT, Drobyski WR, Horowitz MM, & Moulder JE (1997) Long-term results of selective renal shielding in patients undergoing total body irradiation in preparation for bone marrow transplantation. Bone Marrow Transplant 20: 1069-1074.

Leblond V, Sutton L, Jacquiaud C, Item C, Sadoun R, Jaudon MC, Raymond F, Jacobs C, & Deray G (1995) Evaluation of renal function in 60 long-term survivors of bone marrow transplantation. J Am Soc Nephrol 6: 1661-1665.

Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G, & National Kidney Foundation (2003) National kidney foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Ann Intern Med 139: 137-147.

Levey AS, Perrone RD, & Madias NE (1988) Serum creatinine and renal function. Annu Rev Med 39: 465-490.

Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, Jonmundsson G, Mellander L, & Gustafsson G (2003) Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down's syndrome: Results of NOPHO-AML trials. Br J Haematol 122: 217-225.

Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, Jonmundsson G, Mellander L, Siimes MA, Yssing M, Zeller B, Gustafsson G, Nordic Society of Pediatric Hematology and Oncology (NOPHO), & AML Study Group (2005) Long-term results in children with AML: NOPHO-AML study group--report of three consecutive trials. Leukemia 19: 2090-2100.

Lin WY, Liu HC, Yeh TC, Wang LY, & Liang DC (2008) Triple intrathecal therapy without cranial irradiation for central nervous system preventive therapy in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer 50: 523-527.

Luce JK, & Simons JA (1988) Efficacy of mesna in preventing further cyclophosphamide-induced hemorrhagic cystitis. Med Pediatr Oncol 16: 372-374.

Lönnerholm G, Carlson K, Bratteby LE, Backlund L, Hagberg H, Rikner G, Smedmyr B, Oberg G, & Simonsson B (1991) Renal function after autologous bone marrow transplantation. Bone Marrow Transplant 8: 129-134.

Macdonald DR (2003) New frontiers in the treatment of malignant glioma. Semin Oncol 30: 72-76.

Madanat LM, Lähteenmäki PM, Hurme S, Dyba T, Salmi TT, Sankila R (2008) Hypothyroidism among pediatric cancer patients: A nationwide, registry-based study. Int J Cancer 122: 1868-1872.

Maiche AG, Lappalainen K, & Teerenhovi L (1988) Renal insufficiency in patients treated with high dose methotrexate. Acta Oncol 27: 73-74.

Malatack JJ, Consolini DM, & Bayever E (2003) The status of hematopoietic stem cell transplantation in lysosomal storage disease. Pediatr Neurol 29: 391-403.

Mann JR, Gray ES, Thornton C, Raafat F, Robinson K, Collins GS, Gornall P, Huddart SN, Hale JP, Oakhill A; UK Children's Cancer Study Group Experience (2008) Mature and immature extracranial teratomas in children: the UK Children's Cancer Study Group Experience. J Clin Oncol 26:3590-3597.

Marec-Berard P, Philip T (2004) Ewing sarcoma: The pediatrician's point of view. Pediatr Blood Cancer 42: 477-480.

Marky I, Björk O, Forestier E, Jonsson OG, Perkkiö M, Schmiegelow K, Storm-Mathiesen I, Gustafsson G, & Nordic Society of Pediatric Hematology and Oncology (2004) Intensive chemotherapy without radiotherapy gives more than 85% event-free survival for non-hodgkin lymphoma without central nervous involvement: A 6-year population-based study from the nordic society of pediatric hematology and oncology. J Pediatr Hematol Oncol 26: 555-560.

Meyers RL (2007) Tumors of the liver in children. Surg Oncol 16: 195-203.

Miralbell R, Bieri S, Mermillod B, Helg C, Sancho G, Pastoors B, Keller A, Kurtz JM, & Chapuis B (1996) Renal toxicity after allogeneic bone marrow transplantation: The combined effects of total-body irradiation and graft-versus-host disease. J Clin Oncol 14: 579-585.

Miralbell R, Sancho G, Bieri S, Carrio I, Helg C, Brunet S, Martin PY, Sureda A, Gomez De Segura G, Chapuis B, Estorch M, Ozsahin M, & Keller A (2004) Renal insufficiency in patients with hematologic malignancies undergoing total body irradiation and bone marrow transplantation: A prospective assessment. Int J Radiat Oncol Biol Phys 58: 809-816.

Moe PJ, & Holen A (2000) High-dose methotrexate in childhood all. Pediatr Hematol Oncol 17: 615-622.

