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NEUROLOGICAL COMPLICATIONS AFTER STEM CELL TRANSPLANTATION IN CHILDREN

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To all parents,
struggling, worrying, never losing hope,
to all of you who wish to become one and
to Primus, who made my greatest wish come true.

ABSTRACT

Allogeneic haematopoietic stem cell transplantation (HSCT) is a well established method used in the treatment of a number of benign and malignant blood diseases, inborn errors of metabolism and severe congenital immunodeficiency syndromes. Around 60 children are transplanted in Sweden every year. Every HSCT carries a risk of different types of complications for the patient. As the success rate and survival after HSCT increases, the prevention of neurological complications and their long-term sequelae has particular significance in the paediatric patient group.

Paper I describes the acute neurological complications after HSCT in 144 paediatric patients transplanted between 1995 and 2002 at the Karolinska University Hospital-Huddinge. The group of 19 patients (13%) who suffered from neurological complications within three months after HSCT had an elevated risk of death within the first year after HSCT. An increasing number of positive herpesvirus serologies and CMV sero-positivity before HSCT as well as electrolyte-disturbances, high blood pressure and elevated bilirubin during the first three months after HSCT increased the risk of neurological complications. The most common complication was seizures and the most frequent causes of these complications were infection and encephalopathy. In several patients the exact aetiology of the complication could not be determined. Intrathecal chemotherapy is given as prophylaxis to high risk patients after HSCT to lower the risk of CNS relapse of malignant disease. The treatment increases the risk for acute and late onset neurological complications. However the need for this treatment is questioned as advances in primary oncologic treatment before HSCT has substantially decreased the risk for CNS relapse. In Paper II and III we retrospectively compared patients who received intrathecal therapy after HSCT to a group who was not given this treatment. The primary aim was to examine if there was a reduction in CNS relapses in the group given intrathecal chemoprophylaxis. In Paper II 120 patients transplanted 1992 to 2005 were included in the study. In Paper III 397 patients transplanted 1992 to 2006 were studied. Neither of the studies could identify a difference in the prevalence of CNS relapses, other types of relapses, mortality or a difference in the prevalence of neurological complications between the two groups. The study results have resulted in a revision of the clinical protocol for intrathecal chemoprophylaxis after HSCT in many centres.

In Paper IV we addressed the fact that infections are a common cause of neurological complications after HSCT and that the exact cause of many complications are unknown. We aimed to study the prevalence and the clinical symptoms of CNS infections by human polyomavirus (HPyV) within a year after HSCT. We analysed retrospectively the CSF of 20 HSCT patients with neurological complications for five different HPyV; JC-, BK-, KI-, WU-, and MCPyV. JC- and BK-PyV are known neurotropic viruses discovered in the 1970`s. KI-, WU- and MCPyV are more recently discovered viruses where the neurotropic ability is not yet known. The PCR analyses of the 20 CSF-samples were negative for all the five viruses. More studies need to be done to determine the significance of the new HPyV in complications after HSCT.

Conclusion: our studies have contributed with a small piece of knowledge in the struggle to prevent neurological complications after HSCT. Further research is though needed to identify additional risk factors and further improve treatment so that less neurotoxic treatments are needed.

LIST OF PUBLICATIONS

The thesis is based on the following papers, which will be referred to by their Roman numerals (I-IV):

I. Acute neurological complications after hematopoietic stem cell transplantation in children. **Rubin J**, Wide K, Remberger M, Gustafsson B. Pediatric Transplantation 2005; 9 (1), 62-67.

II. Intrathecal chemotherapy after HSCT in children. **Rubin J**, Frost B-M, Arvidson J, Wide K, Gustafsson-Jernberg Å, Gustafsson B. Pediatric Transplantation 2008; 12 (8), 889-895.

III. Use of intrathecal chemoprophylaxis in children after SCT and the risk of central nervous system relapse. **J Rubin**, K Vettenranta, J Vettenranta, M Bierings, J Abrahamsson, A N Békássy, Y Håkansson, B-M Frost, J Arvidson, C Spendilow, J Winiarski and B Gustafsson. Bone Marrow Transplantation 2011; 46 (3), 372-378.

IV. Human polyomaviruses were not detected in cerebrospinal fluid of patients with neurological complications after hematopoetic stem cell transplantation **Rubin J**, Giraud G, Priftakis P, Wide K, Gustafsson B, Ramqvist T, Dalianis T. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ADA	Adenosine deaminase deficiency
aGVHD	Acute graft versus host disease
ALL	Acute lymphoblast leukaemia
AML	Acute myeloid leukaemia
ARDS	Acute respiratory distress syndrome
ATG	Antithymocyte globulin
BFM	Berlin-Frankfurt-Munich group
BM	Bone marrow
BO	Bronchiolitis obliterans
CAST	Centre for Allogeneic Stem Cell Transplantation, Karolinska
CB	Cord blood
cGVHD	Chronic graft versus host disease
CML	Chronic myelogenous leukaemia
CMML	Chronic myelomonocytic leukaemia
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CVD	Cerebrovascular disease
DAD	Diffuse alveolar damage
DCOG	Dutch Childhood Oncology Group
DLI	Donor lymphocyte infusion
DNA	Deoxyribonucleic acid
EBV	Epstein –Barr virus
EEG	Electroencephalogram
FHL	Familial Haemophagocytic Lymphohistiocytosis
FLAIR	Fluid attenuation inversion recovery
GI	Gastrointestinal
GVHD	Graft versus host disease
GVL	Graft versus leukaemia
Gy	Grey
HBV	Hepatitis B virus
HC	Haemorrhagic cystitis
HHV-6	Human herpes virus 6
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPyV	Human polyomavirus
HRT	Hormone replacement therapy
HSCT	Allogeneic haematopoietic stem cell transplantation
HSV	Herpes simplex virus
ICH	Intracranial haemorrhage
JMML	Juvenile myelomonocytic leukaemia
LIP	Lymphocytic interstitial pneumonia
LP	Lumbar puncture

MDS	Myelodysplastic syndrome
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MSD	Matched sibling donor
MUD	Matched unrelated donor
NK cells	Natural killer cells
NOPHO	Nordic Society of Paediatric Oncology and Haematology
Ph+ALL	Philadelphia chromosome positive ALL
PRES	Posterior reversible encephalopathy syndrome
PBSC	Peripheral blood stem cells
PTLD	Post transplant lymphoproliferative disease
PTM	Post transplant malignancies
PyV	Polyomavirus
RIC	Reduced intensity conditioning
RSV	Respiratory syncytial
SCID	Severe combined immunodeficiency
SD	Standard deviation
SOS	Sinusoidal obstruction syndrome
t-AML	Treatment related AML
TBI	Total body irradiation
VOD	Veno-occlusive disease
VZV	Varicellae zoster virus
WAS	Wiskott-Aldrich syndrome
X-linked SCID	X-chromosome-linked severe combined immunodeficiency

INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (HSCT) is a lifesaving method of treatment used in paediatric medicine to treat a number of benign and malign blood disorders as well as certain inborn errors of metabolism and severe congenital immunodeficiency syndromes. However, the procedure is associated with a number of life-threatening complications with the risk of long-term sequelae. Avoiding complications involving the central nervous system is of special importance for developing children. As the success rate of, and survival after HSCT increases, the prevention of neurological complications and their long-term sequelae has particular significance.

HISTORY

The first successful allogeneic stem cell transplantations were performed in 1968 after a few decades of isolated attempts with many failures. Gatti and Bach independently reported two cases of successful transplantations, in the same issue of The Lancet in December 1968. Gatti reported the case of a 5 months old boy with "sex-linked lymphopenic immune deficiency", today known as X-linked severe immunodeficiency, or X-linked SCID. The boy received leucocytes from blood and stem cells from his sister's bone marrow intraperitoneally (BM). He did not receive any pre-treatment. Bach reported the case of a 22 months old boy with Wiskott-Aldrich syndrome (WAS). He was given his sister's BM stem cells without conditioning treatment, resulting in failure of the transplantation. Prior to the next stem cell infusion, he received a four-day course of cyclophosphamide, which resulted in engraftment. (1, 2). In Sweden, the first allogenic stem cell transplantation was performed in a 17 years old patient with aplastic anaemia in 1975 at the Karolinska University Hospital Huddinge (3). During 1975 to 1986 74 paediatric patients were transplanted at the Karolinska University Hospital Huddinge (4).What was an experimental procedure 50 years ago is now a well established treatment method with over 10.000 allogeneic HSCT per year in Europe (5) and 250 per year in Sweden. Out of these 60 are performed on paediatric patients (6). The modern allogeneic HSCT procedure contains two phases; first the conditioning phase when chemotherapy is given, sometimes combined with total body irradiation (TBI), then the patient receive an infusion of stem cells from a donor. Within the following weeks, the stem cells will migrate through the body to attach and proliferate in the bone marrow of the recipient. The engraftment of the new cells, which usually occurs within two to four weeks after the stem cell infusion, is defined as a stable absolute neutrophil count $> 0,5 \times 10^{(9)}$cells/L. The survival rate after HSCT in the scenario with a well matched donor and a benign disease is now very high. As an example, patients diagnosed with thalassemia major transplanted with a fully matched sibling donor has an 8-year overall survival of 94.5% and an 8-year disease free survival of above 80% in Europe (7).The survival rate of children with malignant diseases is lower. This is due to the risk of a relapse in the malignant disease as well as often more aggressive treatment choices, including an anti leukaemic treatment and a more intensive conditioning therapy for relapsing patients. There is also a difference in survival depending on the donor type. The 3 –year survival after HSCT for paediatric patients with leukaemia or myelodysplastic syndrome (MDS) is about 60% with a

matched sibling donor (MSD) and 50% with a matched unrelated donor (MUD) (8). Thanks to the progress of paediatric oncologic treatment and the HSCT procedure the outcome of malignant diagnoses are improving fast. A study from 2010 of a patient group with mixed indications for HSCT, showed a survival rate of 37% in patients who were transplanted 1993-1997 and a survival rate of 53% in patients transplanted 2003-2007 (9).When considering the increasing survival rates of children with malignant diseases, one must take into account the success of paediatric oncologic primary treatment where the children with acute lymphoblast leukaemia (ALL) now have a 90% 5-year survival (10) compared to 70% in the 1980's (11). The consequence of this success is that the patients currently eligible for HSCT have a more advanced malignant disease than previously (9).

The stem cell transplantation procedure contains a number of elements that involve a high mortality risk. All patients go through a period of severe immunosuppression with the risk of mortal infections; there is a risk of severe drug-related side effects and the risk of malignant disease relapse remains for the oncologic patients, although it is diminished after HSCT. For paediatric survivors of HSCT the development of cognitive and psychomotor skills is naturally of the utmost importance. When reviewing literature on neurological complications we can conclude that the risk has decreased due to better treatment options. Radiotherapy for patients < 3 years of age is now used restrictively both in primary oncologic treatment and in the conditioning regimen for HSCT since evidence of more severe neuropsychological sequelae has been found in this age group, compared to older children (12-14). The literature on neurological complications after stem cell transplantation in children, especially on long-term neurological sequelae, is scarce. Although, in recent years, more attention has been brought to the subject (Table 1). Several available studies confirm that there is an increased mortality risk associated with neurological complications after HSCT (15-18) (Fig 1.).

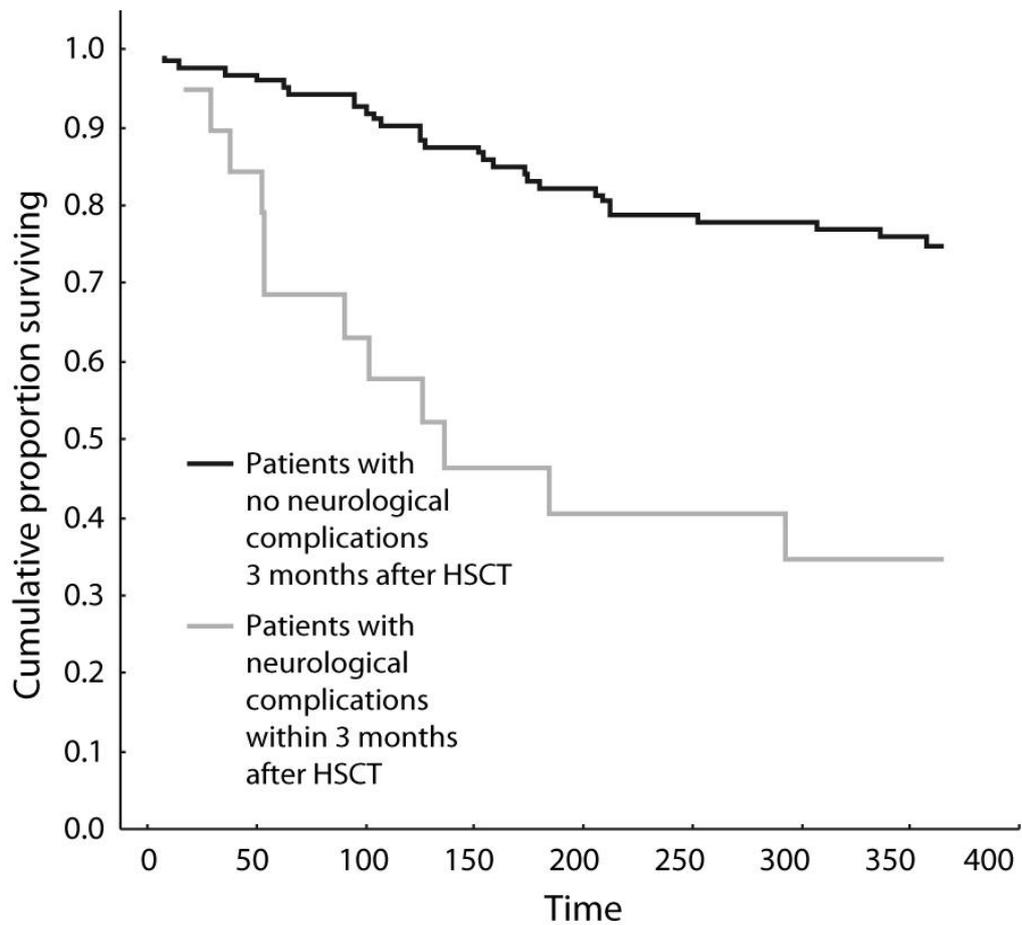Fig. 1. Kaplan Meyer survival curve showing the difference in 1-year survival between patients suffering from acute neurological complications (appearing within three months) after HSCT compared to patients without acute neurological complications (p< 0.01). Data from Paper I, where 144 paediatric patients transplanted between 1995 and 2002 were studied. (Time shown as number of days after transplantation.)

