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VISUAL OUTCOME AND OCULAR FINDINGS, AFTER ALLOGENEIC SCT IN CHILDREN

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200 1810 – 2010 Years

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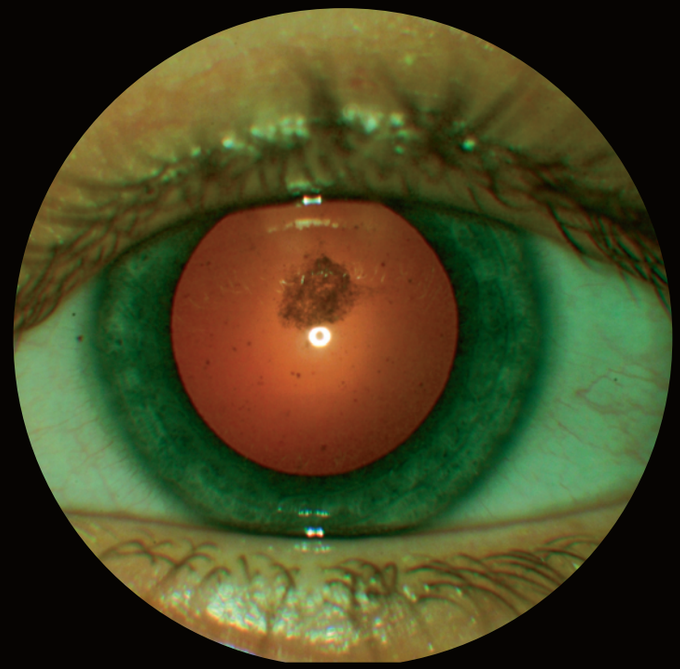

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# ABSTRACT

**Background:** Stem cell transplantation (SCT) offers a chance of cure in children with leukaemia and other life-threatening haematological, immunological, and metabolic diseases that do not respond to conventional treatment. Pre and post SCT, these children receive irradiation, and/or chemotherapy and immunosuppressive agents which like the primary disease may adversely affect the eye, the central nervous system as well as the posterior visual pathways and potentially threaten vision. The most commonly described ocular problems after SCT in childhood are cataract, dry eye syndrome (DES), and conjunctival or corneal graft-versus-host-disease (GVHD).

**Aims:** To determine the visual outcome, the frequency of ocular complications, visual fields, visual evoked potentials, and visual processing skills in a group of children/young adults who underwent SCT in childhood and to investigate the possible impact of the underlying disease, conditioning regimen, and treatment post SCT.

**Subjects and methods:** An ocular examination, including best corrected visual acuity (BCVA), refraction, slit lamp and fundus examination, tear break-up time, Schirmer's test, intraocular pressure; digital fundus photography, Rarebit visual field perimetry, visual evoked potentials, and visual processing skill tests, were performed in 79 children (37 boys, 42 girls; age median 7 years range 2–18) during 2004–2007.

**Results:** A best corrected visual acuity (BCVA) of 0.5 was achieved in 96% of the eyes. There was an increased risk of cataract and cataract surgery after conditioning including irradiation compared to chemotherapy ($p < 0.001$). There was an increased risk of developing cataract earlier if the child received single dose total body irradiation (TBI) compared to fractionated TBI ($p < 0.01$). Cataract development did not correlate with prolonged exposure to corticosteroids or chronic GVHD. Thirty-two percent of the patients showed objective signs of DES, defined as corneal staining with a short break-up time and/or pathological Schirmer. Malignant disease increased the risk of DES in girls. Frequent occasions of high cyclosporine A levels showed a significant association with DES. Single dose TBI and cataract surgery significantly correlated to a lower mean hit rate on visual field examination. Digital measurements of optic discs demonstrated significantly larger optic disc cup areas and smaller rim disc areas than reference material. Pathological visual evoked potentials (VEP) were observed in 15% of patients and were significantly associated with decreased BCVA. VEP may be of clinical use in patients with malignant diseases and/or patients conditioned with TBI. Four children with mucopolysaccharidosis I-Hurler showed a reduction of corneal opacities after early SCT but a decreased BCVA and high hyperopia were still present. In fanconi anaemia (FA) patients small optic discs were observed in six of ten eyes. Visual perceptual skills were subnormal in FA patients.

**Conclusion:** Various ocular complications are common in children treated with SCT. Regardless of the prevalence of anterior and posterior abnormalities, the ultimate visual outcomes were excellent in the majority of the patients. The choice of conditioning regimen but also the character of the underlying disease had an impact on the prevalence of complications. Awareness of and systematic and close follow-up of these sometimes severe complications, as well as early intervention are necessary to preserve good visual perception in this continuously increasing group of patients.

# LIST OF PUBLICATIONS

I. Fahnehjelm KT, Törnquist AL, Olsson M, Winiarski J. Visual outcome and cataract development after allogeneic stem-cell transplantation in children. Acta Ophthalmol Scand. 2007 Nov;85(7):724-33.

II. Fahnehjelm KT, Törnquist AL, Winiarski J. Dry-eye syndrome after allogeneic stem-cell transplantation in children. Acta Ophthalmol. 2008 May;86(3):253-8.

III. Törnquist AL, Olsson M, Martin L, Winiarski J, Fahnehjelm KT. Visual field results and optic disc morphology in patients treated with allogeneic stem cell transplantation in childhood. Acta Ophthalmol. Epub 2010 Jan 8.

IV. Törnquist AL, Martin L, Winiarski J, Fahnehjelm KT. Ocular manifestations in Fanconi Anaemia. Submitted 2010-01-12 to British Journal of Ophthalmology.

V. Fahnehjelm KT, Törnquist AL, Malm Gunilla, and Winiarski J. Ocular findings in four children with mucopolysaccharidosis I-Hurler (MPS I-H) treated early with hematopoietic stem cell transplantation. Acta Ophtalmol. 2006 Dec;84(6):781-5

VI. Törnquist AL, Andersson T, Winiarski J, Fahnehjelm KT. Visual evoked potentials in short and long term survivors after allogeneic stem cell transplantation in childhood. In manuscript