Mojiminiyi OA, Marouf R, Abdella N, Kortom M, & Abdul-Razzak R (2002) Serum concentration of cystatin C is not affected by cellular proliferation in patients with proliferative haematological disorders. Ann Clin Biochem 39: 308-310.

Mott MG, Stevenson P, & Wood CB (1972) Methotrexate meningitis. Lancet 2: 656.

Mulaomerovic A, Halilbasic A, Cickusic E, Zavasnik-Bergant T, Begic L, & Kos J (2007) Cystatin C as a potential marker for relapse in patients with non-hodgkin B-cell lymphoma. Cancer Lett 248: 192-197.

Nachman JB, Sposto R, Herzog P, Gilchrist GS, Wolden SL, Thomson J, Kadin ME, Pattengale P, Davis PC, Hutchinson RJ, White K, & Children's Cancer Group (2002) Randomized comparison of low-dose involved-field radiotherapy and no radiotherapy for children with hodgkin's disease who achieve a complete response to chemotherapy. J Clin Oncol 20: 3765-3771.

Nandagopal R, Laverdiere C, Mulrooney D, Hudson MM, Meacham L (2008) Endocrine late effects of childhood cancer therapy: A report from the children's oncology group. Horm Res 69: 65-74.

Narayanan S, & Appleton HD (1980) Creatinine: A review. Clin Chem 26: 1119-1126.

Nath CE, McLachlan AJ, Shaw PJ, Coakley JC, & Earl JW (2007) Amphotericin B dose optimization in children with malignant diseases. Chemotherapy 53: 142-147.

Nathan PC, Whitcomb T, Wolters PL, Steinberg SM, Balis FM, Brouwers P, Hunsberger S, Feusner J, Sather H, Miser J, Odom LF, Poplack D, Reaman G, & Bleyer A (2006) Very high-dose methotrexate (33.6 g/m^2) as central nervous system preventive therapy for childhood acute lymphoblastic leukemia: results of National Cancer Institute / Children's Cancer Group trials CCG-191P, CCG-134P and CCG-144P. Leuk Lymphoma 47:2488-2504.

Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, Friedman DL, Marina N, Hobbie W, Kadan-Lottick NS, Pape L, Ahlenstiel T, Ehrich JH, & Offner G (2007) Reversal of loss of glomerular filtration rate in children with transplant nephropathy after switch to everolimus and low-dose cyclosporine A. Pediatr Transplant 11: 291-295.

Pang R, & Poon RT (2006) Angiogenesis and antiangiogenic therapy in hepatocellular carcinoma. Cancer Lett 242: 151-167.

Patte C, Auperin A, Gerrard M, Michon J, Pinkerton R, Sposto R, Weston C, Raphael M, Perkins SL, McCarthy K, Cairo MS, & FAB/LMB96 International Study Committee (2007) Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-hodgkin lymphoma in children and adolescents: It is possible to reduce treatment for the early responding patients. Blood 109: 2773-2780.

Patzer L, Ringelmann F, Kentouche K, Fuchs D, Zintl F, Brandis M, Zimmerhackl LB, & Misselwitz J (2001) Renal function in long-term survivors of stem cell transplantation in childhood. A prospective trial. Bone Marrow Transplant 27: 319-327.

Paulino AC, & Okcu MF (2008) Rhabdomyosarcoma. Curr Probl Cancer 32: 7-34.

Perazella MA (1999) Crystal-induced acute renal failure. Am J Med 106: 459-465.

Perrone RD, Madias NE, & Levey AS (1992) Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem 38: 1933-1953.

Picci P (2007) Osteosarcoma (osteogenic sarcoma). Orphanet J Rare Dis 2: 6.

Piepsz A, Colarinha P, Gordon I, Hahn K, Olivier P, Sixt R, van Velzen J, & Paediatric Committee of the European Association of Nuclear Medicine (2001) Guidelines for glomerular filtration rate determination in children. Eur J Nucl Med 28: BP31-6.

Pierrat A, Gravier E, Saunders C, Caira MV, Ait-Djafer Z, Legras B, & Mallie JP (2003) Predicting GFR in children and adults: A comparison of the Cockcroft-Gault, Schwartz, and modification of diet in renal disease formulas. Kidney Int 64: 1425-1436.

Pui CH (1995) Childhood leukemias. N Engl J Med 332: 1618-1630.

Pui CH, & Evans WE (1998) Acute lymphoblastic leukemia. N Engl J Med 339: 605-615.

Pui CH, & Evans WE (2006) Treatment of acute lymphoblastic leukemia. N Engl J Med 354: 166-178.

Pui CH, & Howard SC (2008) Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol 9: 257-268.