Table 1. Overview of published studies on the incidence and causes of neurological complications (NC) after allogeneic stem cell transplantation (HSCT) in children.   (Until May 2011)

Publ year	Author	No of patients	Age at HSCT	Specific subgroup and/or Observation time	Incidence	Most common symptoms	Most common causes	Risk factors for NC if specified	Influence on survival, if specified
1984	Wiznitzer	57	6 m -24 yrs	10 d - 81 months after HSCT	Total 59%. CNS 35% PNS 14% both 10%	Not specified	Infections,CNS leukemia, CVD, metabolic encephalopathy, cognitive	Not specified	All pts 51%,surv median 46 months
1990	van der Berg	23	4-17 yrs	Only ALL patients	17%	leukoencephalopathy, MR, toxoencephalitis	leukoencephalopathy, MR, toxoencephalitis		25% vs 100%
1999	Iguchi	77 (54 allo)	1-17 yrs		15,80%	seizures, headache, ataxia, oculomotor paralysis	CVD 23 st, Bu 3 pt CsA 1 pt, FK506 1 pt, leukoenc 2 pt, SIADH 1pt, TBI 1pt	For allo, mud, gvhd > 2	early NC = early death but over all no difference
2002	Faraci	272 (185 allo)	0.1 - 19.5 yrs	Mean 15 months. (2 d to 15.6 yrs)	13,60%	seizures, impaired consciousness, motitlitydisorder and sensistivity disorder, cortical function,ivolontary movements, visual	CsA tox , irr/chemo, CNS inf, CVD, immune mediated compl	HSCT type, TBI, aGVHD, GVHD > gr 2, CsA	Mortality 30% vs 8.5%
2004	Woodard	405	1-21 yrs	Only studied; encephalopathy	6,40%		TTP; amphoteicin B, seizures, viral encephalopathy, adem ,liver failure, uremia, aspergillosis, CVS, CyA, MOF		17/26 died
2005	Rubin	144	0-18 yrs	78 d (7-90 d)	13%	seizures, alt conc, headache, paresis,	infection, encephalopathy, CVD, unknown.	electrolytes, bilirubin, CsA, viral serologies	6/19, 7/125 32% vs 5%

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Publ year	Author	No of patients	Age at HSCT	Specific subgroup and/or Observation time	Incidence	Most common symptoms	Most common causes	Risk factors for NC if specified	Influence on survival, if specified
2005	Uckan	113	1,5 m-18 yrs	Only life-threathening NC studied	9,70%	seizures, cranial nerve palsy, CVD, headache	CyA, CVD, hypertension, inf, hypomagnesemia, busulphan	GVHD > 2, MUD, AML, PSBC	9% vs 65%
2007	Zaucha-Prazmo	87	all children	171 pts auto+allo	8%	neurotox, CVD, radikulitis	CyA, CMV, EBV radikulitis , CVD		4 of 7 died
2008	Schmidt	904 (allo 328)	all children		8.4% (76 pt, 82 NC) (14.6% 48 pt/ 328)	Seizures, altered conciousness, motor dysfunction.	Infection, toxic, unclear, neoplastic, vascular, methabolic		18/76 died, mortality 23.7%
2008	Weber	165 (54 auto)	3 m-30 yrs	42.5 mo mean	24% NC 40/165, 30.6% 34/111 allo SCT	severe: 12 seizures/headache 2, parkinson like 1, somnolence 2, aphasia 1, slurred speach + vision 1.	Inf 9, drug 4, CVD 2, CNS relapse 2, unknown encephalopathy 1, CsA 1 19 severe and 21lighter symptoms		
2010	Koh	202	0-20 years	Only early NC, observation time 6 months	13,5% (N=27)	seizures 15, altered consciousness 12 , headache 6, motor weakness 2, parestesia, sensory def 1, tremor 1, visual 1.	CyA tox 16, CNS inf 2, CVD 2, TMA 2, methabolic encephalopaty 2, irraiation/chemotherapy 1, encephalitis/unkown 1, myopathy/unkown 1.	aGVHD > gr 2, donor	52.1% 5 yr survival vs 64.9%
2010	Noe	67	all children	Only CyA tox studied. Observation time 1005 days.	6 pt 9%	seizures,blurred vision, confusion	CyA tox		

NC= neurological complication, allo= allogeneic, auto= autologous, CNS= central nervous system,

PNS= peripheral nervous system, CsA ciklosporin, CVD=cerebrovascular disease,
TMA= thrombotic microangophaty, TTP=Thrombotic Thrombocytopenic purpura
d=days, m=months, yr=years, inf=infection

HEMATOPOIETIC ALLOGENEIC STEM CELL TRANSPLANTATION

Indications

The number of diagnoses for which HSCT is a potential rescue or definitive treatment increases continuously (19). In paediatric HSCT the scope is especially wide as a number of congenital diseases are treated in early childhood. The HSCT procedure differs depending on the diagnosis treated. In HSCT for benign blood diseases such as aplastic anaemia, sickle cell disease, thalassemia, Familial Haemophagocytic Lymphohistiocytosis (FHL), Kostmanns disease and severe combined immunodeficiency the aim is to replace the recipients' malfunctioning haematopoietic stem cells with healthy haematopoietic stem cells able to produce healthy functioning blood cells. In inborn errors of metabolism the new haematopoietic stem cells will be able to produce healthy leucocytes which in their turn can produce the enzymes the patient is missing. The conditioning regimen shall eliminate the patient's mal-functioning haematopoietic stem cells with minimum damage to other organ systems and the donor cells shall engraft without graft versus host disease. A mixed chimerism, i.e a bone marrow where some of the patient's cells still survives can be tolerated. In these disorders maximum steps are taken to avoid graft versus host disease (GVHD). Antithymocyte globulin (ATG) is therefore given to all of these patients irrespective of type of donor (20). In malignant blood disorders a more aggressive conditioning treatment is often used to eradicate the malignant disease from the recipient which can result in a higher frequency of complications. Also in these cases, a certain degree of GVHD can be tolerated as a graft versus leukaemia (GVL) effect is wanted. All patients with malignant disorders go through primary oncologic treatment for their disease before proceeding to HSCT-treatment; the only exception is juvenile myelomonocytic leukaemia (JMML) where HSCT is generally considered the only treatment with a chance for cure (21). Children with acute myeloid leukaemia (AML) are transplanted in first remission if they are diagnosed with cytogenetic unfavourable markers. Whereas children in their first remission with ALL, only have HSCT in the absence of morphological remission from induction therapy or if high levels of minimal residual disease (MRD) still remain after three months of therapy or children diagnosed with Phil + ALL (Philadelphia chromosome positive ALL)(22) . For chronic myelogenous leukaemia (CML), HSCT is the only curative option and should be done within six months in affected children as well as children diagnosed with MDS (23, 24). The timing of HSCT depends on the response to primary therapy. HSCT has

presently rarely been reported in paediatric patients with solid tumours. Among the patients, reported as case studies or small case-series, relapsed rhabdomyosarcoma, Ewing sarcoma and neuroblastoma are the most common diagnoses (25-27). In Germany presently there is an ongoing study for children with high-risk metastatic rhabdomyosarcoma using haploidentical HSCT (28). Autologous HSCT is of proved benefit in high-risk neuroblastoma and Hodgkins disease and is under investigation for advanced stages and relapse of Ewing sarcoma (29).

Choosing the donor and stem cell source

A human leucocyte antigen-identical (HLA-identical) sibling donor is the donor of choice for all HSCTs. A closely related donor with a matching HLA type will minimize the risk of GVHD and GVHD related complications after the transplantation. This in turn will substantially reduce the mortality risk after the transplantation (8). If no such donor is available registries of voluntary donors worldwide are searched. At our centre a matched unrelated donor should be matched on a minimum of six of the six most important alleles from the antigen sites HLA-A,-B and DRB1 and molecular high resolution techniques should be used. At many centres HLA C is also included as a necessary allele (30). For children a more extensive matching with an up to 14/14 match (HLA-A,-B,-C, DRB1,DQA1,DQB1 samt DPA1) is presently used at the Karolinska University Hospital (31). In homozygogous thalassemia, HSCT is performed to cure a non-mortal disease. The HSCT with a sibling donor is certainly preferable, due to the higher mortality risk of using another type of donor. However, if the disease is severe, a non-sibling donor can be considered. In these cases, evaluation in clinical protocols is essential. In aplastic anaemia the treatment of choice is HSCT with a matched sibling donor, if available. If a sibling donor is not available, immunosuppressive therapy is tried before searching for a matched unrelated donor (19). In severe combined immunodeficiencies (except for children with adenosine deaminase deficiency, ADA, where enzyme therapy is an option (32)) and metabolic diseases there is no alternative to HSCT for cure and the HSCT has to be performed, sibling or no sibling available. A syngenic (monozygotic twin-) donor is rare but if available it is the optimal donor in treating non-malignant disease, due to the practically non-existent risk of GVHD (33, 34). A sibling donor is available in approximately 30% of all paediatric HSCTs. In absence of an HLA matched sibling donor or a well-matched unrelated donor, a relative with not fully matching HLA type might be used –

which is referred to as a haplo-identical donor. For most transplantations, the donor used is a MUD from one of the donor transplantation registries worldwide (35) .

Initially, stem cell transplantation was equal to bone marrow transplantation, as this was the stem cell source used. Now peripheral blood stem cells (PBSC) and cord blood (CB) has emerged as alternative sources of stem cells for transplantation. BM is still the primary choice for transplantation of benign disorders (19). BM is also the primary choice for paediatric patients, where transplantation with PBSC has shown a higher frequency of chronic graft versus host disease (cGVHD) and higher mortality risk than transplantations with BM. PBSC is the stem cell source of choice for adults as the concentration of lymphocytes is much higher than in BM which results in easier mobilisation and faster hematopoietic recover (36). CB is the third stem cells source used. With CB as stem cell source, some patients will receive CB stored from their HLA –matched siblings, whereas others get an unrelated CB donation from a cord blood bank. The haematopoietic recovery is slower with CB compared to BM or PBSC (37) and there is the disadvantage that there will be no cells left for a potentially required donor lymphocyte infusion (DLI). A DLI - treatment is given to the recipient as supportive therapy to enhance the engraftment or in the case of threatening rejection of the donor cells by the recipient. The DLI can also be given with the intent to prevent or treat relapse of malignant disease (19). CB is the only option for patients where a sibling or a MUD is not available as the donor pool expands with the tolerance of one or two HLA mismatches when CB is given (38, 39). CB transplantation also has a lower risk of latent virus transmission and a lower risk of acute GVHD (39-41). The risk for infections, most importantly viral infections, has though been reported to be higher in CB transplantation than in PBSC and BM transplantation. This is linked to the limited capacity of the CB transplant T-cells and the slow recovery of T-cell function (21, 42, 43). In severe combined immunodeficiencies (SCID) and metabolic diseases there is no alternative to HSCT for cure and the HSCT has to be performed sibling or no sibling available.