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Amplitude of accommodation |
| AGU | Aspartylglucosaminuria |
| aGVHD cGVHD | Acute (a) and chronic (c) graft versus host disease |
| ALD | Adrenoleukodystrophhy |
| ALL | Acute lymphoblastic leukaemia |
| Amega | Congenital amegakaryocytic thrombocytopenia |
| AML | Acute myeloid leukaemia |
| ATG | Antihymocyte globulin |
| BCVA | Best corrected visual acuity |
| BMT | Bone marrow transplantation |
| Bu | Busulfan |
| BUT | Break-up time |
| CA | Cup area |
| CAT | Cone adaptation test |
| CGD | Chronic granulomatous disease |
| CML | Chronic myeloid leukaemia |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| Cy | Cyclophosphamide |
| CyA | Cyclosporine A |
| D | Dioptres |
| DA | Disc area |
| DEM | Developmental eye movement |
| DES | Dry eye syndrome |
| EBV | Epstein-Barr virus |
| ERT | Enzymatic replacement therapy |
| FA | Fanconi anaemia |
| GAG | Glucosaminoglycans |
| GH | Growth hormone |
| GI | Gastrointestinal |
| HLA | Human leukocyte antigen |
| HLH | Haemophagocytic lymphohistiocytosis |
| HRR | Hardy Rand Rittlers |
| HSC | Haematopoietic stem cell |
| HSCT | Haematopoietic stem cell transplantation |
| HZO | Herpes zoster ophthalmicus |
| ICD | Inner canthal distance |
| IOL | Intraocular lens |
| IOP | Intraocular pressure |
| IQ | Intelligence quotient |
| JMML | Juvenile myelomonocytic leukaemia |
| LCH | Langerhans cell histiocytosis |
| MDS | Myelodysplastic syndrome |

| | |
|---|---|
| MHR | Mean hit rate |
| MPS I-H | Mucopolysaccharidosis I-Hurler |
| MRD | Matched related donor |
| MSD | Matched sibling donor |
| MTX | Methotrexate |
| MUD | Matched unrelated donor |
| OCD | Outer canthal distance |
| ONH | Optic nerve hypoplasia |
| pD | Prism dioptres |
| PFL | Palpebral fissure length |
| PFW | Palpebral fissure width |
| PSC | Posterior subcapcular cataract |
| QOL | Quality of life |
| RA | Rim area |
| RAF | Royal Air Force |
| RB | Rarebit |
| SAA | Severe aplastic anaemia |
| SCA | Sickle cell anaemia |
| SCID | Severe combined immunodeficiency |
| SCT | Stem cell transplantation |
| SD | Standard deviation |
| SE | Spherical equivalent |
| s-TBI f-TBI | Single (s) and fractionated (f) total body irradiation |
| TVPS-R | Test of visual perceptual skills revised |
| UMRD | Upper eye lid margin to the corneal reflex distance |
| VEP | Visual evoked potential |
| WAS | Wiskott-Aldrich syndrome |

# 1 INTRODUCTION

## 1.1 BACKGROUND

### 1.1.1 Stem cell transplantation

***Stem cell transplantation*** (SCT) is a procedure that offers a chance of survival/cure in children with leukaemias that do not respond to conventional treatment and in children with other severe haematological and inborn errors of metabolism and immunodeficiencies.[1-4] ***Haematopoietic stem cells*** (HSCs) are cells defined by the ability to self-renewal, keeping a stable number of stem cells; the ability to produce at the single cell level differentiated young cells; and the ability to functionally reconstitute a given tissue in vivo.[5] HSCs from the bone marrow, peripheral blood, or cord blood are used as the stem cell source and are responsible for producing all mature blood cells, during an organism's life.[6] ***Transplantation*** is a medical treatment that replaces a recipient's diseased organ with a healthy organ from a donor. In haematopoietic SCT intravenous infusion of HSCs following a conditioning regimen is used with the intention of restoring the haematopoietic function in patients with damaged or defective immune system.[6] ***Conditioning regimen*** is the procedure used to prepare a patient before SCT takes place. It may be based on chemotherapeutic agents, antibody therapy, and total body irradiation (TBI). The main purposes are to allow the growth of new blood stem cells, to prevent rejection of the transplanted cells, and to eliminate any remaining malignant cells in the body.

The initial trials of bone marrow transplantation (BMT) were made at the end of 19$^{th}$ century, but it was not until 1957 that Thomas[7] reported the first infusion of bone marrow to patients who had received radiation and chemotherapy. These human BMTs were, for the most part, unsuccessful and the patients died early due to poor engraftment. In 1958, the first human leukocyte antigen (HLA) was described.[8-10] The present knowledge is the outcome of several investigations.[11-14] Thomas also reported the use of TBI and syngeneic transplantation for treatment (between monozygotic twins) of leukaemia.[15]

In the early 1960's there was a better understanding of the HLA proteins found on the leukocytes that make the tissue unique, which showed the way to use allogeneic (genetically different individuals) sibling donors for transplantations. During this decade the emphasis was placed on the areas of histocompatibility, conditioning regimen, and prevention and treatment of graft-versus-host-disease (GVHD). Survival rates were still poor.[16]

During the second half of 1960's high-dose conditioning regimens were incorporated. These consisted of the maximum tolerated doses of TBI/chemotherapy which not only killed the cancer cells but also helped in the suppression of the host immune system in order to avoid rejection of the graft.[17,18] In the late 1960s human SCT began to use siblings who were genotypically identical with their recipient. In 1968, the first SCT performed in a 5-month old male diagnosed with severe combined immunodeficiency

disease (SCID) was reported.[19] Even though he was not perfectly matched with his sister, who was the best available donor, the SCT was performed and was successful and the baby achieved a full correction. Due to the histocompatibility mismatch, he developed a severe GVHD but survived.

In the 1970's the attempts were made to improve supportive care measures and more effective conditioning regimen. The introduction of cyclosporine A (CyA) occurred[20] and the survival rate improved. A few years later Thomas[21] reported the use of allogeneic SCT from an HLA identical sibling after TBI and cyclophosphamide (Cy). It was concluded that for better results the transplantation should be performed early in the course of the disease and under good health conditions. In the late 1970's it was reported that patients who had undergone SCT and had developed GVHD had less risk of suffering cancer relapse. In the 1980's the interest in performing SCT increased and by 1986 about 5000 transplants were performed each year at different transplant centres all over the world.[22] At this stage the most important thing was to establish the criteria for performing SCT, patient risk factors, disease status, type of transplant, and presence of infections. During this period busulfan (Bu) and Cy came into use to replace the use of TBI.[23] By the late 1980's the use of stem cells collected from peripheral blood for allogeneic SCT was introduced.[24] In 1990's the implementation of SCT as a treatment for the management of malignant and non-malignant diseases grew. Genomic techniques were introduced allowing increased use of unrelated donors as a graft source for SCT.

During the 21$^{st}$ century improvement of the prevention and treatment of GHVD continues and the criteria for diagnosis and management of chronic GVHD (cGVHD) have been developed.[25]

In the 2009 report, the CIBMTR (Centre for international blood and marrow transplantation research)[26] database included data from more than 500 centres in 54 countries worldwide. An estimated 50–60,000 haematopoietic SCTs are undertaken annually worldwide. Approximately 45% of all allotransplants performed worldwide are from unrelated donors. One-year survival rates after transplantation have generally improved in the last two decades. The 3-year probabilities of survival in patients under 20 years of age depend on the donor type and the underlying disease. In HLA-matching sibling donor (MSD) the survival rate is between 24–61% and in HLA-matched unrelated donor (MUD) is 19–46%. In each underlying disease survival possibility depends on the severity of the disease. For acute myeloid leukaemia (AML) with a matching sibling the survival rate is between 17–52% and with an unrelated donor is of 19–43%. Myelodysplastic syndrome (MDS) survival rate is approximately 43–68%. For acute lymphoblastic leukaemia (ALL) with a HLA-MSD the survival probabilities are between 23–65% and for ALL with a HLA-MUD 20–57%. The annual numbers of patients undergoing allotransplantation for the most common indications have changed over the past decade. While allotransplantations for AML and ALL have steadily increased, allotransplantations for chronic myeloid leukaemia (CML) have decreased. Survival probabilities for CML patients are approximately 70%. Allogeneic SCT is the treatment of choice for young patients with severe aplastic anaemia and an available

HLA-MSD. For severe aplastic anaemia (SAA) with a HLA-MSD the survival rate is of 85% and with an MUD is between 61–63%.