Pui CH, Robison LL, & Look AT (2008) Acute lymphoblastic leukaemia. Lancet 371: 1030-1043.

Pääkkö E, Harila-Saari A, Vanionpää L, Himanen S, Pyhtinen J, & Lanning M (2000) White matter changes on MRI during treatment in children with acute lymphoblastic leukemia: Correlation with neuropsychological findings. Med Pediatr Oncol 35: 456-461.

Pöge U, Gerhardt T, Bökenkamp A, Stoffel-Wagner B, Klehr HU, Sauerbruch T, & Woitas RP (2004) Time course of low molecular weight proteins in the early kidney transplantation period--influence of corticosteroids. Nephrol Dial Transplant 19: 2858-2863.

Rahn KH, Heidenreich S, & Bruckner D (1999) How to assess glomerular function and damage in humans. J Hypertens 17: 309-317.

Ramu K, Fraiser LH, Mamiya B, Ahmed T, & Kehrer JP (1995) Acrolein mercapturates: Synthesis, characterization, and assessment of their role in the bladder toxicity of cyclophosphamide. Chem Res Toxicol 8: 515-524.

Randers E, Krue S, Erlandsen EJ, Danielsen H, & Hansen LG (1999) Reference interval for serum cystatin C in children. Clin Chem 45: 1856-1858.

Raney RB,Jr, Crist WM, Maurer HM, & Foulkes MA (1983) Prognosis of children with soft tissue sarcoma who relapse after achieving a complete response. A report from the intergroup rhabdomyosarcoma study I. Cancer 52: 44-50.

Ranke MB, Schwarze CP, Dopfer R, Klingebiel T, Scheel-Walter HG, Lang P, Niethammer D, & PDWP of the BMT (2005) Late effects after stem cell transplantation (SCT) in children--growth and hormones. Bone Marrow Transplant 35 Suppl 1: S77-81

Rask C, Albertioni F, Bentzen SM, Schroeder H, & Peterson C (1998) Clinical and pharmacokinetic risk factors for high-dose methotrexate-induced toxicity in children with acute lymphoblastic leukemia--a logistic regression analysis. Acta Oncol 37: 277-284.

Rehling M, Moller ML, Thamdrup B, Lund JO, & Trap-Jensen J (1984) Simultaneous measurement of renal clearance and plasma clearance of 99mTc-labelled diethylenetriaminepenta-acetate, 51Cr-labelled ethylenediaminetetra-acetate and inulin in man. Clin Sci (Lond) 66: 613-619.

Remuzzi G, & Bertani T (1990) Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? Kidney Int 38: 384-394.

Risch L, Herklotz R, Blumberg A, & Huber AR (2001) Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. Clin Chem 47: 2055-2059.

Risch L, Saely C, Reist U, Reist K, Hefti M, & Huber AR (2005) Course of glomerular filtration rate markers in patients receiving high-dose glucocorticoids following subarachnoidal hemorrhage. Clin Chim Acta 360: 205-207.

Roebuck DJ, & Perilongo G (2006) Hepatoblastoma: An oncological review. Pediatr Radiol 36: 183-186.

Roos JF, Doust J, Tett SE, & Kirkpatrick CM (2007) Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children--a meta-analysis. Clin Biochem 40: 383-391.

Rossi R, Helmchen U, & Schellong G (1992) Tubular function and histological findings in ifosfamide-induced renal fanconi syndrome--a report of two cases. Eur J Pediatr 151: 384-387.

Rossi RM, Kist C, Wurster U, Kulpmann WR, & Ehrich JH (1994) Estimation of ifosfamide/cisplatinum-induced renal toxicity by urinary protein analysis. Pediatr Nephrol 8: 151-156.

Rossi R, Kleta R, & Ehrich JH (1999) Renal involvement in children with malignancies. Pediatr Nephrol 13: 153-162.

Sand TE, & Jacobsen S (1981) Effect of urine pH and flow on renal clearance of methotrexate. Eur J Clin Pharmacol 19: 453-456.

Sanders JE (1997) Bone marrow transplantation for pediatric malignancies. Pediatr Clin North Am 44: 1005-1020.

Sandlund JT, Downing JR, & Crist WM (1996) Non-hodgkin's lymphoma in childhood. N Engl J Med 334: 1238-1248.

Sato T, Kobayashi R, Toita N, Kaneda M, Hatano N, Iguchi A, Kawamura N, & Ariga T (2007) Stem cell transplantation in primary immunodeficiency disease patients. Pediatr Int 49: 795-800.