Conditioning treatment

The treatment before the infusion of stem cells- the conditioning treatment- differ with underlying disease, age of the patient, treatment centre and the overall health condition of the patient. If the patient is elderly, has an impairment of kidney or lung function or

the disease progression is not so severe that it justifies the toughest conditioning regimen, a reduced intensity conditioning (RIC) can be chosen. In general, children will receive full myeloablative conditioning with the maximum chance for cure and a higher risk of complications. This is due to the fact that despite the difficult disease they are being treated for, the children are generally in good health. CAST, the Centre for Allogeneic Stem Cell Transplantation at the Karolinska University Hospital Huddinge where the paediatric patients from our centre, participating in all four studies in this thesis, are transplanted, follow the international protocols regarding the choice of conditioning regimens for HSCT. Before transplantation the children are treated according to the NOPHO (Nordic Society of Paediatric Oncology and Haematology) protocols for children. For the conditioning myeloablative protocols containing total body irradiation 3 Grey (Gy) x 4 and cyclophosphamide 60 mg /kg are chosen for ALL in adults and, at the time for these studies also in children. A full myeloablative treatment for children with ALL today consists of TBI 3 Gy x 4 days, etoposide 60 mg/kg x1, 1 day. If there has been a CNS involvement of ALL intrathecal methotrexate 1x1 during 2 days is given (44). Children < 3 years are given a regimen without TBI. Busulphan and cyklophosphamide is used for AML, CML, MDS, JMML and chronic myelomonocytic leukaemia (CMML). Melphalan is added for children < 18 years old, with MDS and JMML(45). Reduced intensity regimens of busulphan/fludarabine and cylophosphamide/fludarabine are given to, as mentioned above, adult patients with a certain few malignant disorders, and to patients with contraindications against full myeloablative conditioning. When busulphan is used in the conditioning treatment, seizure prophylaxis with clonazepam is given during the conditioning phase (46). A RIC treatment can be used in children with diagnosis as Fanconis aplastic anaemia and FHL (47, 48). A paediatric RIC treatment may consist of Flu 30mg /m2 for five days followed by Cy 10 mg/kg for two days(49) .Two intrathecal injections of methotrexate are included in the conditioning regimen for patients with previous CNS AML or ALL leukaemia and for all infant ALL patients (44, 50, 51). Other European transplantation centres, follow different protocols. The Utrecht centre, participating in study III, follow the Dutch Childhood Oncology Group (DCOG) and the Berlin-Frankfurt-Munich treatment protocols with slightly different primary treatment and conditioning regimens. As an example the myeloablative conditioning for ALL for children > 2 years of age, included 2x2 Gy TBI during three consecutive days and thereafter one day with a dose of etoposide 60 mg/kg/day, plus local irradiation of the respective areas, if involvement of testes or CNS (52). In preparing the studies we concluded that these

differences were not sufficient to interfere with the results when studying the effect on isolated CNS relapses of intrathecal therapy after HSCT.

Prophylaxis against GVHD

To reduce the risk of GVHD ATG-treatment is given the last days before the infusion of stem cells in benign disorders and when the donor is a MUD or a HLA mis-matched donor. It is also given in some RIC protocols (20). GVHD-prophylaxis is given to all patients from day –1. The most common drugs used are cyclosporine in combination with methotrexate. During the six to twelve months after HSCT the patients are seen regularly for monitoring of blood cell counts, infections prophylactic treatment, re-vaccination and observation for and treatment of GVHD. The patient will stay on immunosuppressive treatment as primary prophylaxis against GVHD, for up to twelve months after HSCT depending on donor, disease and symptoms of GVHD (53).

Prophylaxis against infectious complications

All patients receive infectious prophylaxis against P jiroveci with cotrimoxasole three days per week as standard drug until the day of transplantation. Cotrimoxasole is then re-introduced after engraftment and continued until six months after HSCT – or if later- until the end of immunosuppressive treatment or resolution of GVHD disease. If the patient is toxoplasma gondii antibody positive a daily dose of cotrimoxasole is given. After conditioning, during the neutropenic phase, ciprofloxacin is used as antibacterial prophylaxis and nystatin and fluconazole as fungal prophylaxis. This treatment is started after the conditioning phase and continued until engraftment. Additional viral prophylaxis is prescribed if the patient is herpes simplex virus (HSV) positive with a HSV titre of > 1000 or hepatitis B virus (HBV) positive. If there was a previous deep fungal infection, fungal prophylaxis with voriconazol during minimum three months is prescribed. Varicellae seronegative patients shall receive prophylactic acyclovir treatment for three weeks following exposure to varicella zoster virus (VZV). Anti-pneumococcal prophylaxis is a lifelong preventive treatment for all asplenic patients. In case of acute GVHD, fungal and viral prophylaxis shall be given if the patient receives a high-dose steroid treatment. In cGVHD, fungal and herpes simplex prophylaxis shall be given for as long as the immunosuppressive treatment continues. All patients are fully re-vaccinated after HSCT. The revaccination is

initiated six months after the procedure. There are no differences in the infectious prophylaxis strategy between children and adults (54, 55).

Prophylaxis against CNS relapse: Intrathecal therapy

At some centres, intrathecal chemotherapy is given during the follow up to prevent relapse of malignant disease in the CNS. The data on the efficacy of these invasive treatments originate from work by Thompson et al. in 1986, where intrathecal methotrexate after HSCT reduced the risk of CNS relapse in ALL from 19% to 4% (56). Since 1986 the paediatric oncologic treatment has made great progress in primary treatment and the conditioning regimens for paediatric HSCT have changed and become more efficient. Today the routines and recommendations regarding intrathecal chemoprophylaxis to children after HSCT differ among international centres (57).

At our transplantation clinics; CAST and the department for Haematology and Oncloogy at the Astrid Lindgren's Children's Hospital at the Karolinska University Hospital Huddinge we had the routine to give six intrathecal injections to patients with a high risk of CNS-relapse after HSCT until 2008. The patients considered high risk were all ALL patients, AML 4 and 5 and AML M0 –M3 if they had previous extramedullary engagement, high presenting leucocyte count or slow response to primary treatment. The intrathecal treatment schedule prescribed one injection given every two weeks, starting on day + 32 after HSCT. Patients with CNS leukaemia had a prolonged treatment regime with intrathecal injections every eight weeks for 18 months after HSCT. The prolonged treatment schedules varied in time from patient to patient depending on side-effects, other diseases and complications. Intrathecal injections were not given if platelet count < 50 x10^9/L or leukocyte count < 1.0 x10^9/L. Low platelet count, infections or other complications would often delay the start of intrathecal therapy, until the second or third month after HSCT. The usual practice for patients was to receive cytarabine, but in presence of GVHD methotrexate was used as an alternative. The dose was age-dependent: < 1 year of age 16 mg cytarabine or 6mg methotrexate, at 1-2 years of age; 20 mg cytarabine or 8 mg methotrexate; at 2-3 years of age 26 mg cytarabine or 10 mg methotrexate and over 3 years of age; 30 mg cytarabine or 12 mg methotrexate. Despite the delays in starting intrathecal therapy, often due to low platelet counts or other complications, patients were generally given

their first intrathecal injections within the first three months after HSCT (58, 59). (II, III)

Complications following HSCT

The weakened immune system during the first year after HSCT causes a high risk of infectious complications of a wide range, which in turn can lead to further complications. The donated stems cells ability to react against the patient's own cells and tissue is called graft versus host disease, GVHD, and is in itself a major risk factor for death after HSCT. GVHD can also lead to secondary complications and the chronic form of GVHD may disable the patient for years after the transplantation. The conditioning and transplantation puts all the organ systems in the body at risk. Complications due to HSCT and pharmacological treatment can occur in most organ systems of the body. Below, the most common and potentially serious complications are described more thoroughly. Other complications, not described in detail here are acute respiratory distress syndrome (ARDS), a rare often fatal respiratory condition (60) and veno-occlusive disease (VOD, also called sinusoidal obstruction syndrome, SOS), a likewise serious condition due to damage of small blood vessels and stagnations of the blood flow in the liver (61). In addition there are complications in the skeletal system in the form of osteonecrosis (62, 63) in the eyes (64), the kidneys (renal failure often due to nefrotoxic drugs) (65) and in the vascular system (thrombosis) (66).

Graft versus host disease

Despite excellent matching with new technologies (high resolution DNA typing) the risk for GVHD is 30% in a matched related donor transplantation, and 45% in a MUD transplantation in children (8). The GVHD is the reaction of the donors T-lymphocytes against the tissues of the patient. The donor T-lymphocytes are highly functioning immune cells designed to protect the body from foreign material and – if infused to a foreign body- the recipient- they react to the recipient's body as they would to a foreign tissue or infection (53). The treatment of GVHD is to suppress the already weak immune system to avoid the heavy attack on the recipient's body. This leads to an even higher risk of infections and also to secondary complications from the GVHD drugs. High dose steroids- the treatment most commonly used that is very effective, causes cushingoid symptoms, gastritis, severe mood changes, increased appetite, reduced bone

density and overweight. Long-term treatment with high doses of steroids can also reduce height (67) . If the GVHD is severe, a wide range of immunosuppressive drugs can be used to incapacitate the T-cell function, with a risk of infectious complications and with varying results. As an example it is well known that there is an increased risk of CMV infection/reactivation if GVHD is present (68). Acute GVHD (aGVHD) is traditionally defined as GVHD appearing within three months (100 days) after HSCT. Symptoms still present more than three months after HSCT is chronic GVHD (cGVHD). However, in 2005 another categorization was proposed where aGVHD is categorized by symptoms and not by the time passed after HSCT and is now standard guidelines (69).

Table 1.
Classification of GVHD. According to National Institute of Health Consensus Development Project 2005 (68).

Cathegory of GVHD	Subcathegory	Timing of symptom after HSCT or DLI	Presence of Acute GVHD features	Presence of Chronic GVHD features
Acute	Classic acute	≤ 100 days	Yes	No
	Persistent, recurrent or late onset	>100 days	Yes	No
Chronic	Classic Chronic	No time limit	No	Yes
	Overlap syndrome	No time limit	Yes	Yes

As the severity of aGVHD is an important prognostic factor, accurate clinical staging of GVHD is essential. The staging is commonly expressed as grades I-IV (70). Grade I -II are mild and moderate aGVHD, grade III is severe and grade IV is life-threatening. Involvement of liver or the GI- tract is directly considered as a moderate aGVHD. Therefore aGVHD of stage one in the liver and GI, is considered a grade II in severity. Skin GVHD is graded depending on the percentage of skin affected and the type of rash/erythroderma. Liver GVHD is graded by the degree of bilirubin elevation and gastrointestinal (GI) GVHD is graded by the volume of diarrhoea and presence of absence of abdominal pain/ileus. Severe aGVHD has a very poor prognosis. In a large study from 2002 of CML patients, the patients with aGVHD grade III had a 25% long

term survival compared to 63% for the patients with grade I aGVHD (71) . Chronic GVHD is one of the major causes of death in children after HSCT. Around 25% of paediatric patients develop cGVHD, compared to 30-50% of adult patients (16, 72). Chronic GVHD is generally graded as limited or extensive. The cGVHD can affect the GI tract, the lungs, the skin, the musculoskeletal system, the liver and the eyes. Skin is the organ most commonly affected. A long-term chronic skin GVHD can cause severe psychological strain. The treatment-approach should be multidisciplinary with pharmacological treatment of corticosteroids and other immunosuppressive treatments such as T-lymphocyte "depletion" (with ATG), extracorporeal photophoresis and physiotherapy (16, 53, 72). The cGVHD can manifest itself in the lungs as bronchiolitis obliterans (BO) in 8% to above 20% in different studies of HSCT patients (5, 73, 74). BO is a fibrous scarring in the bronchioles, which manifests later than three months after HSCT and most often with other manifestations of chronic GVHD. Risk factors in the recipient are older age and poor lung function before HSCT as well as the choice of donor; cord blood has a low risk, and PSCB a higher risk than BM. Recently published data suggest that certain genetic and biochemical factors that may predispose patients to contract BO (75-77). Respiratory infections in the 110 days following HSCT, also increase the risk of BO. The symptoms of progressing BO may be discrete. Long standing cough and dyspnoea with normal chest X ray and spirometry showing new onset of airway obstruction are important signs. Correct diagnosis is performed by High Resolution Computer Thomography (HRCT) (78). The treatment is, as for other symptoms of GVHD, corticosteroids, immunosuppressive therapy and also bronchodilators, infectious prophylaxis and sometimes anti-reflux medication. Similar pulmonary GVHD manifestations involve the alveolae (diffuse alveolar damage, DAD) or take the form of lymphocytic interstitial pneumonia (LIP), bronchiolitis obliterans organizing pneumonia (BOOP) or lymphocytic bronchiolitis/bronchitis (77, 79).

Chronic GVHD presenting as symptoms from the CNS are rare. Neuropathological findings have shown focal cerebral vasculitis and perivascular lymphocytic infiltration/immune-mediated encephalitis in a few published cases (80).There are also reports of demyelinating disease of the CNS (81). Among the peripheral neurologic manifestations of cGVHD myositis and polymyositis is often listed as these symptoms are regarded as neuromuscular in origin. Myositis presents as muscle weakness and pain in about 2% of HSCT patients. It is often treatable with corticosteroids and methotrexate. Immune mediated neuropathies such as myasthenia gravis and

Guillaume-Barré- like symptoms is very rare (occurs in about 1% of patients) (53, 81-83). Paediatric chronic GVHD survivors have been reported to have a higher risk of cataract and also muscle weakness, in long term follow-up (84).

The graft versus tumour effect

The donor T-cells' ability to react against recipient tissue does not only result in GVHD, but also in a reaction against the patients' malignant cells. This is called the graft versus tumour or graft versus leukaemia (GVL) effect. Multiple studies have been done on how to maximise GVL and minimize GVHD. It is probable that a CD8+cell depleted graft would be beneficial, resulting in fewer cases of GVHD with sustained GVL and engraftment, but no definite conclusion has been reached. The timing for DLIs (donor lymphocyte infusions) after HSCT and blocking or administrating different cytokines have also been investigated, but more studies need to be done regarding the optimal timing for these interventions (85). The most successful use of the GVL-effect with DLI is reported in CML (86) .

Infectious complications

During the first two- four week period after the stem cell infusion, neutropenia (neutrophil count < 0.5 x$10^{(9)}$cells/L) increases the risk of infections. The patients are therefore placed in isolation. The risk of infectious complications - contagious infections from the surrounding environment or reactivation of previously acquired latent infections remains elevated during the first six to twelve months or more after the HSCT. This is due to the fact that the patient is receiving immunosuppressive drugs and the new immune system is under development. It is known that the natural killer cells (NK cells) reconstitute within the first 100 days, but that the B- and T- cell function only gradually normalises during the first year after HSCT. The factor most important for the immune reconstitution is the presence or absence of GVHD and subsequent immunosuppressive therapy (16, 82, 87).