At Karolinska University Hospital, Huddinge Sweden, the first allogeneic SCT performed was in 1975 and the first unrelated donor SCT was performed in 1984. Since then, more than 450 children had received allogeneic SCT. Currently more or less 50 children a year are transplanted in Sweden and almost half of these take place at the Karolinska University Hospital Huddinge.

### 1.1.2 Sources of stem cells and donors

Haematopoietic stem cells (HSCs) are progenitors to all cells of the blood system and have the ability to self-renew. Allogeneic haematopoietic SCT involves the transfer of both immature and mature blood cells from the bone marrow, peripheral blood or cord blood transferred from one individual to another.[27]

In order to find an appropriate donor, a study of similarity or histocompatibility must be made. Histocompatibility is determined by the presence of compatible HLA present in the surface of the cells. The degree of graft rejection is related to the histocompatibility between the donor and the host. In order to minimize allogeneic responses, the HLA of the donor and the recipient are HLA matched as closely as possible. Since only monozygotic twins can be completely HLA-identical, immunosuppressive drugs are used to prevent or stop the allogeneic response.[28] There are several types of donors:

#### *1.1.2.1 Syngeneic SCT*

Syngeneic SCT is the transfer of HSCs between individuals who have identical genotypes, i.e identical twins.[29] The severe immunological reactions, like GVHD will not occur. Leukaemia after SCT is greater in recipients of syngeneic transplants than in those of HLA-MSD transplants.[30] Even though syngeneic SCT is rare it has a good prognosis when performed as soon as the first remission (malignant cells have disappeared after treatment) has been achieved. It is rarely performed in leukaemia patients today.[31]

#### *1.1.2.2 Autologous SCT*

Autologous SCT is the removal, storage and re-infusion of a patient's own HSCs after high dose chemotherapy.[29] These cells are frozen at temperatures below -120°C and can be used for up to several years later. Mortality is considered lower than with allogeneic SCT but the lack of GVHD reduces its efficacy.[32] The most common diseases treated with autologous SCT are presented in Table 1. Autologous SCT is predominantly used in adult patients for indications not generally seen in children. The total number of auto-transplants is overall higher than the allogeneic SCTs. In children, on the contrary, only few autologous are performed, mainly in advanced neuroblastoma and medulloblastoma patients.

#### *1.1.2.3 Allogeneic SCT*

In allogeneic SCT the HSCs come from genetically different individuals, the donor can be a sibling, a relative, or an unrelated donor.[29] Less than 30% of potential recipients of HSCs have HLA-identical siblings. In Sweden, the Tobias Registry is the national Swedish registry of healthy donors of blood-forming stem cells. It was established in 1992 and nowadays lists about 40,000 individuals who have registered as potential donors. Allogeneic SCT is used after first relapse in patients who have a good prognosis with chemotherapy. It is the only option in patients with CML. Recipients from unrelated donors are at a higher risk of developing GVHD. The most common diseases treated with allogeneic SCT are presented in Table 1.

**Table 1. Diseases commonly treated with haematopoietic stem cell transplantation (mainly non-paediatric indications*)**

| Autologous transplantation | Allogeneic transplantation |
|---|---|
| Malignant | Malignant |
| Multiple myeloma* | Acute myeloid leukaemia |
| Non-Hodgkins lymphoma* | Acute lymphoblastic leukaemia |
| Hodgkins's disease* | Chronic myeloid leukaemia |
| Acute myeloid leukaemia* | Myelodysplastic syndromes |
| Advanced neuroblastoma | Myeloproliferative disorders* |
| Ovarian cancer* | Non-Hodgkins lymphoma* |
| Germ-cell tumours* | Hodgkins's disease* |
| | Chronic lymphocytic leukaemia * |
| Other diseases | Multiple myeloma* |
| Autoimmune disorders | Juvenile chronic myeloid leukaemia |
| Amyloidosis* | |
| | Other diseases |
| | Aplastic anaemia |
| | Paroxysmal nocturnal hemoglobinuria* |
| | Fanconi Anaemia |
| | Blackfan-Diamond Anaemia |
| | Thalassaemia major |
| | Sickle cell anaemia |
| | Severe combined immunodeficiency |
| | Wiskott-Aldrich syndrome |
| | Inborn errors of metabolism |

### 1.1.3 The stem cell transplantation procedure – an overview

Before SCT the patient undergoes through a series of tests including endocrinologic, pulmonary, neurocognitive, dental, and ophthalmologic evaluation to determine if he or she is healthy enough to tolerate the transplantation. Once the suitable donor has been identified the patient goes through five well defined stages: conditioning regimen,

transplant phase, neutropenic phase, engraftment phase, and the post engraftment phase.

#### *1.1.3.1 Conditioning regimen phase*

The conditioning regimen phase lasts approximately one week. During this period TBI and/or chemotherapy are given for several purposes: to eliminate any existing disease, to give appropriate immunosuppression for the engraftment of the donor HSCs, prevent graft rejection, and to create space for new HSCs.[32-34] SCT together with TBI and chemotherapy are mainly recommended for ALL. On the other hand, TBI is the cause of both immediate and long-term side effects, especially in infants.[33] For AML, the most usual conditioning regimen is chemotherapy alone with Bu and Cy. [33] For non-malignant diseases the protocol of Bu and Cy has proven to be successful in eliminating the need of TBI.[35] More recently other chemotherapeutic agents, including fludarabine, and combinations have been introduced to reduce the toxicity of the conditioning regimens.

#### *1.1.3.2 Transplantation phase*

Before the actual transplantation a day of rest is given after chemotherapy to make sure the cytotoxic agents have been eliminated from the body. Thereafter the stem cells are slowly injected intravenously into the body, through a central vein, to eliminate the possibility of an anaphylactic reaction. This procedure takes from 1 to 5 hours. Frequent problems are hypertension, bad mouth taste and renal dysfunction.[33] After entering the bloodstream, the stem cells travel to the bone marrow, where they begin to produce new white blood cells, red blood cells, and platelets in a process known as "engraftment."