Schellong G, Potter R, Bramswig J, Wagner W, Prott FJ, Dorffel W, Korholz D, Mann G, Rath B, Reiter A, Weissbach G, Riepenhausen M, Thiemann M, & Schwarze EW (1999) High cure rates and reduced long-term toxicity in pediatric hodgkin's disease: The german-austrian multicenter trial DAL-HD-90. the german-austrian pediatric hodgkin's disease study group. J Clin Oncol 17: 3736-3744.

Schröder O, & Stein J (2003) Low dose methotrexate in inflammatory bowel disease: Current status and future directions. Am J Gastroenterol 98: 530-537.

Schwartz CL, Leisenring W, Robison LL, & Childhood Cancer Survivor Study (2006) Chronic health conditions in adult survivors of childhood cancer. N Engl J Med 355: 1572-1582.

Schwartz GJ, Haycock GB, Edelmann CM,Jr, & Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58: 259-263.

Schwartz GJ, & Gauthier B (1985) A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 106: 522-526.

Schwartz GJ, & Furth SL (2007) Glomerular filtration rate measurement and estimation in chronic kidney disease. Pediatr Nephrol 22:1839-1848.

Shankar SM, Marina N, Hudson MM, Hodgson DC, Adams MJ, Landier W, Bhatia S, Meeske K, Chen MH, Kinahan KE, Steinberger J, Rosenthal D, & Cardiovascular Disease Task Force of the Children's Oncology Group (2008) Monitoring for cardiovascular disease in survivors of childhood cancer: Report from the cardiovascular disease task force of the children's oncology group. Pediatrics 121:e387-96.

Simonetti GD, Santoro L, Ferrarini A, Crosazzo-Franscini L, Fossali E, Bianchetti MG, & the CHIld Project (2007) Systemic hypertension and proteinuria in childhood chronic renal parenchymal disease : Role of antihypertensive drug management. Paediatr Drugs 9: 413-418.

Skinner R (2003) Chronic ifosfamide nephrotoxicity in children. Med Pediatr Oncol 41: 190-197.

Skinner R, Sharkey IM, Pearson AD, & Craft AW (1993) Ifosfamide, mesna, and nephrotoxicity in children. J Clin Oncol 11: 173-190.

Skinner R, Pearson AD, English MW, Price L, Wyllie RA, Coulthard MG, & Craft AW (1998) Cisplatin dose rate as a risk factor for nephrotoxicity in children. Br J Cancer 77: 1677-1682.

Skinner R, Pearson ADJ, Price L, Coulthard, & Craft AW (1992) The influence of age on nephrotoxicity following chemotherapy in children. Br J Cancer 66:S30-S35.

Skärby T, Jonsson P, Hjorth L, Behrentz M, Bjork O, Forestier E, Jarfelt M, Lönnerholm G, & Hoglund P (2003) High-dose methotrexate: On the relationship of methotrexate elimination time vs renal function and serum methotrexate levels in 1164 courses in 264 swedish children with acute lymphoblastic leukaemia (ALL). Cancer Chemother Pharmacol 51: 311-320.

Skärby TVCh, Anderson H, Heldrup J, Kanerva JA, Seidel H, & Schmiegelow K (2006) High leukovorin doses during high-dose methotrexate treatment may reduce the cure rate in childhood acute lymphoblastic leukemia. Leukemia 20:1955-1962.

Sleijfer DT, Smit EF, Meijer S, Mulder NH, & Postmus PE (1989) Acute and cumulative effects of carboplatin on renal function. Br J Cancer 60: 116-120.

Smeland E, Fuskevag OM, Nymann K, Svendesn JS, Olsen R, Lindal S, Bremnes RM, & Aarbakke J (1996) High-dose 7-hydromethotrexate: Acute toxicity and lethality in a rat model. Cancer Chemother Pharmacol 37: 415-422.

Snyder RL (2007) Resumption of high-dose methotrexate after methotrexate-induced nephrotoxicity and carboxypeptidase G2 use. Am J Health Syst Pharm 64: 1163-1169.

Spitz MR, Sider JG, Johnson CC, Butler JJ, Pollack ES, & Newell GR (1986) Ethnic patterns of hodgkin's disease incidence among children and adolescents in the united states, 1973-82. J Natl Cancer Inst 76: 235-239.

Stabuc B, Vrhovec L, Stabuc-Silih M, & Cizej TE (2000) Improved prediction of decreased creatinine clearance by serum cystatin C: Use in cancer patients before and during chemotherapy. Clin Chem 46: 193-197.

Stanton BA, & Koeppen BM (1990) Renal system. In: R. M. Berne and M. N. Levy, eds. Principles of Physiology. Wolfe Publishing Ltd, United States of America, pp 416.