A common categorisation of infectious complications after HSCT is:
- The pre-engraftment period; 0-30 days after HSCT. In this period bacterial and fungal infections dominate.

- The early post-HSCT period – 30 to 100 days after HSCT. During this period aspargillus is a threat, together with cytomegalovirus (CMV) reactivation and pneumocystis jiroveci.
- The late phase - 100 days after HSCT and onwards. In the late period the patient is still at risk for CMV reactivation and, especially in the case of GVHD and encapsulated bacteria. The patients are also increasingly exposed to community acquired infections.

Infection is one of the most common causes of neurological complications (88) but it is also a heavy disease burden for the HSCT patients without neurological complications.

Bacterial infections

Bacterial infections during the first month after HSCT are most often caused by gram positive bacteria; coagulase negative staphylococci (89) coryneibacteriae and alpha haemolytic streptococci (90). Infection with gram positive bacteria is associated with GI mucosal damage. The gram negative bacterial infections are associated with central lines and severe mucosal damage. Among these, E-coli, Klebsiella and Pseudomonas dominate (90). The gram negative infections are often severe and associated with high morbidity and mortality (90, 91). Bacterial CNS infections are rare (92) . The average prevalence of bacteraemia after HSCT is around 40% (91, 93, 94). In chronic GVHD patients the opsonization is impaired and the patients are at increased risk of rapidly progressive infections with encapsulated bacteria (N Meningitidis, S pneumonia) (95) .After the introduction of reduced intensity conditioning (RIC) regimens a reduction in severe bacterial infections during the first months (96) was seen in adult RIC patients. However, this not been observed in fungal or viral infections (97) . Pathogens with antibiotic resistance are among HSCT patients a growing problem, as it is in overall health care. A high incidence of resistance has been reported against ciprofloxacin which is widely used as bacterial prophylaxis in the neutropenic phase (89).

Parasitic infections

Among parasitic infections, pneumocystis jirovecii and toxoplasma gondii are the most common infections. These are known common agents also in other immunosuppressed patients with T cell defects such as HIV-positive patients. All patients receive pneumocystis prophylaxis with cotrimoxasole due to the risk of the highly mortal pneumocystis jiroveci pneumonia. With prophylaxis the risk is significantly reduced, from more than 10% to below 1% (98) . Toxoplasmosis encephalitis is the most

common manifestation of toxoplasmosis in this group and occurs in a few percent of HSCT patients. Toxoplasmosis in HSCT patients has, despite treatment, a very high mortality of nearly100%. Often, the diagnosis is not confirmed or found, until after death (99).

Fungal infections

Risk factors for invasive fungal infections in paediatric patients after HSCT are granulocytopenia, indwelling central venous catheters, mucositis and long term high dose treatment with corticosteroids (100).Superficial fungal infections are common especially due to steroid treatment in both acute and chronic GVHD. Up to 15% of the HSCT patients develop systemic fungal infections (35, 90, 100). Aspergillus- and candida spieces are the most common systemic fungal agents. Due to fluconazole prophylaxis aspergillus has become relatively more common. The invasive fungal infections occur in two peaks; one before engraftment and one after three to four months (100).Fungal CNS-disease can be caused by Nocardia asteroides which can cause brain abscesses, but more commonly, fungal CNS disease manifests as septic emboli of aspergillus infections, with a primary infection in the lungs. Aspergillus CNS infection is 100 % fatal. The most common cause of fungal meningitis reported in immunosuppressed patients, is Cryptococcal neoformans. Most of the cases are HIV-positive patients, but as Cryptococcus meningitis mainly affects patients with a defect T-cell function, HSCT patients are at risk as well. The most common site for Cryptococcus infection is though the lungs, followed by the CNS (101). Zygomycetes infection in the CNS results in a vascular invasion leading to thrombosis. The treatment is both surgical and pharmacological (with amphotericin B and posaconazole) (101). Late fungal infection affects the lungs or sinuses and is caused by aspergillus (95).

Viral infections

Vigilance and protection against reactivation of herpes viruses is important after HSCT, why prophylaxis is given in high risk situations. (see above; Prophylaxis against infectious complications). Before transplantation all patients and donors are screened for HSV, cytomegalovirus, Epstein –Barr virus (EBV) and VZV.

Herpes simplex virus

HSV is the herpes virus that reactivates first after HSCT, within a few weeks (102). The prevalence of HSV is about 50% in adolescents and increasing with age (103).

HSV reactivation in HSCT patients may present as mucositis, oesophagitis, hepatitis, pneumonia and encephalitis (102). HSV encephalitis is highly fatal despite aciklovir treatment, not only in immunocompromized patients. HSV positive recipients are on HSV prophylaxis with aciklovir which has reduced the risk of disease substantially (104). In case of CNS HSV infection MRI (magnetic resonance imaging) is recommended for imaging to visualise early HSV signs and for an early diagnosis. EEG is useful as 90% of the patients present with EEG changes in the early phase (101).

Cytomegalovirus

Reactivation of CMV and EBV are the most feared viral infections. After HSCT, the patients are screened continuously with CMV and EBV-PCR, for increased numbers of DNA copies of CMV and/or EBV in blood. CMV infection usually occurs early in life with a mild fever and rash, or entirely unnoticeable. At least 50% of the adult population is seropositive. Severe CMV disease occurs only in immunosuppressed patients and can involve the retina, liver, brain, gastrointestinal tract and lungs when reactivated after HSCT. CMV reactivation occurs in 30-70% (102) after HSCT if the donor/or recipient was CMV-positive prior to transplantation. Despite the high prevalence of reactivation, manifest CMV disease is fortunately only seen in 1-6% of patients (90). CMV PCR titre is followed every week until three months after HSCT and thereafter depending on risk factors for disease. CMV reactivation with a PCR of 2000 copies/ml shall be treated. This is called pre-emptive therapy, treating the reactivation before symptoms manifest themselves. The first choice of therapy is intravenous gangciklovir. Due to the potential severity of CMV disease, CMV status is a factor in the choice of donor. For the CMV-negative recipient a CMV-negative donor is preferred. If the patient is CMV positive a CMV positive donor is preferred (90). CMV CNS disease is a late onset disease (median time > 200 days post HSCT). It is rare but highly fatal, despite treatment. The afflicted patients have often undergone multiple pre-emptive CMV treatments, and gangciklovir resistant CMV is common (105).

Epstein-Barr Virus

EBV is known as the "kissing disease", often transmitted in adolescens giving the patient enlarged cervical lymph nodes and fever. The liver may also be mildly affected with elevated liver enzymes. About 60% of 9-12 year old children are seropositive

(106). The complication of importance related to EBV in HSCT patients is posttransplant lymfoproliferative disease (PTLD), a malignant EBV-driven proliferation of lymphoid tissue. EBV is followed by PCR every two weeks the first three months following HSCT in patients with increased risk for EBV reactivation/infection. The risk factors are: EBV serological mismatch between recipient and donor, HLA mismatch- one A, B or DR antigen, cord blood donor, lymphoma or congenital immunodeficiency as reason for HSCT (102). If an increasing number of EBV DNA copies are detected, a decreased immunosuppression can be considered. With further increased titers; rituximab treatment is recommended. Rituximab is an antibody towards CD20 positive lymphoid cells. Treatment with this antibody has shown a reduction of risk of PTLD (107). Rituximab is also the treatment for manifest PTLD disease where not many treatment options are available. EBV specific T lymphocytes, or DLI, can be used in life threatening cases (108). PTLD occurs in about 1% of HSCT patients and most commonly in the first 6 months after HSCT (95). CNS involvement of PTLD is rare but believed to lead to a poorer prognosis (109) . EBV, like CMV, affects the choice of donor. For an EBV-negative recipient an EBV-negative donor is chosen if possible. If an EBV-positive donor has to be used, T-cell depletion of the donor cells may be considered (110).

Varicellae-Zoster virus

VZV is the last herpes virus to reactivate after HSCT, at a median of five months after HSCT. Reactivation occurs in about 40% of the paediatric patients during the first 5 year period after HSCT (111, 112). Limited dermatomal zoster is the clinical presentation in the majority of cases, and disseminated cutaneous involvement in 20%. Visceral involvement is seen in 5-10% (113, 114).VZV can also cause meningitis, meningoencephalitis, myelitis and Guillaume Barrée, as well as paralysis of brain nerves. CNS involvement is feared but rare ($<$ 1% of VZV cases). Aciklovir is a well known treatment which in severe cases can be combined with foscarnet (101, 115-117). Post-herpetic neuralgia after the acute infection is common in adults but less frequent in children (112).

Human herpes virus 6

Another herpes viruses with importance in the HSCT setting is human herpes virus 6 (HHV-6). Primary infection with HHV-6 occurs in early childhood, presenting to the paediatrician as the familiar clinical picture of exantema subitum. Above 75% of the 6-

year olds are HHV-6 positive (118). As CMV and EBV, the HHV-6 stays latent in the body and may be reactivated during immunosuppression (119). Risk factors for reactivation are CB donors, an HLA mismatch between donor and recipient, as well as low anti-HHV-6 IgG titer in the recipient before HSCT. Reactivation of HHV-6 occurs early after HSCT (approximately after 3-4 weeks) and is believed to cause delayed engraftment and is associated to increased mortality (120). HHV-6 is the most common cause of encephalitis in HSCT patients. It has an early onset, within 100 days after HSCT, and high mortality (101).The treatment used is foscarnet or ganciklovir (17, 119, 121).

Polyomavirus

Polyomavirus (PyV) are neurotropic viruses and more specifically, human PyV (HPyV) are known to cause neurological complications in the immunosuppressed patient (122-127). Primary infection by the two first discovered HPyV, BKPyV and JCPyV (128, 129) give mild respiratory illness or no symptoms in immunocompetent individuals (130). Primary infection is followed by a lifelong latency in the kidneys but also in B-lymphocytes (131) and in the case of JCPyV, in the brain (132). The mean seroprevalence for BKPyV and JCPyV together is around 60% in people aged 60 or older. Reactivation of BKPyV is frequently observed in the context of HSCT and renal transplantation, both in urine and serum. In some allogenic HSCT patients BKPyV can induce haemhorragic cystitis (HC) (133) while in some renal transplant patients it can cause BKPyV-associated nephropathy (134). Reactivation of JCPyV in the urine is often seen in healthy individuals. In the context of immunosupression, such as HIV-infection, patients with lymphoproliferative disease, during transplantation or chemotherapy as well as in immunodeficiencies JCPyV can cause progressive multifocal leukoencephalopathy (124, 126). There are also a few reports of CNS disease due to BKPyV (122, 123, 125, 127) . KIPyV, WUPyV and MCPyV (Merkel cell PyV) are more recently discovered HPyV and data on whether these viruses are capable of infecting the CNS are scarce. So far their presence has not been identified in childhood brain tumours (135). However, in one study published in 2009, WUPyV was suggested to be linked to PML in a patient with HIV (136). KI- and WUPyV were originally found in nasopharyngeal aspirates (137, 138) and MCPyV in Merkel cell carcinoma (MCC) (139). The seroprevalence of KI and WUPyV is reported to be high already in childhood (140, 141) . Existing data suggests a mild initial infectious event upon primary infection, which is mediated by respiratory or faecal-oral transmission

during childhood (141). The presence of KI-, WU- and MCPyV has been studied in the respiratory tract, blood and lymphoid tissue of immunocompromised individuals, and to a lesser extent in their urine, CSF and faeces. Generally they are found more often in these patients than in healthy controls (142-147). In 2010 and in February 2011 discoveries of four new human PyV were published. These viruses; PyV 6, 7, 9 och TSV PyV were found exclusively on skin. Weather these viruses are of any importance for HSCT patients is yet unknown, but as they belong to the PyV family, neurotropic abilities and importance for immunocompromized patients must be studied in the coming years (148-150) .

Other viral infections

Viral contagious infections of importance are respiratory syncytial (RSV) -, adeno-, entero-, rhino-, parainfluensa B, metapneumonovirus and gastroenteritis - viruses.

More attention than previously has been given to the risk of adenoviral infection after HSCT as the infection becomes more common due to potent immunosuppressive therapy and the use of T cell depletion (151, 152) . Adenovirus infection in the immunocompetent host is most often a harmless upper respiratory tract infection, or a GI-infection. However, disseminated untreated adenovirus infection in immunosupressed patients has a mortality of 60% (151-153). Adenoviral meningoencephalitis is reported after HSCT in children (154). Generally, adenoviruses infect children between 6 months and 5 years of age, why paediatric HSCT patients are at high risk. During the last ten years, cidofovir has emerged as an effective treatment (155, 156).

Parvovirus is another "paediatric virus"- where the subtype B19 is known as the cause of exantema infectiosum or "fifth disease". Parvovirus infects mainly school-aged children and young adults, during seasonal "outbreaks". In elderly people, over 85% are parvovirus seropositivity (157). The virus can in patients with underlying haematological disorders, cause bone marrow depression and sudden onset of severe anemia (pure red cell aplasia) (158, 159). In HSCT – medicine the virus can be the cause of unexpected decrease in bone marrow function and/or slowed haematological recovery after the transplantation (160).

Respiratory syncytial virus is a seasonal respiratory infection, widespread in all children under 6 years of age. The infection can be severe for younger and premature children, but presents as a mild upper respiratory tract infection in most children. In HSCT patients up to 35% have been found infected. A RSV-lower respiratory tract infection can be life threatening. RSV has been shown to increase the mortality risk after HSCT by 60%. Ribavirin, orally or intravenously is the treatment at hand, although it is not always effective (161).