#### *1.1.3.3 Neutropenic phase*

The neutropenic phase lasts between two and four weeks. During this phase the immune system is completely depressed and as a consequence of the conditioning regimen the normal mucous and skin barriers are disrupted allowing the entrance of bacteria. Broad-spectrum antibiotics and antifungal therapy are given empirically (the patient contracts fever of unknown origin) to reduce the risks of infections.[33]

#### *1.1.3.4 Engraftment phase*

During the engraftment phase, the mucosa starts healing, the infections resolve and acute GVHD (aGVHD) develops. Graft rejection after SCT may be manifested as either the lack of initial engraftment or the development of pancytopenia and marrow aplasia. [36] Normally, GVHD primarily involves skin, liver, and the gastrointestinal (GI) tract.[33] In humans, rejection, drug toxicity, sepsis, and viral infections can cause graft failure.[36] At this time the immune function is not fully recovered and there is a high risk of infections. Prophylactic treatment is often applied to prevent the development of viral and fungal infections.[33]

#### *1.1.3.5 Post engraftment phase*

This period lasts approximately from 3–12 months after the transplantation. The immune function slowly starts the recovery process and graft tolerance occurs.[33] This process might take up to two years or up until the patient recovers full health.

### 1.1.4 Conditioning regimens

Several studies have shown that the choice of conditioning regiment had a significant impact on survival and the incidence of relapse.[37-40] The chosen conditioning regimen depends on the patient's age, prior irradiation, time and sites of relapse and donor availability.[39] One advantage of a chemotherapy regimen is to avoid such long-term effects of TBI as cataracts, sterility, second malignancies, and growth and development problems in children.[41]

#### *1.1.4.1 Myeloablative conditioning regimens*

Myeloablative conditioning regimens are used to eliminate the underlying disease or malignant cells and to suppress the patient's immune system, high-dose chemotherapy with or without TBI is given, followed by SCT.[42] The advantage is that this reduces the probability of relapse; on the other hand, it increases therapy-related toxicity and mortality. The use of TBI or high-dose chemotherapy also has an important impact on the development of GVHD.[1] The most common combinations are Cy with TBI (Cy/TBI) and Bu with Cy (Bu/Cy).

##### 1.1.4.1.1 Radiotherapy

Irradiation is used as a systemic agent and its most important functions are for immunosuppression and malignant cell eradication.[43] Some of the positive aspects of irradiation are (1) it does not react with another agent, (2) there is no sanctuary sparing, like the testes, and (3) a given dose of irradiation may be homogeneous independent of blood supply. The downside is that it affects normal organs, like the GI tract, lung, and lens which are at risk for late complications.[43]

TBI was formerly an important modality for conditioning in autologous and allogeneic SCT for patient with haematological diseases. TBI is still used in conditioning regimens primarily in ALL-patients. In most other disorders chemo-based regimens are used today. The radiation combines TBI with chemotherapeutic agents most often cyclophosphamide, cytarabine or etoposide. Two types of TBI have been used, single dose TBI (s-TBI) and fractionated dose TBI (f-TBI). The TBI dose varies between 1000 cGy (10 Gy) to 1400 cGy (14 Gy) Fractionation decreases toxicity and increases tolerability.[44] The outcome of radiation can be improved by changing the dose, treatment time, and fractionation in order to decrease toxicity in the normal tissues, increase tolerability, to increase malignant cell kill, and to increase immunosuppression.[43] Toxicities related to TBI can be classified as acute or delayed. Acute manifestations include gastrointestinal symptoms, alopecia, decreased

production of saliva and tears, and rashes. The delayed problems include pulmonary and endocrine dysfunction, cognitive impairment, cataracts, and secondary malignancies.[45,46]

#### 1.1.4.1.2 Cyclophosphamide

This was the first drug used as part of the conditioning regimen for SCT. Cy is mainly used together with other chemotherapy agents in the treatment of haematological, autoimmune disorders and some solid tumours. It is the most widely used drug in SCT conditioning regimens because of its broad range of antineoplastic activity and for its immunomodulatory properties.[47] The maximum tolerated dose, as a single agent, is approximately 200 mg/kg. Regardless of its toxicity, it does not destroy stem cell compartments and patients do not die of pancytopenia if engraftment does not occur.[48] High-dose of Cy together with Bu is the chosen conditioning regimen for patients with AML or CML. Neurotoxicity is rare at conventional doses, but at higher doses haemorrhagic cystitis, haemorrhagic myocarditis, reproductive defects, blurred vision, hepatic abnormalities, and pneumonitis may occur.[48]

In 1978 the first ocular toxicity induced by Cy was reported.[49] The major complaint was blurred vision within minutes to 24 hours after administration of Cy. The symptoms resolved within hours to 2 weeks after cessation of Cy. Several cases of blepharoconjunctivitis have also been described.[50]

#### 1.1.4.1.3 Busulfan

Bu is a cytostatic drug with strong myeloablative properties mostly used in SCT conditioning of myelogenous leukaemia. It is used as part of the conditioning regimen before allogeneic SCT instead of radiation therapy[41,51] for patients who cannot be given TBI, either because of their age or because they have already received high irradiation doses.[39] The usual total dose is usually 16 mg/kg given over 4 consecutive days.[51] The paediatric patients need higher doses of Bu than older patients due to their high metabolism.[48] Side effects from Bu are common and are partially reversible after treatment is completed. The most common side effects are myelosuppression, seizures, venoocclusive disease, haemorrhagic cystitis, and alopecia.[48]

### *1.1.4.2 Non-myeloablative conditioning regimen*

A non-myeloablative or reduced intensity conditioning regimen uses several chemotherapeutic agents without TBI, which is instead replaced with additional chemotherapeutic agents followed by immunosuppression. The main objective of this approach is to reduce toxicity and mortality.[52] It is commonly used in patients with non-malignant diseases, especially haemoglobinopathies.[53]

#### 1.1.4.2.1 Fludarabine phosphate

Fludarabine (Flu) is increasingly used as a part of a non-radiation or non-myeloablative regimens. It is particularly useful due to its immunosuppressive effect and has fewer side effects. It has been demonstrated that the use of Flu is sufficiently myeloablative and immunosuppressive to allow engraftment of allogeneic cells and to reduce toxicity

of the transplant treatment.[54] Some side effects associated with Flu are pancytopenia, autoimmune hemolytic anaemia, neurotoxicity (leukoencephalopathy, seizures, cortical blindness, confusion, and coma), and gastrointestinal problems, like nausea, vomiting, and diarrhoea.[48,55]

### 1.1.5 Prophylaxis and management of graft-versus-host-disease

GVHD is a complication post SCT that requires medical treatment to control it. The medical management uses medications for prophylaxis and treatment of GVHD. If GVHD develops after SCT, corticosteroids in combination with cyclosporine A (CyA) are administered. The success rate is approximately between 50–75% for aGVHD and between 25–50% for cGVHD.[56] CyA is also used in combination with methotrexate (MTX). Today other drugs, such as tacrolimus and sirolimus are often substituted for CyA.

#### *1.1.5.1 Cyclosporine A*

Cyclosporine A (CyA)[9,57,58] is an immunosuppressive used for prophylaxis and treatment of GVHD and transplant rejection. The most important function is inhibition of T-cell activation. The major adverse effects of CyA are anaemia, thrombocytopenia, erythrocytosis, and thromboembolism. Neurological consequences may include seizures, tremors, confusion, lethargy, tinnitus, speech disorders, coma, haemiplegia, and encephalopathy with impaired consciousness and convulsions. CyA toxicity can cause visual loss associated with white matter abnormalities, cortical blindness, and microangiopathy. Usually, these manifestations are reversible after discontinuing CyA.