Suarez A, McDowell H, Niaudet P, Comoy E, & Flamant F (1991) Long-term follow-up of ifosfamide renal toxicity in children treated for malignant mesenchymal tumors: An International Society of Pediatric Oncology Report. J Clin Oncol 9:2177-2182.

Svendsen AL, Feychting M, Klaeboe L, Langmark F, & Schuz J (2007) Time trends in the incidence of acute lymphoblastic leukemia among children 1976-2002: A population-based nordic study. J Pediatr 151: 548-550.

Taub JW, & Ge Y (2005) Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer 44: 33-39.

Tenstad O, Roald AB, Grubb A, & Aukland K (1996) Renal handling of radiolabelled human cystatin C in the rat. Scand J Clin Lab Invest 56: 409-414.

Thomas H, Boddy AV, English MW, Hobson R, Imeson J, Lewis I, Morland B, Pearson ADJ, Pinkerton R, Price L, Stevens M, & Newell DR (2000) Prospective validation of renal function-based carboplatin dosing in children with cancer: A United Kingdom Children's Cancer Study Group Trial. J Clin Oncol 18:3614-3621.

Thyss A, Milano G, Kubar J, Namer M, & Schneider M (1986) Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. Lancet 1: 256-258.

Timmermann B, Kortmann RD, Kuhl J, Rutkowski S, Meisner C, Pietsch T, Deinlein F, Urban C, Warmuth-Metz M, & Bamberg M (2006) Role of radiotherapy in supratentorial primitive neuroectodermal tumor in young children: Results of the german HIT-SKK87 and HIT-SKK92 trials. J Clin Oncol 24: 1554-1560.

Tonini GP, Lo Cunsolo C, Cusano R, Iolascon A, Dagnino M, Conte M, Milanaccio C, De Bernardi B, Mazzocco K, & Scaruffi P (1997) Loss of heterozygosity for chromosome 1p in familial neuroblastoma. Eur J Cancer 33: 1953-1956.

Treon SP, & Chabner BA (1996) Concepts in use of high-dose methotrexate. Clin Chem 42:1322-1329.

Tsubaki T, Goodin S, Leader WG, & Chandler MH (1993) Estimation of creatinine clearance in

patients with gynecologic cancer. Clin Pharm 12: 685-690.

Vandell AG, & DiPiro JT (2002) Low-dosage methotrexate for treatment and maintenance of remission in patients with inflammatory bowel disease. Pharmacotherapy 22: 613-620.

Van Rossum LK, Mathot RA, Cransberg K, Zietse R, & Vulto AG (2005) Estimation of the glomerular filtration rate in children: Which algorithm should be used? Pediatr Nephrol 20: 1769-1775.

Van Why SK, Friedman AL, Wei LJ, & Hong R (1991) Renal insufficiency after bone marrow transplantation in children. Bone Marrow Transplant 7: 383-388.

Varan A (2008) Wilms' tumor in children: An overview. Nephron Clin Pract 108: c83-90.

Wasen E, Isoaho R, Mattila K, Vahlberg T, Kivela SL, & Irjala K (2003) Serum cystatin C in the aged: Relationships with health status. Am J Kidney Dis 42: 36-43.

Widemann BC, & Adamson PC (2006) Understanding and managing methotrexate nephrotoxicity. Oncologist 11: 694-703.

Womer RB, Pritchard J, & Barratt TM (1985) Renal toxicity of cisplatin in children. J Pediatr 106: 659-663.

Yetgin S, Olgar S, Aras T, Cetin M, Duzova A, Beylergil V, Akhan O, Oguz O, & Saracbasi O (2004) Evaluation of kidney damage in patients with acute lymphoblastic leukemia in long-term follow-up: Value of renal scan. Am J Hematol 77: 132-139.

Ylinen EA, Ala-Houhala M, Harmoinen AP, & Knip M (1999) Cystatin C as a marker for glomerular filtration rate in pediatric patients. Pediatr Nephrol 13: 506-509.

Zager RA, O'Quigley J, Zager BK, Alpers CE, Shulman HM, Gamelin LM, Stewart P, & Thomas ED (1989) Acute renal failure following bone marrow transplantation: A retrospective study of 272 patients. Am J Kidney Dis 13: 210-216.

Zuelzer WW, Inoue S, Thompson RI, Ottenbreit MJ (1976) Long-term cytogenetic studies in acute leukemia of children; the nature of relapse. Am J Hematol 1: 143-190.

Äärimaa T, Arola M, & Salmi TT (1997) CNS tumours in south-western Finland: High location, high incidence. Acta Paediatr 86: 1074-1076.