The recent epidemic of H1N1 influenza reached the HSCT-wards. H1N1 in HSCT patients presented with fever and respiratory symptoms. A third of the patients developed pneumonia and treatment in Intensive Care Units (ICU) was common (15%). The mortality is around 20% (162-165). Ozeltamavir is the treatment of choice and appears to decrease mortality if initiated early in the disease process. Seasonal influenza/Influenza B affects about 1% of the HSCT population. Parainfluensa viruses also contribute to morbidity and mortality after HSCT, although to a lower extent than RSV.

Looking specifically at CNS infections, toxoplasma and fungal infections are the most common CNS infections in patients with malignancies. The risk of CNS infections in the HSCT group is highest in the first three months after HSCT. Regarding viral infections; in a study of 2,628 patients, 32 had viral encephalitis (1.2%) and the most common agents were HHV-6, EBV, HSV, JCPyV and CMV, VZV as well as adenovirus. Bacterial CNS infections are most often caused by staphylococci-, gram negative bacteria or Listeria monocytogenes and later in time after HSCT; Cryptococcus. CNS infections affect around 2% of HSCT patients (92, 101)

Relapse after HSCT

Malignant relapse after HSCT is naturally the most feared outcome. Although, HSCT is not today, as earlier, the very last treatment to offer a patient with relapsing malignant disease. DLI can be given in an attempt to induce GVT effect. A second and even a third HSCT is sometimes done (58, 86). The outcome after HSCT has improved substantially over time, and thus the relapse risk has decreased. In the 1970's the relapse risk for ALL after HSCT was over 40% (166). Gooley et al reports that the risk of relapse or progression to malignant disease decreased by 21% comparing patients (of

all ages and mixed diagnoses) transplanted 1993-1997 to patients transplanted 2003-2007 (9). The relapse risk after HSCT for haematological malignancies is now around 30% in children (18, 167). The risk differs depending on leukaemia type and risk group. For children with a positive MRD before transplantation the prognosis is poor; a 50% risk of relapse and death within one year has been seen in this group (168). Most common are relapses in BM only; about 60% of relapses (169, 170). About 20% are combined extramedullary and BM relapses and 20% isolated extramedullary relapses. The most common sites for isolated extramedullary relapses are testes, cns, skin and the head and neck area. Relapse is rarely seen in lymph nodes, bones, kidneys or breast (171, 172).

The incidence of isolated CNS relapse after HSCT is difficult to estimate, as there are only a few published studies, which have all been done on small patient groups. Two studies published in the eighties (56, 173) reported that the overall risk of isolated CNS relapse for ALL patients after HSCT was between 11% and 13%. In patients with previous leukemic CNS involvement the risk was significantly higher (>25%) than in patients with no previous CNS involvement (5–7%) (56, 173, 174). A more recent larger study on CNS relapse after HSCT of adult HSCT patients with ALL and AML showed a 3.2% prevalence of relapse in the CNS relapse combined with other relapse after HSCT. ALL patients, patients with prior CNS-leukemia, patients with active disease at HSCT had an increased risk for combined CNS relapse. The risk of combined CNS relapse if the patient had prior CNS involvement of leukaemia was 21.3% and the risk without was 1.3%. Interestingly chemoprophylaxis against CNS relapse after HSCT was also a risk factor for combined CNS relapse. Isolated CNS relapse was seen in 0.9% of patients. In these patients ALL as diagnosis, the absence of HLA mismatch, use of CB or PCSB, prior CNS disease and intrathecal chemoprophylaxis after HSCT increased the risk for isolated CNS relapse (175).

To reduce the risk of CNS relapse of leukaemia, intrathecal chemotherapy is given after HSCT. The efficacy of intrathecal chemotherapy against CNS relapses has proven to be very effective in primary leukemia treatment. When introduced in the 1970s', it reduced the CNS relapse frequency with 50%, from 50% to 23% (176). Thompson et al. completed in 1986 the only study to clearly compare regimens with or without intrathecal methotrexate after HSCT (56). Treatment with intrathecal methotrexate had a protective effect against CNS relapse in ALL patients after HSCT. The effect was

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particularly strong in patients with previous CNS disease. There was no demonstrable effect of intrathecal methotrexate on CNS relapse in AML. The effect of intrathecal therapy on CNS relapse has not been confirmed in other larger studies and the intrathecal treatment itself has been associated with many neurological complications (173, 175, 177-179). Thompson et al and Oshima K 2008 noted in their studies a higher risk for leucoencephalopathy in the intrathecally-treated patients (56, 175). In a small study of six patients, symptoms ranging from mild headache to sacral radiopathy with irreversible cauda equina syndrome was seen in one patient and progression of a pre-existing leucoencephalopathy in another after intrathecal chemotherapy. The two patients with the most severe complications did have a previously known CNS diagnosis, a prior subarachnoidal haemorrhage and leucoencephalopathy respectively (180). Intrathecal cytarabine has proven to be safe without major complications in other studies (181, 182).

The treatment for CNS relapse after HSCT is individual depending on diagnosis and prior CNS treatment burden. The treatment often includes intrathecal chemotherapy, DLI and sometimes a third HSCT. The numbers are small and outcome differs between the case reports and studies published. The outcome was reported a "surprisingly good" in the study of Oshima K from 2008 with three patients out of seven with isolated CNS relapses who were alive and leukaemia free for over a year after CNS relapse (175).

Secondary malignancies

The data concerning secondary malignancies (post transplant malignancies, PTM) after HSCT is accumulating as follow up time increases with prolonged survival. Risk factors for PTM are previous radiotherapy, chemotherapy (especially alkylating agents) and cGVHD (16). Post-transplant MDS and AML are associated with the carcinogenic effect of previous chemotherapy. Several studies have shown that the risk of PTM is increased 5 times (all cancers) and 2- 3 times for solid tumours which gives a cumulative risk of about 3% after HSCT in children (95, 183-185) The risk seems to be inversely correlated to the age of the patient at HSCT (186, 187).The PTM can be divided in two major groups; haematological; AML/MDS diagnoses generally occurring during the first decade after HSCT (183).The main solid tumours seen are skin-cancer, oropharyngeal cancer, thyroid-, and breast- cancer (16).The risk is especially high for brain/CNS and thyroid cancer in young children. The appearance of

these tumours was often preceded by treatment with CNS irradiation (186, 188). The PTLD is a third group of malignant disease after HSCT, this was described previously under infectious complications, as it is an EBV driven disease. Survival after a solid secondary tumour and it's treatment is about 60% after 5 years (189).The survival after treatment related AML (t-AML)/MDS is poor with a median survival of 6-12 months (190).

Endocrine complications

The treatment included in the HSCT procedure can affect endocrine function in several ways. The previously frequently given single dose of TBI was associated with a higher risk than the present fractioned TBI- regimens (191, 192). A great concern for the patients is the almost 100% risk of gonadal dysfunction and infertility after HSCT. The risk is higher for women than men, and higher with busulphan- and TBI- containing regimens than with Cy alone. If HSCT is done before puberty the chance of restored gonadal function is better. Few men need hormone replacement therapy (HRT) while most females will require HRT both to induce puberty, to maintain menstrual cycles and to support bone mineralization/turnover. In the group of children with malignant diseases who are aggressively treated, fertility is as low as 3% in women and, at most, 20% in men. The option of freezing ovarian tissue prior to HSCT is developing and for men cryopreservation of sperm is a well known option which can increase the chance of conceiving biologically after HSCT (87). Growth failure is another common complication however it is likely of multifactorial origin. Younger age at HSCT, TBI and cranial radiation results in higher risk for growth retardation (95). The growth retardation seen is generally about -1 standard deviation (SD) in height compared to expected height without HSCT. One of the most common late HSCT complications is hypothyroidism (87). Patients treated with TBI are most at risk, with a 15% risk of TBI-related hypothyroidism, if fractioned TBI has been given. With single dose TBI, which was used earlier, the risk was as high as 50%. A busulphan/cyclophosphamide conditioning reduces the risk to 11% (95).

Neurological complications

The neurological complications seen within the first months after HSCT are related to the pancytopenic-state (infections due to neutropenia, bleedings due to thrombocytopenia) and to neurotoxic effects of some of the drugs given. Later the

sequelae from CNS infections and the irradiation damage to the central nervous system become apparent, often in the form of decreased cognitive functions. In an overview of available studies on neurological complications after HSCT in children (Table 1) we see that the most common CNS symptoms are seizures, followed by altered consciousness and headache. The underlying causes, excluding relapse of malignant disease in CNS, are infections, drug toxicity (cyklosporin causing most complications), cerebro vascular disease (CVD) and metabolic encephalopathy. There are also a number of incidences where the underlying cause is unknown. Peripheral neurological complications are rarely reported. Polymyositis/myopathy related to GVHD is, although rare, the one most commonly described. Only one study reports a high incidence (14 %) of peripheral neurological symptoms, where infections caused several cases of cranial nerve palsy (174) (Table 1).

Six of the studies examining risk factors generally agree on an increased risk for neurological complications in transplantations where an unrelated donor is used, cyklosporin is used as GVHD prophylaxis or where the recipient suffers from GVHD > gr 2 (Table 1). In other studies the risk of neurological complications after HSCT is shown to increase when both CNS irradiation and intrathecal therapy is given before HSCT (56). It is probable that intrathecal therapy given after HSCT adds to that risk. Five of the listed studies confirm the increased mortality risk for patients having suffered from neurological complications (Table 1).

Drug-induced neurotoxicity is one of the major causes of neurological complications after HSCT. Busulfan, cytarabine and etoposide are drugs given in the conditioning treatment that can cause seizures, encephalopathy and confusion. Ciklosporin – very commonly used as GVHD prophylaxis- can cause neurologic toxicity in up to 28% of recipients (193). Some of the antibiotics and other supportive drugs used can also cause complications involving the CNS; high dose corticosteroids are known to cause severe mood swings, cephalosporins can cause seizures, while there's a risk of developing parkinsonism and in rare cases progressive leukoencephalopaty, with the anti-fungal drug amphotericin B (194).The drug related complications are most often immediately reversible with reduction of the drug dose. However, the myopathy which can be seen as a peripheral neurological/muscular complication from high doses of steroids, can take months to reverse after cessation of steroids.

Cerebrovascular disease after HSCT includes cerebral haemhorrages, thrombosis and vasculitis. Among cerebral haemhorrages, intrachranial and subarachnoidal haemhorrages are most common, as well as associated with the worst prognosis(195, 196). Factors increasing the risk of intrachranial haemhorrage (ICH) are systemic infection, low platelets, GVHD and VOD. Known vascular risk factors as high blood pressure, diabetes mellitus and thrombocytopenia contribute to the risk of cerebrovascular disease after HSCT. Subdural haemhorrages occur less frequently after HSCT (88, 196, 197).

Infection is the third of the major causes, responsible for up to 25% of neurological complications after HSCT (15, 18, 174). CNS infections are though rather rare. Viral encephalitis, with a median onset time of three months after HSCT, occur in about 1% of patients with a 1-yr survival of 55% (101). Late CNS infections occur in about 7% of the patients and foremost in the patients on continuous treatment with immunosuppressive cGVHD treatment. See above; Infectious complications.

Metabolic disturbances involving the CNS, often called metabolic encephalitis, may be caused by renal failure, electrolyte imbalance, hypoxia or infections (119). One large study of 405 paediatric patients found that 6.4% of the patients had encephalopathy after HSCT (198). The incidence is though difficult to assess, due to the poor characterisation of this diagnosis. The EEG changes are most often a diffuse slowing of the EEG pattern, on MRI cerebral atrophy and focal lesions can be seen. In over half of the encephalopathy patients in the above mentioned study, there were leukoencephalopathy changes visible on the MRI. Often no distinct cause is found. In these patients, a combination of many risk factors may be responsible.

Leucoencephalopathy is a feared complication most commonly seen in heavily CNS-treated patients. CNS irradiation and intrathecal chemotherapy combined, results in a high risk of leucoencephalopathy. Thompson et al found in 1986 a prevalence of 7 % of leucoencephalopaty in a group of patients that received CNS-therapy both before and after HSCT. Leucoencephalopathy was not seen in any of the patients who only had CNS-therapy prior to, or after HSCT. In a study from 2007, six of 138 paediatric HSCT patients developed leucoencephalopaty and two of the six patients died during follow-up. The cause of leucoencephalopaty is unkown, but all patients in this study were receiving cyklosporin, which can be a contributing factor. JCPyV is believed to be one

of the causative agents involved in the development of leucoencephalopathy, which has been described earlier (132).

Posterior reversible encephalopathy syndrome (PRES) is a syndrome defined by clinical and radiological findings. These include headache, altered consciousness, visual disturbances, seizures and predominantly subcortical white matter imaging changes, posteriorly or in the parieto-occipital area. The changes can also include grey matter areas. The radiological findings are bilateral, showing low attenuation of the posterior and occipital lobes on CT scans. On magnetic resonance imaging (MRI) they are pathognomonic with hyperintensity in T 2 weighted images and typical lesions on FLAIR (fluid attenuation inversion recovery) images. PRES can be described as cerebral oedema and microinfactions, caused by hypertension (more commonly in adults than in children) or renal insufficiency together with chemotherapy and/or immunosuppression and other not yet described factors. PRES is despite its name not always reversible. Immediate treatment including withdrawal of suspected causative agents, aggressive blood pressure treatment and anticonvulsants is essential to avoid progression and death. PRES has been reported in 56 paediatric patients undergoing cancer treatment (199) but PRES is likely to be the true diagnose in many patients where the neurological complications after HSCT are diagnosed as "seizures of unknown cause" or "encephalopathy" without further specification. The diagnosis was first recognised in 1996, after the introduction of MRI into wider clinical practice.