It has been shown that early administration (about 7 days before SCT) of CyA may reduce the risk of developing GVHD[59] while others state that there is no difference on the outcome if given CyA earlier.[60]

#### *1.1.5.2 Corticosteroids*

Corticosteroids, such as prednisolone, are commonly used in the prevention and treatment of aGVHD and cGVHD. Methylprednisolone is a synthetic corticosteroid used for its anti-inflammatory effects but also has analgesic properties is useful in the treatment of pain in the management of cancer.[61] The administration of long-term corticosteroids has several side effects, including weight gain, osteoporosis, psychosis, glaucoma and cataract. The addition of corticosteroids to the combination of CyA and MTX increases the incidence of bacterial and fungal infection during the first months after transplantation.[62] This occurs because glucocorticoids reduce the migration of monocytes to sites of inflammation and inhibit the production of mast cells and macrophages.

#### *1.1.5.3 Methotrexate*

Methotrexate is often used to potentiate the immunosuppression system during the first weeks after SCT in addition to CyA.

## 1.2 INDICATIONS OF SCT IN CHILDREN

The children in this study underwent SCT for malignant and non-malignant diseases, which are described below.

### 1.2.1 Malignant diseases

#### *1.2.1.1 Leukaemia*

The most common type of cancer in children is ALL, representing more than 75% of all paediatric cases of leukaemia.[63] Each year about 200 children and adolescents (< 18 years of age) are diagnosed with ALL in the five Nordic countries: Denmark, Finland, Iceland, Norway, and Sweden.[64] The peak incidence is between 2 and 10 years of age. Age at diagnosis has strong prognostic importance, in young children (1–9 years of age) disease-free survival is better than older children, adolescents, or infants.[65,66] In some studies, the prognosis for girls with ALL is slightly better than for boys.[67,68]

Acute myelogenous leukaemia (AML) is the less common leukaemia in children, while it is the predominant acute leukaemia in adults.[69]

Despite improvements in conventional therapy, there is a group of patients with ALL and AML at risk of relapse for whom allogeneic SCT in first remission is the best chance of cure.[70] In these cases most paediatric patients receive HSCs from either a matched sibling donor (MSD) or matched unrelated donor (MUD).

An uncommon type of leukaemia in the paediatric population is chronic myeloid leukaemia (CML), which accounts for 2%.[71] For CML, SCT is still very relevant in children in spite of the availability of tyrosine kinase inhibitors which are used in adults to treat CML. Tyrosine kinase inhibitors are currently the first treatment option for adult patients with newly diagnosed CML and allotransplantation is reserved for patients who fail such therapy.

#### *1.2.1.2 Myelodysplastic disorders*

In this group of clonal myeloid haematological disorders two diseases are included:

1.2.1.2.1 Juvenile myelomonocytic leukaemia

Juvenile myelomonocytic leukaemia (JMML)[72] is a myeloproliferative malignant disorder characterized with hepatosplenomegaly (90%), lymphadenopathy (25–75%), infections in the respiratory system, pallor, fever, skin rashes, petechia (small red dot caused by a minor hemorrhagae), ecchymoses, xantomas (cholesterol deposits that appear as nodules), and eczema lesions. The incidence is between 1–17%, more frequent in boys than in girls (2:1) and in children < 2 years of age. JMML can only be cured by SCT and is not treatable by conventional chemotherapy.

#### 1.2.1.2.2 Myelodysplastic syndrome

Myelodysplastic syndrome (MDS)[73,74] includes a group of malignant haematopoietic disorders characterized by impaired mutation of haematopoietic cells, increased apoptosis leading to progressive peripheral cytopenias and tendency to progress into AML. MDS is a rare condition in the paediatric population, the incidence is of 9% and presents in children < 15 years of age. There are two treatment alternatives, the first one includes only chemotherapy or TBI plus chemotherapy and the second consists of allogeneic SCT with a MSD, MUD, or matched related donor (MRD).

### 1.2.2 Non-Malignant diseases

The non malignant group includes several groups of diseases, such as metabolic diseases, haemoglobinopathies, acquired bone marrow failure, inherited bone marrow failure syndrome and primary immunodeficiencies. In 1980 Hobbs used Bu and Cy as a conditioning regimen before transplantation in patients diagnosed with Hurler's disease to avoid the use of irradiation. This protocol was very successful and has been used as a conditioning regimen for other genetic disease transplants.[75]

#### *1.2.2.1 Metabolic diseases*

The metabolic diseases are a diverse group that include mucopolysaccharidosis, leukodystrophies, and disorders of glycoprotein metabolism. Haematopoietic SCT is considered the only therapy or the most effective long-term treatment for some metabolic diseases. The main objectives of SCT are: to prolong survival; to improve somatic and neurophysiological development; and to enhance quality of life.

#### 1.2.2.1.1 Mucopolysaccharidosis I-Hurler

Mucopolysaccharidosis I-Hurler (MPS I-Hurler)[76-78] is a rare life-threatening, autosomal, recessive, inborn error of the lysosomal metabolism. Systemic characteristics of MPS I-Hurler are skeletal deformities, mental retardation, hydrocephalus, hearing loss, hepatosplenomegaly, short stature, cardiac problems, and developmental delay. Ocular manifestations include protrusion of the bulbi, hypertelorism, atypical eyebrows, corneal clouding, retinal dystrophies, glaucoma, chronic papilloedema, optic atrophy, and posterior visual pathway or cortical problems. Treatment with SCT is recommended in children $\leq 2$ years of age with normal cardiopulmonary function and normal intelligence. A myeloablative regimen and immunosuppressive therapy is administered before SCT.

#### 1.2.2.1.2 Gaucher's disease

Gaucher's disease[79] is an autosomal recessive, glycolipid storage disorder characterized by the accumulation of glucosylceramide. It is classified in three different types based on the presence and severity of neurological impairment. Type 1 is the most common and it differs from type 2 and 3 because there is no central nervous system (CNS) involvement. Type 2 is of early onset and death may happen within the first 2 years of

life. Type 3 presents neurological involvement of later onset and shows aspects of chronicity. Gaucher's disease has no cure and SCT as a treatment modality was used before enzyme replacement therapy (ERT) became available in the 1990s. Treatment is mainly used to alleviate manifestations.

#### 1.2.2.1.3 Aspartylglucosaminuria

Aspartylglucosaminuria (AGU)[80,81] is a rare, lysosomal, inherited storage disorder, caused by a defective activity of the enzyme aspartylglucosaminidase. Its main features are progressive mental retardation, short stature, coarse features, upper respiratory infections, joint laxicity, hernias, and hypertelorism. It is a very common condition in Finland. Different treatment modalities have been tried, like Bu/Cy or Cy/TBI follow by SCT and CyA and MTX as prophylaxis therapy; ERT, and gene therapy. The results of SCT are controversial.

#### 1.2.2.1.4 Adrenoleukodystrophy

Adrenoleukodystrophy (ALD)[82,83] is a peroxisomal, sex-linked storage disorder, affecting primarily males, in which very long-chain fatty acids accumulate in plasma and in the CNS. In childhood is malignant, causing death by the end of the first decade of life if left untreated. It manifests with progressive neurological deterioration, including early dementia, and adrenal insufficiency. ALD can be controlled with a restricted diet and ingestion of supplements. Improvement is seen within months after SCT. SCT may be successful when there still is only very early evidence of demyelination without a progressive neurological disability.

#### 1.2.2.1.5 Langerhans cell histiocytosis

Langerhans cell histiocytosis (LCH)[84] is a rare idiopathic disorder with manifestations ranging from relative benign bone lesions to multiorgan involvement, dysfunction, and death. The organs mainly involved are the liver, lungs, CNS, and haematopoiesis. The calculated incidence in the Nordic countries is of 3.5–7 cases per million per year. The suggested conditioning protocol is Bu followed by SCT, CyA and MTX. Flu has recently been used as part of the treatment.

### *1.2.2.2 Haemoglobinopathies*

Haemoglobinopathies are defined by the presence of qualitative and/or quantitative abnormalities affecting the globin chain. Qualitative abnormalities lead to the production of an abnormal structure of haemoglobin, causing for example sickle cell anaemia (SCA). Haemoglobin quantitative abnormalities result from either reduced or absent synthesis of α- or β-globin chains, defining the thalassaemias.[85]

#### 1.2.2.2.1 Sickle cell anaemia

Sickle cell anaemia (SCA)[53,85] is an autosomal, recessive, inherited disorder mainly found among blacks in which the abnormally sickle-shaped red blood cells appear in the blood stream. Anaemia, vasoocclusion, severe pain, several organ dysfunctions, stroke, and acute chest syndrome are the most significant clinical manifestations of

SCA. It is not often symptomatic in children younger than months. The management includes pain control, hydroxyurea, blood transfusion, antibiotics, and SCT in selected cases with an HLA-MSD. Before SCT a myeloablative conditioning regimen (Bu and Cy) is given.

#### 1.2.2.2.2 Thalassaemia major

Thalassaemia[85-87] consists of a group of inherited disorders, affecting the production of haemoglobin leading to more or less severe anaemia, depending on zygosity. Homozygous beta-thalassaemia major first presents in children before 6 months of age. Complications are related to whether the patient receives adequate transfusion and chelating therapy or not. Secondary hemosiderosis, cardiomegaly, hypersplenism, and severe bone malformations can appear. Allogeneic SCT may be offered to patients that are transfusion dependent and have the severe major form. The conditioning regimen includes Bu and Cy and the donor is preferably an HLA-MSD. The outcome is good especially in young patients who do not have advanced clinical manifestations with an overall survival rate of 95%.

### *1.2.2.3 Bone marrow failure syndromes*

Bone marrow failure syndromes are a heterogeneous group of genetic disorders that share inability of the bone marrow to produce an adequate number of blood cells.[88] These syndromes are divided into acquired and inherited.

#### 1.2.2.3.1 Severe aplastic anaemia

Severe plastic anaemia (SAA)[89-92] is a rare, acquired, potentially fatal bone marrow failure that occurs mainly in children and young adults. It is characterized by pancytopenia, and hypocellular marrow. It has been associated with hepatitis, chemicals, and some drugs like nonsteroidal anti-inflammatory agents, anticonvulsants, and sulfonamides. The management protocol is based on supportive care which includes transfusion, and prevention and treatment of infections and immunosuppressive therapy (Cy) together with antihymocyte globulin (ATG) followed by SCT and CyA. SCT is considered the best treatment for younger patients with SAA and by using a HLA-MSD the current disease-free survival rate is of 90% after 5 years of SCT. For children without a sibling donor and not responding to immunosuppressive therapy, a SCT with an HLA-MUD is an acceptable alternative.

#### 1.2.2.3.2 Fanconi Anaemia

Fanconi anaemia (FA)[93-95] is a rare, autosomal, recessive, inherited syndrome with excess chromosomal breakage and can develop haematological non-haematological malignancies, and aplastic anaemia. It normally develops by seven years of age. The systemic clinical manifestations include abnormal skin pigmentation (café au lait and hypopigmented spots), short stature, congenital skeletal malformations (thumb malformations), (Fig 1) renal, cardiac, genitourinary, gastrointestinal, endocrine, and neurodevelopmental abnormalities. The most common ocular findings reported are short and/or almond shape palpebral fissures, ptosis, microphthalmia, hypo-,

hypertelorism, and epicanthal folds. Marrow failure usually appears between 5 and 10 years of age. Conditioning regimens for SCT have been modified due to the chromosomal instability and much reduced doses of chemotherapy are used. This also reduces the risk of developing leukaemia, while the risk of other non-haematopoietic malignancies, such as squamous cell carcinoma remains. Irradiation should be avoided because of the higher risk of developing malignant diseases. Well tolerable conditioning regimen by FA patients includes low-dose Cy, Flu, and ATG. The overall survival rate is 82%.

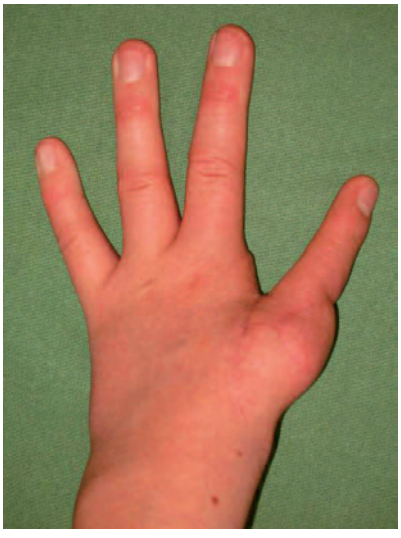

***Fig 1.*** *A characteristic and in part surgically corrected thumb malformation in an eleven year old boy diagosed with Fanconi`s aplastic anaemia at seven years of age. Missing or dysplastic thumbs are the most common malformations in fanconi anaemia.*

1.2.2.3.3 Congenital amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (Amega)[95,96] is an autosomal, recessive disorder and is the rarest of the inherited bone marrow failure syndromes. It develops during early childhood and it is characterized by thrombocytopenia, bleeding, infections, and haemorrhages. Approximately 50% of the patients develop marrow aplasia, and some develop MDS or leukaemia. In general this disease has poor prognosis or survival without SCT. The only curative treatment is an early SCT with either a sibling or unrelated matching donor. The conditioning regimen prior SCT consists of Bu and Cy.

### *1.2.2.4 Primary immunodeficiency diseases*

Primary immunodeficiency diseases[97] are a group of genetic disorders in which essential cells of immune system, mainly T- and/or B-lymphocytes are missing or dysfunctional causing vulnerability to opportunistic infection increasing the vulnerability to infectious diseases, autoimmunity and malignancy.