Long-term neurological consequences are not sufficiently studied. In Clarke 2010, a long-term follow up study of paediatric patients after HSCT the only long-term neurological sequelae listed were visual disturbances (200). The same study showed lower health related quality of life (QOL) score compared to non-transplanted paediatric cancer survivors. A Swedish study from 2005 did on the contrary not see a lower QOL score in HSCT patients compared to the norm (201). That study revealed though that the HSCT survivors had a higher risk of pain related problems. Long-term impact on parameters as IQ, achievement, memory and fine motor functioning are seen in some studies (202) especially in patients with previous cranial irradiation, TBI and in those patients who were very young at the time of CNS-directed treatment (13, 14, 203, 204). There is data supporting a decline of cognitive function after HSCT in patients that have received CNS irradiation. Patients who have not undergone CNS irradiation may have a decline in cognitive functioning at one year

after HSCT, but functioning has often improved at three year follow up controls (205).

Late peripheral neurological complications are not common. Polyneuropathy of Guillaume-Barré type as well as chronic demylenating polyneuropaty are considered to be caused by GVHD and/or the neurotoxic effect of calcineurin inhibitors and other drugs. Pain and muscle weakness is more common in HSCT long-term survivors than others (64, 200). Polyneuropathy causing weakness, muscle spasm, muscle cramps and similar peripheral symptoms are a manifestation of cGVHD (81).

PRIMUS NOV 2009

AIMS OF THE THESIS

The overall aim of the thesis was to identify neurological complications after paediatric HSCT and their possible causes and thereby, in the future prevent this type of complications by different interventions.

Paper I: To study the incidence of, and contributing factors to acute neurological complications after HSCT in children.

Paper II: To study effect and complications of intrathecal chemoprophylaxis against isolated CNS relapse of leukaemia in children after HSCT.

Paper III: To study effect and complications of intrathecal chemoprophylaxis against isolated CNS relapse of leukaemia in children after HSCT with a larger material than in the previous study, in order to form recommendations regarding intrathecal chemoprophylaxis.

Paper IV: To study whether BK-, JC-, KI-, WU and MCPyV DNA was detectable in CSF from immunocompromised patients with neurological complications after HSCT in order to learn more about the fairly newly discovered polyomaviruses KI-, WU and MC and their role after HSCT. We also wanted to find out whether some of the unexplained neurological complications after HSCT can be explained by polyomavirus reactivation/infection in the CNS.

PATIENTS, MATERIAL AND METHODS

PATIENTS AND MATERIAL

Patients and material, Paper I

One hundred and forty four paediatric (< 18 yrs of age) patients who underwent HSCT at the Karolinska University hospital (Huddinge) between 1995 and 2002 were included in the study. The patient material reflected the mixed group of patients who are eligible for paediatric HSCT with varying preceding treatments, donor types, conditioning regimen and background disorders (malignant; 108 patients, and benign; 36 patients). Age-median was 8.9 years. The background factors studied were: age at transplantation, sex, pre-HSCT herpes virus serologies for CMV, EBV, HSV, VZV, transplantation related diagnosis and it's treatment, other diagnoses, previous neurological diagnosis/symptom, previous treatment for epilepsy and exposure to pre-HSCT irradiation. The transplant related factors registered were: conditioning regimen and whether this included TBI and/or etoposide, type of donor, type of GVHD prophylaxis (methotrexate or not) and presence of GVHD. In the search for factors contributing to neurological complications blood pressure level, the level of creatinine, haemoglobin, bilirubin, platelets, leukocytes and electrolytes (potassium, sodium, magnesium, calcium) at admittance and the number of aberrant (high or low, compared to age-adjusted reference values) values of these parameters throughout the study period, the first three months period after HSCT were registered. The number of ciklosporin concentrations > 250 ng/L throughout the three months period and weight at admittance and lowest and highest weight during the three months period were also registered. If a neurological complication occurred the symptoms and the results of all investigations done to determine the cause (EEG, neuroradiology, blood and CSF cultures) the vital parameters and the laboratory results on the day of the complication were registered. Death and cause of death and study time for each patient were also registered. The data was collected from original paper- and computerized patient chart as well as microfilms in a de-identified database.

Patients and material, Paper II and III

A total of 120 paediatric HSCT patients transplanted either at the Karolinska University Hospital or Uppsala Academic Children's Hospital between 1992 and 2005 were included in the study for paper II. Eligible for intrathecal

chemoprophylaxis and therefore for inclusion in the study, regardless of whether they had received intrathecal prophylaxis or not, were all ALL patients, all AML M4 and AML M5 patients as well as the AML patients with AML M0-M3 with high risk factors such as a very high presenting leukocyte count or another high risk factor such as extra-medullar involvement or slow response to primary treatment. Patients who died or relapsed with malignant disease before three months had passed after HSCT were excluded since the effect of the intrathecal treatment could be evaluated three months after HSCT, the earliest. In Paper III the study was enlarged to include patients transplanted between 1992 and 2006. The patients' charts from Study II were re-reviewed as new parameters were included (specification high risk factors for ALL and AML) in study III and patients from the two remaining Swedish paediatric HSCT centres in Gothenburg and Lund were added as well as patients transplanted during the same period in Helsinki, Finland and Utrecht in The Netherlands. In total 397 patients were included. The data was collected from original paper- computerized patient charts and microfilm. Information about background factors, disease and HSCT procedure was recorded (see above, Paper I regarding the background and transplantation related parameters recorded) as well as outcome parameters such as relapse, type of relapse, time of relapse, neurological complications and death. The information collected regarding intrathecal chemotherapy was; the number of both pre- and post HSCT intrathecal chemotherapy injections, type of drug given in the post-HSCT injections, and at what day after HSCT the intrathecal therapy was started. All data was collected in a de-identified database. Data regarding HSCT complications with focus on neurological and cognitive complications, relapse in the CNS, relapse of any type and death after HSCT was also collected. Information from Gothenburg, Lund, Uppsala and the Helsinki and Utrecht centres were collected at the respective site by the co-authors on site after which it was gathered and analyzed in Stockholm.

The standard intrathecal treatment was six injections starting one month post HSCT and then given every two weeks. Patients with previous CNS leukaemia had a prolonged treatment regimen of 18 months where intrathecal injections were given every eight weeks. The drugs used were most commonly cytarabine and methotrexate. Cytarabine was most often used as the standard intrathecal drug but in patients presenting with GVHD, methotrexate was preferred. For some patients with an individual sensitivity to, or side-effects from cytarabine, methotrexate was used

and vice versa. This resulted in a mixed treatment course where both drugs were given in 23% of the patients in Paper II and 30% of the patients in Paper III. The average number of intrathecal injections was six in both studies and all of the patients with any kind of post-HSCT intrathecal regimen were analyzed together as one group, the "intrathecal group".

Patients and material, Paper IV

To retrospectively analyze the presence of polyomavirus DNA in immunosuppressed patients with neurological complications during the first year after HSCT, CSF samples from patients transplanted between the years 2000-2008 was collected form storage at the Department for Virology, Clinical Microbiology, Karolinska University Hospital Huddinge. During the study period 598 patients had undergone 635 allogeneic haematopoietic stem cell transplantations at the Centre for Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital Huddinge. CSF was available in sufficient amount for analyses from 20 of 46 patients where a lumbar puncture (LP) had been performed, and viral analyses ordered during the first year post HSCT. A bio-bank was set up for storage of the selected samples according to the rules of the Karolinska Institute and the Karolinska University Hospital. The LP's had been performed between five and 313 days post HSCT (median time 68 days). The CSF was in the clinical setting immediately analyzed depending on clinical suspicion and thereafter the leftover CSF was stored at -20 ^{0}C. The 20 patients in whom we were able to perform analysis were of all ages (1 to 60 years old, mean 31 years old) with five children < 18 in the group. Seventeen were transplanted due to a malignant disease and the remaining three were transplanted due to sickle cell disease, thalassemia and FHL. Six patients received a reduced conditioning regimen. Data regarding the diagnosis, conditioning, neurological symptoms and the outcome for these patients was collected from computerized charts and registered in a de-identified database together with the results of the virological analyses.

METHODS

All the studies had a retrospective approach; hence background data as well as data concerning risk factors and outcome were collected retrospectively from patients' charts. Data were registered in Exel ® files. Statistics were computed with the statistical software Statistica ®. The level of statistical significance was set at p< 0,05 in all studies.

Methods, Paper I

In Paper I background factors, the treatment of and the outcome for patients with acute neurological complications occurring within the first three months after HSCT (n=19) were compared with the same parameters for patients without neurological complications in the first three months (n=125). Blood pressure, electrolytes, ciklosporin concentration levels, creatinine and bilirubin levels were collected from daily/weekly registrations for all patients for the first three months after HSCT. Blood pressure, and creatinine above standard age-adjusted reference values was registered as high, for ciklosporin > 250 ng/L was registered as high and electrolytes were registered as "high" or "low" depending on their accordance with standard reference values. Bilirubin was "high" if above 20 μmlol/L of bilirubin. Haemoglobin was registered as low below 80 g/L, platelets < 20 x 10(9) /L and low leukocytes was defined as poly < 0.5x10(9)/L. The patients' baseline viral serology status on herpes viruses; CMV, EBV, HSV and VZV, which are routinely registered in HSCT-care were also documented and analysed as potential risk factors. The neurological complications were categorized as belonging to either of the following groups of symptoms; altered consciousness, seizures, headache/nuchal rigidity or paresis and to belong to one of the following diagnosis groups; encephalopathy, seizure, infection, meningitis or CVD. The follow up period was from transplantation to death or lost to follow-up four years (mean). Statistical analyses were performed with the chi-squared test, and if the numbers were small, with Fischers' exact test. We used the Wiklcoxon matched pairs test in order to analyze the aberrations in laboratory parameters before and after the neurological complication. The Mann Whitney U test was applied to compare the number of positive herpes viralserologies in donors and recipients in the two groups, i.e. the group with neurological complications and the group without. A logistic regression model was applied to analyze the risk for a neurological complication considering the serological status of CMV and HSV infection, in the recipient and donor respectively. A Kaplan Meier survival analysis was performed.

Methods, Paper II and III

In Paper II and III the outcome for patients receiving intrathecal chemoprophylaxis post HSCT, was compared to patients who did not receive this treatment. In paper II there were 74 patients receiving intratheal therapy vs 46 patients not given intrathecal therapy and in paper III; 136 vs 261 respectively. In both studies there were a

difference, (although not statistically significant in Study II) in the background data between the proportion of children who had received pre-HSCT CNS irradiation with a larger proportion in the group treated with intrathecal therapy. The groups were comparable in all other aspects considered. The primary end-point was isolated CNS relapse, but also mortality and relapse overall as well as parameters to detect neurological complications secondary to the intrathecal treatment were registered and analysed. A neurological complication was defined as one of the following conditions: seizures, cerebrovascular disease, peripheral neuropathy, altered consciousness, visual disturbances, serious/repeated headaches or cognitive difficulties. These were then classified as either cognitive or non-cognitive in nature. Two additional parameters were collected as measures of estimated cognitive function in the patients; the grade the in school the patient was in, in relation to his/her age and an activities of daily living – score (ADL-score) at one year after HSCT and repeated at the end of follow up. For the ADL –score the Lansky performance scale was used for the patients < 16 years of age and the Karnofsky performance score was used for patients > 16 years of age (206, 207) . The study period for Paper II was 1st of January 1992 to June 30th 2007 (mean follow-up time four years) and for Paper III 1st of January 1992 to 31 aug 2008 (mean follow-up time 4,2 years).

Statistical considerations: The study would require 1500 subjects per group to detect a difference of 1% in CNS relapse after HSCT between the groups, as the outcome is very rare. It is estimated to be 2-5% without previous CNS involvement and 11-27% with prior CNS involvement. However, the study size was determined by the number of patients who were available for inclusion. With 397 patients included in study III, the power of the results to detect an absolute difference in outcome of 5%, between the groups studied, is 80%. Study II had a yet more insufficient number but was done, with this in mind, as a pilot study with available patients at the two participating centres.

Methods, Paper IV
The CSF samples of the 20 patients were analysed for the presence of PyV DNA. Data regarding background factors, disease, conditioning treatment, GVHD prophylaxis and clinically diagnosed PyV infections were collected from patients' charts and registered in a data base. The registry also included other data on other viral infections and their treatment as well as data regarding the neurological symptoms at the time of LP and data on the outcome of the HSCT.

The BK- and JCPyV were analysed with a nested PCR which detects both BK- and JCPyV. This PCR can detect approximately 10 genomic copies of BKPyV plasmid DNA and 5-10 copies plasmid JCPyV DNA (208-210). The CSFs (10 µl) were heated at 94°C for 9 min for denaturation before added to the PCR mix as previously done by Bogdanovic et al (210). The KI and WU PyV were also analysed with a PCR which detects both viruses. This PCR can detect around 10 copies of a KI PyV VP1 gene containing plasmid (133). Similarly, the CSFs (4 µl) were heated at 94°C for 9 min for denaturation before added to the PCR mix (210).The MCPyV was analysed with a new PCR assay. The 4 µl CSF sample had a 9 min denaturation period and was then added to the PCR mix for 40 cycles of 30 sec at 95°C, 30 sec at 53°C and 45 sec at 72 °C. DNA from a MCPyV positive MCC was used as positive control. The primers, 137-MCPyV573.F and 138-MCPyV739.R generating an amplicon of 177 bp from the early part of LT: 137-MCPyV573.F; GTCTCGCCAGCATTGTAGTCT and 138-MCPyV739.R; GCAGTAAGCAGTAGTCAGTTTC. This PCR assay had a detection limit of 10 MVPyV genomes.