#### 1.2.2.4.1 Severe combined immunodeficiency

Severe combined immunodeficiency (SCID)[98,99] is a heterogeneous group of lethal, congenital immune disorders with x-linked or autosomal heredity that result in missing or non-functional T and B lymphocytes. SCID usually presents during infancy between 3–8 months of age. The clinical manifestations include failure to grow, thrush, yeast infection, diarrhoea, and opportunistic infections such as pneumocystis jiroveci and cytomegalovirus (CMV). In general, SCT is the treatment of choice in patients diagnosed with SCID, but in selected cases gene therapy and enzyme replacement can also be an option. The long-term survival rate may reach 80–100% under optimal circumstances. Average survival has been poorer historically due to complicated infections and late diagnosis.

#### 1.2.2.4.2 Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome (WAS)[99] is an x-linked disorder, characterized by thrombocytopenia, bleeding, immunodeficiency, recurrent bacterial pulmonary infections, and eczema. The suggested treatment is early SCT. The mean survival rate is high particularly with a sibling donor.

#### 1.2.2.4.3 Chronic granulomatous disease

Chronic granulomatous disease (CGD)[100,101] is a rare, often x-linked syndrome caused by a defective neutrophil function. The median presentation age is 7 years (range 0.4–15 years). Clinical systemic manifestations include recurrent bacterial and fungal infections, lymphadenitis, hepatic abscesses, and formation of granulomas in vital organs. In the eye it presents with retinal granulomas. The most common management protocol is non-myeloablative conditioning prior to SCT. The published survival rates after SCT have been high, above 90%.

#### 1.2.2.4.4 Haemophagocytic lymphohistiocytosis

Haemophagocytic lymphohistiocytosis (HLH)[100,102] is a rare immunoregulatory disorder with a defective lymphocyte and NK-cell cytotoxic and apoptotic activity resulting in excessive accumulation of tissue macrophages and organ dysfunction or enlargement. It presents with hepatosplenomegaly, haemophagocytosis, cytopenias, lymphadenopathy, neurological changes, and seizures. The management protocol is primarily cytotoxic chemotherapy and immunosuppressive agents (corticosteroids, CyA) followed by SCT.

#### 1.2.2.4.5 Wegener and Non-Hodgkins Lymphoma

Wegener's granulomatosis[103] is a necrotising, granulomatous, small vessels vasculitis. The most relevant clinical manifestations are granulomatous inflammation of the respiratory tract, necrotising vasculitis, glomerulonephritis, conjunctivitis, scleritis, uveitis, and proptosis. It presents at approximately 6 years of age. It is managed with corticosteroids, cytotoxic (Cy) and immunosuppressive drugs. In case of developing Non-Hodgkins lymphoma SCT is an option.

## 1.3 STEM CELL TRANSPLANTATION RELATED COMPLICATIONS

One of the major objectives of SCT is to provide the highest curative outcome with the least toxicity. Since the survival rate after SCT has improved during the past decades, quality of life in surviving patients has become increasingly important and the number of studies of long-term complications has increased[104-107] The outcome of SCT is dependent on the patient's age, diagnosis, prior treatment, type of donor, conditioning regimen, and prophylaxis protocol.[108] SCT related complications can be classified into early and late. A list of complications in children is presented in Table 2.

### 1.3.1 Early complications

#### *1.3.1.1 Mucositis*

Mucositis (inflammation of the mucous membranes)[109,110] is one of the most frequent adverse effects of chemotherapy and radiotherapy. The incidence of oral and gastrointestinal mucositis is approximately 63–95%. Factors that contribute to the development of mucositis are primarily the conditioning regimen (chemotherapy and TBI), and GVHD prophylaxis (MTX as well as fungal and viral infections, eg herpes simplex). It can involve the entire GI tract, specially the mucosa of the oral cavity and the oropharynnx. The clinical presentation consists of mucosal burning, erythema, and oedema, with progression to ulceration. The main goal of the management is to prevent or reduce the direct damage, promote rapid healing, and eliminate the risks of infections.

#### *1.3.1.2 Graft-versu-host-disease*

GVHD was first described in 1955 by Barnes and Loutit[111] as "secondary disease", which consisted of diarrhoea, weight loss, skin changes, and liver abnormalities in irradiated mice after allogeneic SCT. GVHD is the most common complication after allogeneic SCT. It is a consequence of donor cells recognizing the patient's antigens as foreign,[1] and the development of GVHD represents a key factor in the outcome after allogeneic SCT. The pre-transplant conditioning regimen and the post-transplant immunosuppression and compliance with the prophylactic regimen are important factors since they affect susceptibility of the recipient to develop GVHD. By increasing the conditioning regimen the immunosuppression decreases.[112] The major disadvantage of reducing the risk of GVHD by T-cell depletion is the increased risk of graft failure and recurrent leukaemia.

GVHD can manifest as an early (acute) or late (chronic) complication after SCT.

**Table 2. Early and late complications in children after SCT***

| **Early** |
|---|
| 1. Mucositis |
| 2. Acute GVHD, including ocular GVHD |
| 3. Haemorrhagic cystitis |
| 4. Venoocclusive disease |
| 5. Transplantation-related infection |
| 6. Transplantation-related lung injury |

| **Late** |
|---|
| 1. Endocrine abnormalities: |
| Delayed puberty |
| Short stature |
| Hypogonadism |
| Hypothyroidism |
| Infertility |
| 2. Chronic GVHD, including ocular GVHD |
| 3. Cataracts and decreased lacrimal function |
| 4. Skin and mucosal lesions |
| 5. Dental abnormalities |
| 6. Skeletal problems: |
| Non-traumatic avascular necrosis |
| Osteochondromas |
| Osteoporosis |
| 7. Lung function impairment |
| 8. CNS damage: |
| Impairment in cognitive capacity |
| Leukoencephalopathy |
| 9. Secondary malignancies: |
| Leukaemia and solid tumours |
| 10. Psychosocial problems |

**Cohen A, Faraci M, Nathan PC, Tabbara KF.*[104-107]*

#### 1.3.1.2.1 Acute GVHD

Acute GVHD[113-115] is a common complication after allogeneic SCT and it describes a specific syndrome characterized by dermatitis, gastroenteritis, and hepatitis that occurs within the first 100 days after SCT, frequently between 20 and 40 days. Despite immunosuppressive prophylaxis the incidence of GVHD can vary from 16–78%. There are different factors that affect the development of GVHD, for example if the donor is an identical sibling and the recipient is young, the risks of developing GVHD decline substantially. Other risk factors are listed on Table 3.

Additionally, lower relapse rates have been noted in patients who developed aGVHD. The first-line therapy includes a combination of immunosuppressive agents and corticosteroids.