ETHICAL PERMISSIONS AND CONSIDERATIONS

The research group has had the standpoint that information about new studies and especially those who might question treatment already given might cause distress to patients who have already undergone a difficult treatment and especially cause distress in those families where the patient has deceased. This has to be weighed against the individual patient's right to privacy and the right to decide whether his/her hospital charts may be scrutinized for research. We applied therefore in a similar manner to the Board of Ethical permissions concerning the patient information and consent regarding all the four studies. In Paper I and II we were excused from asking families of deceased patients, and patients of unknown fate and of current address living abroad, about participation. The willingness to participate in the studies among the patients asked about participation was high; 144/147 and 120/122 respectively. Study III was an extension of study II and on the basis of the results from Paper II, study III was regarded as a follow up study of clinical results and no extra consents needed to be sought from the patients of the children transplanted in Stockholm or Uppsala. For the children transplanted in Gothenburg, Malmö, Utrecht and Helsinki a general consents for participation in research studies were standard routine at each of these centres. In study IV ethical permission for the study did not dictate that information about or

consent for the study had to be communicated to/attained from the participants. In Sweden all patients have to be asked consent for storage and further use of biological material from clinical procedures. None of the eligible patients in the study had refused such use of their samples.

RESULTS

Results, Paper I

The study included 144 paediatric patients with varying diagnoses and varying treatment burden before HSCT. Nineteen patients (13%) developed neurological complications during the first three months. The background factors of statistically significant importance for the development of acute neurological complications were the number of positive baseline serologies for herpes viruses (CMV, EBV, VZV, HSV) in the recipient and also CMV-positivity alone in the recipient (pre-HSCT was associated with an increased risk). The post-transplant parameters of importance for the development of neurological complications were high or low potassium and sodium, as well as low calcium (registered as "high" = above or "low" = below standard reference values). High levels of bilirubin (>20 Umlol/L) and blood pressure above standard age-adjusted reference values were also associated with an increased risk. The symptoms of the neurological complications found were seizures (n=10), altered consciousness (n=5), headache with nuchal rigidity (n=3) and paresis (n=1). The incidents were diagnosed as infectious (n=7), encephalopathy (n=7) and CVS (n=3). In two cases the underlying cause of the neurological symptom (seizures in two patients) was unknown and the exact cause of the encephalopathy was uncertain in several cases. The encephalopathy diagnosis was given if the patient had symptoms and EEG and/or neuroimaging results indicating encephalitis/encephalopaty but no other exact cause could be found. All the seven patients with encephalopathy had EEG changes indicating encephalitis. Only one of the seven patients had CT/MRscan pathology, in the remaining sex patients the neuroimaging result were normal (5 patients) or missing/not performed (1 patient). The mortality risk for the patients suffering from acute neurological complications was significantly higher than for the group without neurological complications.

Results, Paper II and III

In study II the group treated with intrathecal therapy consisted of 74 patients; 56 ALL patients and 18 AML patients, with a mean age at HSCT of 9.7 yrs (range 0.5 to 18 yrs). The group not given intrathecal therapy comprised 46 children, 36 children with ALL and ten children with AML, with a mean age of 9.2 yrs (range 1-18 yrs). Fifteen percent of the patients given intrathecal therapy had previous CNS leukaemia, compared to twenty percent in the other study group. The difference in CNS

leukaemia before the procedure was not significant. A similar proportion of the patients in the two groups were in bone marrow complete remission (CR), with < 5 % blasts, before HSCT. In study II, one patient in each group was identified with primary CNS-relapses during the transplant follow up.

In study III the group given intrathecal treatment consisted of 136 patients; 107 ALL patients and 29 AML patients, with a mean age at transplantation of 9.2 yrs. The group without intrathecal treatment comprised 261 children, 166 with ALL, 89 children with AML and six children with AUL. The mean age was 8.7 years. Twenty-three percent of the patients in the group receiving intrathecal therapy had CNS leukaemia before HSCT, compared to 13% in the other study group. The difference, although noteworthy, was not statistically significant. Patients in complete bone marrow remission (CR), with < 5 % blasts, before HSCT was equally distributed between the groups. Isolated CNS relapses were observed in four patients; two (1.5%) patients from the group given intrathecal prophylaxis and two (1%) from the group without not intrathecal treatment.

In study II, the patients with CNS-relapses had high risk pre-B ALL (intrathecal therapy group) and AML M1"high risk" (presenting with a high leukocyte count and extramedullar, mastoid, relapse before HSCT) respectively. None of the patients had CNS involvement of their leukaemia before the HSCT treatment. Both patients were given TBI in their conditioning regimen. The pre B-ALL patient received six intrathecal injections after HSCT, but despite this, he developed an isolated CNS relapse nine months after transplantation. This patient had also been given CNS radiotherapy according to his leukaemia treatment program before HSCT. The AML patient suffered a relapse in the CNS within a year of transplantation. Similarly, none of the four patients who suffered CNS relapse in study III, had CNS leukaemia before HSCT. Three patients had pre-B-ALL HR and one pre-B-ALL IR. Two of the patients were given CNS radiotherapy and one received TBI, in the conditioning regimen. The time to CNS relapse was 9, 27, 38 and 29 months (mean 26 months for all the patients; mean for the intrathecally treated patients group: 18 months, mean for the group: 34 months). For one patient in the group not given intrathecal chemotherapy after HSCT, the isolated CNS relapse was his/her third relapse after HSCT.

With one case reported in each group in study II, and two cases in each group in study III, neither of the studies showed a statistically significant difference in the incidence of CNS-relapse between the groups (p> 0.05). Nor did intrathecal therapy have any influence on other types of relapses or survival. The time to CNS –relapse as not affected by intrathecal therapy.

As intrathecal therapy is often recommended to patients with a CNS involvement of leukemic disease before HSCT (even at centres where it is not given to any other patients), the outcome in this subgroup is interesting. Study III included 67 patients with CNS involvement before HSCT. Thirty-three of these patients received intrathecal therapy after HSCT and 34 patients did not. There were no isolated CNS relapses among these patients why we were unable to compare the groups regarding this outcome. However it is interesting to notice that despite the absence of intrathecal treatment none of these 34 patients had an isolated CNS relapse during follow up. When we studied differences in the incidence of overall relapse and death there were no differences between the groups.

When assessing neurological complications between the group who was given intreathecal therapy after HSCT, and the group that did not receive this treatment, we found an equal proportion of neurological complications. In study II, 20% (15 patients) versus 15% (7 patients) developed neurological complications. In study III, 11% versus 10% of children had neurological complications during follow up. Both studies also made the attempt to detect more subtle sequelae of cognitive type after HSCT. These were registered as neurological complications of "cognitive type", a parameter including data on whether the patient was in a school class corresponding to his/her age as well as an assessment of ADL score. "Cognitive complications" were registered in very few cases; 4 of the 15 patients who had neurological complications in the intrathecally treated group in study II. In the untreated group 1/7 patients had these complications. In study III the corresponding numbers were 7/14 in the treated group and 8/25 in the untreated group. In both studies the majority of the children attended a school class that corresponded to their age both at one year after HSCT and at the end of follow-up. There were no differences between the two groups. The Lansky/ Karnofsky scores for the surviving children were high in both studies with the lowest mean figure of 88% at one year after HSCT in the intrathecally treated group of study II. In the other patient groups the scores were

above 90% in study II and III. None of these three parameters indicated any difference in cognitive function between the study groups in these two studies. Furthermore, there was no evidence that intrathecal therapy caused an earlier onset of complications or that the advent of potential complications was delayed by using intrathecal-therapy.

Results, Paper IV

The symptoms and causes of neurological complications in the 20 patients studied were in accordance with other studies; headache and seizures were common (18, 211) and drug toxicity, suspected or verified, was a frequent cause of neurological complications (three suspected drug reactions, and one verified). There were eleven patients (55%) were no definite cause was documented regarding the neurological symptoms exhibited. Headache with or without fever was the most frequent neurological symptom documented (35%), followed by seizures (20%) and confusion/hallucination (20%). In four patients, JCV was analyzed in the clinical setting in the CSF drawn, and in one patient JCPyV was analyzed in blood at the time of neurological complications. All of these tests were negative. In the remaining fifteen patients none of the known PyV's were found at the time of neurological complications. In three patients, BKPyV was analysed at another time point, after HSCT due to symptoms of HC. One of the patients had a positive blood sample and was treated with cidofovir. All the 20 CSF samples, analyzed retrospectively with PCR, were negative for BK- , JC-, KI-, WU och MC PyV DNA. One sample could not be processed for JC/BK virus DNA, as the CSF was cloudy and the liquid part of the sample evaporated during the denaturation phase. Nine of the 20 patients survived until the end of study period with a mean follow up time of 3,4 years. This confirms the reduced overall survival after HSCT for patients with neurological complications, as seen in other studies (18).The neurological complications were in two cases related to the cause of death, where the neurological complication presented as a symptom of leukemic relapse, and cranial herniation respectively.

DISCUSSION

Nineteen patients, 13% of the 144 patients in Paper I, suffered from neurological complications during the first three months after HSCT. This figure was expected as previous studies have shown that neurological complications are common. Since the group studied represented several diagnoses and underlying conditions it reflected the composition of the patient group at a paediatric transplant centre well. The factors that were found to be associated with neurological complications may not increase the risk for neurological complications one by one, yet we need to be attentive to all these potential risk factors. When coinciding they may be responsible for an elevated risk of neurological complications. Electrolyte disturbances and elevated blood pressure are known risk factors associated with neurological complications. This was confirmed in our study. The results underline the importance of close monitoring of all vital parameters to protect the patients from potentially severe neurological complications. Other studies have found GVHD and the use of cyklosporin to be risk factors for neurological complications (16, 193). This was not supported by our study. As cyklosporin is a well known risk factor, it may very well have been a contributing factor to the neurological complications seen in our study. It can be argued that our definition of a high concentration (> 250 ng/L) was set too high. We should consider that it is not necessarily the high peaks in concentration that create the elevated risks of neurological complications. There could indeed be other factors of cyklosporin use, as exposure to near toxic doses during a longer time of use, that are responsible for these findings. The definition of a high bilirubin level used in the study; 20uM/L, is very close to the normal reference value, and might seem low. We chose this level not knowing what to expect, as this parameter had not been studied in relation to neurological complications before. The bilirubin level increases with liver failure, for example due to veno-occlusive disease (VOD). Our interest in studying bilirubin-levels relates to the possible link between neurological complications and severe complications, such as VOD and GVHD. It has been seen that symptoms from the CNS can be the first symptom of multi-organ failure (212, 213). Neither low haemoglobin, nor low platelet levels were risk factors in our study, even though CVD was among the complications seen. This may be explained by the fact that low haemoglobin and platelet levels are rapidly treatable by transfusion and are not allowed to be abnormal in the clinical setting for long. Electrolytes on the other hand, can be more difficult to correct, with intravenous nutrition or orally administered substitution. Increased risk

was seen with a rising number of positive viral serologies of the four herpes viruses tested before HSCT. This fact that several positive herpes virus serologies combined, pose an increased risk for neurological complications was a previously never reported finding. However, the mechanism behind it is probably not surprising; positive herpes simplex serology requires aciklovir prophylaxis, and reactivation of viruses calls for yet more anti viral treatment, which in turn likely contributes to complications.

In seven patients no other diagnosis or cause of the symptoms than "encephalopathy" could be determined, neither in the clinical setting nor in the retrospective study of the charts. Neuro-imaging was done in six of the seven patients but only abnormal in one patient. As described above; some diagnoses have very distinct changes on MRI of the brain. Choosing MRI over CT and/or repeated neuroradiological examination might have facilitated the search for a more precise diagnosis.

In half of the patients who died in the first 90 days in the neurological complications' group, the neurological complications were the cause of death (3/6 cases). In two of the three remaining cases the neurological complication was CVD, where the direct cause of death was multiorgan failure, which could in large part be due to the CVD. This lead to the major finding of the study, one that is confirmed in several other studies as well: that there is an increased mortality risk for patients with neurological complications. This result should motivate further efforts to reduce neurological complications in paediatric patients after HSCT treatment.

Intrathecal chemotherapy is a treatment which has proved to be essential for primary treatment of leukemia in children, largely reducing the CNS relapse rate. Rapid progress is made in the field of paediatric oncology and over the last ten years survival has increased from 80% to 90% for paediatric ALL (214, 215) Despite this progress there is a risk of CNS relapse after HSCT which cannot be disregarded, especially in children with previous CNS leukemia. The risk for CNS relapse in these children is 17-36% compared to 2-10% in patients without previous CNS leukemia (2-10% in ALL and 2-8.8% in AML patients (216, 217)). In the 80s' Thompson et al showed the beneficial effect of intrathecal therapy after HSCT (56). Post-HSCT intrathecal therapy has subsequently been used frequently, although irregularly, among HSCT centers in Europe (57). In study II and III we wanted to examine the effect of intratheal therapy after HSCT in patients with modern primary leukemic

treatment. The median number of intrathecal injections given after HSCT at the centres in the study giving this treatment was six injections. This represents a notable increase in exposure to the risks of intrathecal chemotherapy when considering the number if injections given in standard NOPHO and other treatment protocols for primary leukemia. The NOPHO protocols contain six intrathecal-injections for children diagnosed with AML and 16-18 injections for children diagnosed with ALL, in primary treatment (22, 218). Our results showed that six intrathecal injections do not appear to have the intended protective effect against leukemic relapse in the CNS after HSCT. The same year as the publication of paper II, another study was published which addressed the same question, and found likewise, that intrathecal chemotherapy after HSCT did not have a protective effect against CNS-relapse. In the study, written by Oshima et al, the average number of post-HSCT intrathecal injections was though only two which may have decreased a potential effect of the treatment (175).

Our study material came from six different transplantation centres in Europe where different treatment schedules were used, both for leukemia and for the HSCT procedure. In examining the differences we concluded that they were not large enough to result in bias in the study. Background factors among the patients receiving and not receiving intrathecal prophylaxis were evaluated and found comparable. The proportion of patients not in complete remission at HSCT, a factor suggested to be a risk factor for relapse, was similar in both groups.

In the intrathecally treated group a higher proportion of patients received CNS irradiation before HSCT. This difference was significant in study III. This could lead to a potential bias with more heavily treated patients in the intrathecal group which would falsely strengthen the "effect" of intrathecal therapy. However no effect of intrathecal therapy was seen and the risk of bias is thus not relevant for the primary end point. The excess complications after intrathecal administration might be higher in that group, not due to intrathecal therapy but due to previous CNS irradiation.

One interesting subgroup in the studies was of course the patients with CNS leukemia before HSCT, as several small studies recommend intrathecal prophylaxis to this group. This recommendation is based on case reports and clinical experience (58, 59, 173, 175, 178). In our study III 67 patients had CNS leukemia before HSCT and 34 of them

did not receive intrathecal chemotherapy. The study sample was unfortunately too small to analyze the implications of intrathecal chemotherapy in this group.The fact remains though that there were no isolated CNS relapses in the patients with previous CNS disease seen during follow up neither for them with intrathecal therapy nor for the patients without intrathecal therapy.

The advantages of not giving intrathecal therapy after HSCT are many, both practical and psychological. Lumbar punctures cause pain and anxiety in a majority of patients, why it should be performed under general anesthesia. When given, the intrathecal injection involves the risk of bleeding into the CSF a with a risk of transmitting leukemis cells into the CSF well as a risk for CNS infection and subdural haematoma.. (219, 220). A study by Menesis *et al* showed that general anesthesia for short duration during painful procedures in children undergoing treatment for malignancies, is safe when carried out by trained professionals in outpatient clinical surgery units (221). Children who have undergone a stem cell transplantation generally have a longer history of chemotherapy and having gone through a heavy conditioning therapy, they are more vulnerable to both infections and toxicity, than the average pediatric oncology patient. Apart from the acute complications of injection, the long-term sequeale of intrathecal methotrexate are well-documented. A higher risk for leukoencephalopathy has been seen when intrathecal methotrexate is given after HSCT (56).Cytarabine, currently the most common drug used, is not considered to be associated with as many complications as methotrexate (182).However, all the injection-related risks still apply.

In our studies, we retrospectively tried to detect cognitive complications as well as other neurological sequelae, through studying the patients' charts. The methods used might seem unspecific; however, we concluded that the report of a child attending a school class without support and in his/hers correct age group is a relevant parameter when estimating cognitive function. The Lansky and Karnofsky scores that were used are well recognized. The medical chart in combination with a full medical exam was a good basis for assessing a Lansky/Karnofsky score. Although it might not be fully applicable to the assessment of neurological sequelae, a patient seriously affected by neurological sequelae, would be recognizable (206, 207) Studying these parameters, we were not able to identify any difference in the cognitive outcome between the two groups, despite the fact that in the intrathecal group 21% received CNS irradiation

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before HSCT, compared to 9 % in the non-intrathecal group. However, the mean observation periods in both studies extended to only four years. The Lansky/Karnofsky score showed no differences between the two groups nor were there a difference in the number of neurological complications recorded during the study period.

In summary, our studies demonstrate a lack of support for the hypothesis that intrathecal therapy reduces the incidence of CNS-relapse. The power of the results is limited to 80% to detect an absolute difference in outcome of less than 5% between the two groups studied. Considering the documented risks of intrathecal therapy, the results are strong enough to not support the use of intrathecal therapy for all ALL patients and high risk AML patients after HSCT. Each patient should undergo individual assessment of suitability and necessity for intrathecal therapy. Finally our data indicates that intrathecal-therapy does not increase the risk of cognitive complications after HSCT.

Immunosupression is a risk factor for reactivation of viruses such as polyomaviruses to which a majority of the population is seropositive since childhood. The three new viruses; KI-, WU- and MCPyV have been very scarcely studied in the CNS. Even though our sample collection was small, we retrieved CSF samples from 20 patients, and this has brought us one step closer to understanding these new viruses. The study size was limited by the available amount and number of samples in storage at the Department of Virology, Clinical Microbiology, Karolinska University Hospital Huddinge. The methods used for detection of PyV DNA are well known and have shown reliable results in other studies. The capability of detecting small amounts of DNA was good (detection level JC/BKPyV PCR; 10 copies of BKPyV plasmid DNA and 5-10 copies of JCPyV DNA, MCPyV PCR: 10 MVPyV copies, KI/WUPyV PCR: 10 copies). The MCPyV method was newly developed with experience from extensive research on PyV in collaboration with Prof Tina Dalianis Research Group, at the Department of Oncology-Pathology, Karolinska Institutet, Cancer Center Karolinska, the Karolinska University Hospital. A denaturing period had previously been used (210) for CSF samples to ensure free viral DNA (if present) in the samples before the polymerase chain reaction sequence was started. Primers and positive controls were tested in trial-PCR runs previous to tests on our study-samples. As seen in our first study (I) and several other studies (17, 88) the causes of neurological complications are

often unclear. The cause is often multifactorial but there may also be causative agents not yet known or sought for. In order to decrease the number of neurological complications, preventable and treatable causes of neurological complications need to be studied. In 2007-2008 three new HPyVs were described, and already one of these viruses, WUPyV, has been suggested to be linked to the development of PML in an HIV patient. If the new HPyV have similar abilities as JCPyV and BKPyV to infect the CNS they might be a part of the missing answers behind undiagnosed neurological complications in immunosuppressed patients. The patients in our study were all immunosuppressed; all LP samples were taken within one year after HSCT (median time for LP day +68 after SCT), a majority of the patients had episodes of acute GVHD, while a third of them had cGVHD, and was subsequently on immunosuppressive drugs. The polyomaviridae family continues to be of interest for HSCT clinicians and researchers. The oncogenic potential of MCPyV is highly interesting. Data on four new human PyV have been published recently; in late 2010 and early 2011. The viruses, called PyV 6, 7, 9 and TSV PyV have so far been found exclusively on skin but as they belong to the PyV family- we plan to study these viruses in the CNS of immunocompromized patients.

CLOSING REMARKS AND FUTURE PERSPECTIVES

To conclude, owing to devoted paediatric oncologists worldwide and extensive work on international protocols and registries - the progress of paediatric oncology and HSCT has made remarkable improvements, both regarding survival statistics and the prevention of sequelae. However, more studies on the causes of and prevention of neurological complications after HSCT are needed, especially in children. More resources and perseverance in determining causes of CNS-symptoms would be of great value to our current patients, and the patients of tomorrow. A widened use of documentation with neuro-imaging as well as a broad search for possible viral agents in CSF would be of great value. The results presented in this thesis have led to a restrictive use of intrathecal therapy after HSCT in our hospital. This will reduce the treatment burden, the individual suffering and possibly even the risk of neurological complications for the patients concerned. If so, our goal has been achieved. For now.

SVENSK SAMMANFATTNING

Hematopoetisk stamcellstransplantation (HSCT) är en väl etablerad behandling av ett flertal benigna och maligna blodsjukdomar, vissa medfödda ämnesomsättningssjukdomar och svåra medfödda immunbristsjukdomar. I Sverige stamcellstransplanteras årligen cirka 60 barn. Vid en allogen stamcellstransplantation erhåller patienten, recipienten, stamceller från blod, benmärg eller navelsträngsblod från en besläktad eller obesläktad givare. Patienten får vid så gott som alla HSCT en kraftig cellgiftsbehandling med eller utan strålning före infusionen av stamcellerna. Behandlingen skall slå ut patientens sjuka stamceller i benmärgen så att de nya stamcellerna skall kunna ersätta dem. I efterförloppet efter en HSCT finns risk för flera olika typer av komplikationer. Allvarligast är graft versus host reaktioner där de nya stamcellerna reagerar immunologiskt mot patientens vävnader. Andra komplikationer är infektioner, störningar i hormononsättningen, sekundära cancersjukdomar och lungsjukdom. Komplikationer i det centrala nervsystemet (CNS) förekommer hos ca 15% av patienterna och kan leda till långvariga symptom så som nedsatt kognitiv förmåga. Komplikationerna i CNS kan vara orsakade av cytostatika eller andra läkemedel, strålning, infektioner och cerebrala blödningar. Allt eftersom överlevnaden efter stamcelltransplantation ökar blir förebyggande åtgärder för att minska neurologiska komplikationer och deras sena effekter av allt större betydelse särskilt i den pediatriska patientgruppen.

Artikel I: Prevalensen av, och orsaker till, akuta neurologiska komplikationer efter HSCT studerades i en grupp bestående av 144 pediatriska patienter transplanterade mellan 1995 och 2002 på Karolinska Universitetssjukhuset -Huddinge. De 19 patienter (13%) som insjuknade i neurologiska komplikationer inom tre månader efter HSCT hade en ökad risk för död inom det första året efter HSCT. De riskfaktorer för akuta neurologiska komplikationer som identifierades var CMV-seropositivitet hos recipienten före HSCT, en ökande risk med ett ökande antal positiva herpesvirus (CMV, EBV, HSV och VZV) serologier hos patienten före HSCT, samt elektrolytrubbningar, högt blodtryck och förhöjda bilirubinnivåer under de tre första månaderna efter HSCT. Det vanligaste neurologiska symptomet var kramper och den vanligaste orsaken var infektioner och encephalopati. I flera fall kunde den exakta orsaken till komplikationen inte fastställas.

Artikel II och III: Cytostatika given i ryggmärgsvätskan, intratekalt, ges efter HSCT för att förebygga leukemiska återfall i centrala nervsystemet hos högriskpatienter. Denna behandling kan dock ge upphov till både akuta och sena neurologiska komplikationer. Varje lumbalpunktion kan också upplevas som ett trauma för patienten. Ingreppet kan även medföra en risk för spridning av leukemiska celler till centrala nervsystemet. Effekten av intrathekal behandling efter HSCT visades i en studie gjord 1986. Det är dock sannolikt att efter de framsteg som gjorts inom pediatrisk onkologi de senaste 20 åren har gjort intrethekal behandling efter HSCT överflödig för många patienter. I artikel II och III jämförde vi därför retrospektivt risken för återfall i CNS och frekvensen av neurologiska komplikationer efter HSCT mellan två grupper av pediatriska patienter som erhållit intrathecal behandling efter HSCT respektive ej erhållit denna behandling. I artikel I studerades 120 patienter vilka

var transplanterade i Uppsala och Stockholm mellan 1992 och 2005. Vi fann mellan dessa grupper ingen skillnad i leukemiska återfall efter HSCT. Denna studie följdes av en utvidgad studie med 397 patienter från Sverige, Finland och Nederländerna transplanterade mellan 1992 och 2006. Inte heller i denna studie fann vi en ökad risk för isolerade CNS återfall om intrathekal behandling ej givits. Risken för neurologiska komplikationer var inte större för de patienter som fått intrethekal behandling jämfört med dem som ej fått denna. Trots att ingen riskökning angående neurologiska komplikationer framkom, kvarstår det faktum att dessa injektioner kan vara mycket traumatiska för patienten och är resurskrävande för vården. På basen av resultatet av dessa studier har därför rutinerna för intrathekal behandling efter HSCT ändrats på vårt och på flera andra sjukhus.

Artikel IV:
I studie I såg vi ett samband mellan positiva virusserologier och en ökad risk för neurologiska komplikationer samt att flera fall av neurologiska komplikationer saknade definitiv bakomliggande diagnos. Två typer av polyomavirus (PyV), JC- och BKPyV är neurotropa virus vilka kan orsaka neurologiska symptom hos immunosupprimerade patienter. PyV -gruppen har vuxit snabbt under senaste åren och nu finns åtta typer av PyV. I studie IV ville vi studera förekomsten av PyV i CNS hos tre av de nya typerna av PyV samt av JC- och BKPyV samt deras symptom och prognos vid PyV infektion i CNS. Vi analyserade sådeles förekomsten av JC-, BK-, KI-, WU- och MCPyV i liquor hos 20 patienter vilka haft neurologska komplikationer efter HSCT. Studien genomfördes i samarbete med professor Tina Dalianis och doktorand Geraldine Giraud, vid Institutionen för onkologi-patologi/ Cancer Centrum Karolinska, Karolinska Institutet/Karolinska Universitetssjukhuset-Solna. Liquor analyserades med PCR. Ingen av de fem typerna av PyV kunde påvisas i liquor hos studiepatienterna. Att studera PyV efter HSCT är trots detta av fortsatt intresse. Flera nya PyV typer finns också vars neurotropa potential är helt okänd.

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