The most common ocular clinical manifestations reported in children due to aGVHD are severe DES, acute pseudomembranous conjunctivitis, viral keratitis, like herpes zoster ophthalmicus, and atrophic disturbances of the cornea.[116,117]

**Table 3. Risk factors for the development of aGVHD***

| |
|---|
| Histoincompatibility |
| Allosensitization of donor |
| Patient age |
| Donor age |
| Gender mismatch of donor and recipient (female to male) |
| Omission of GVHD prophylaxis |
| Intensity of conditioning regimen |

** Deeg HJ, Yamaguchi M.*[119]

#### *1.3.1.3 Haemorrhagic cystitis*

Haemorrhagic cystitis (inflammation of the bladder)[118] presents with dysuria (pain or difficult urination) and haematuria (blood in urine). The incidence is between 10–20%. It has two presentations; the early form is mainly associated with conditioning regimens, especially with Cy and the later form with virus infections. Other factors that might trigger this disorder are the patient's physiology and genetics, graft characteristics, degree of immunosuppression, and GVHD. The main goal of management is to control symptoms and prevent long-term consequences.

#### *1.3.1.4 Venoocclusive disease*

Venoocclusive disease[120,121] of the liver is a frequent and sometimes fatal complication of SCT. Fatality rates are low in children. It is considered a toxic presentation of the chemo-based conditioning regimen, especially with Bu. The most common symptoms are jaundice or painful hepatomegaly, thrombocytopenia, and weight gain secondary to fluid accumulation in the abdomen. The symptoms that develop within the first three weeks after SCT are conditioning related and those developed after (> 20 days) are related to complications of SCT, like GVHD and infections. The incidence after SCT varies from 1–54%. Reduction of the risk factors will improve SCT outcomes. The main goal of the treatment is to prevent mortality.

#### *1.3.1.5 Transplantation-related infections*

The immune system is suppressed when donor cells are transplanted causing the suppression of the immune function of leukocytes, leading to increase in the risk of

infections. There are common infections caused by bacteria, fungi, viruses, or other parasites. The occurrence is determined by different factors such as the patient's history before SCT, underlying disease, type and intensity of conditioning regimen as well as the infection prevention treatment used.

##### 1.3.1.5.1 Bacterial infections

Bacterial infection[122] is a frequent complication after SCT. Important clinical risk factors are the presence of mucositis in the mouth and GI tract, indwelling central lines, and the duration of neutropenia. In the eye bacterial infections manifest as purulent conjunctivitis and herpes simplex keratitis.

##### 1.3.1.5.2 Protozoal infections

Protozoal infections[123] are rare except for the pneumocystis jiroveci infection. Risk factors include treatment with corticosteroids or CyA. The most common clinical manifestation is intersticial pneumonitis. Another less common protozoal infection is toxoplasmosis, caused by the toxoplasma gondii which can develop in immunusuppressed patients. Clinical manifestations are due to involvement of the CNS, lungs, eyes (chorioretinitis), and heart. Less common protozoal infections are malaria, amebiasis, and trichomoniasis among others.

##### 1.3.1.5.3 Fungal infections

Fungal infections[124] are opportunistic infections affecting immune compromised patients causing sometimes serious or lethal infections. They are more common in allogeneic SCT than in autologous SCT and may be due to the presence of GVHD. The most common fungal infections are caused by Candida followed by Aspergillus species. The neutropenic phase increases the risk of acquiring fungal infections since the skin and mucosal membranes of the respiratory, GI, and urinary tracts are disrupted by chemo and radiotherapy. Organs mostly affected by Candida are the kidneys, brain, heart, lung, eyes, skin, skeletal muscle, liver, spleen, bone, and joints.

##### 1.3.1.5.4 Viral infections

The most common viral infections[125] seen after SCT are cytomegalovirus (CMV), herpes simplex virus and varicella-zoster. One of the main risk factors is the development of GVHD due to natural immune suppression of the disease and from the medications given as treatment. Some systemic clinical manifestations of viral infections include pancytopenia, pharyngitis, pneumonitis, enteritis, and hepatitis; while the ocular manifestations are chorioretinitis and ocular infections.

#### *1.3.1.6 Early pulmonary complications*

The onset of these early pulmonary complications[126] are within the first few weeks after SCT. One of the earliest pulmonary complications is the rapid onset of pulmonary oedema. There are two main factors, first an abnormal renal and cardiac function due to chemotherapy and second the large amount of fluids administered during the procedure. The incidence has been reported in 43% of the patients

undergoing SCT. Other early pulmonary problems are the inflammation of the oral and nasopharyngeal mucosa, pulmonary vascular disease, and lymphocytic bronchitis.

### 1.3.2 Late complications

Many late complications are caused by radiation or chemotherapy. Patients given a single higher dose s-TBI, are at higher risk of earlier toxicities.[127] In order to reduce toxicity other treatment modalities have been implemented in the past years, like f-TBI (for those for whom radiation is the only option), high-dose chemotherapy combinations or immunosuppressive non-myeloablative agents among others.[38,128,129]

#### *1.3.2.1 Chronic graft versus host disease*

Chronic GVHD is an important complication after SCT. It normally occurs more than 100 days after the transplantation and can occasionally be fatal.[130] The incidence of cGVHD is greater in patients older than 15-20 years of age and with a history of a prior aGVHD.[131] In children the incidence of cGVHD is almost half of that reported in adults (children 22–29%: adults 40–50%). Other risk factors are female donor/male recipient, donor age $\geq$ 5 years of age, use of TBI, HLA disparity in patients undergoing MUD, diagnosis of haematologic malignancies, shorter duration of CyA prophylaxis, and latent infections in either the donor or the recipient.[131,132] Many organs and systems can be involved, such as the skin (scleroderma), liver (cholestasis and cirrhosis), eyes, mouth (mucosal thickening), lungs (fibrosis), GI tracts, vagina, oesophagus, and neuromuscular system.[115,133] The most common treatment for cGVHD is the long-term use of immunosuppressive therapy, such as CyA, and sometimes it is combined with corticosteroids.[130] Chronic GVHD may have a very poor prognosis if it is extensive and also affect the patient's quality of life.[134]

#### *1.3.2.2 Endocrine effects*

Endocrine insufficiencies[135,136] are among the most common long-term complications. Cytotoxic therapy and irradiation damage endocrine glands causing very specific clinical manifestations such as impaired growth velocity, impaired growth hormone secretion, gonadal failure, and hypothyroidism.

Growth hormone (GH) secretion is mainly affected by chemotherapy. The incidence of GH deficiency varies from 11–80%.[137-139] Growth velocity may be affected by irradiation by means of reducing production of insulin-like growth factor and skeletal growth if liver and bones are irradiated. Other possible factors are untreated hypothyroidism, lack of gonadal production, continuing GVHD, poor nutrition and corticosteroids therapy.[140]

Gonadal failure has mainly been associated with TBI and to a lesser extent with Cy and Bu, which seem to be more gonadotoxic than other chemotherapeutic

agents.[141,142] Some manifestations are delayed puberty development, girls who already had menstrual cycles before the cancer treatment will have amenorrhea in case of ovarian damage, and permanent damage in the majority of men, reducing spermatogenesis and increasing gonadotropine concentrations. [104,108]

Hypothyroidism[143] is the most common thyroid complication after SCT and very much related to the use of TBI. Other risk factors[104] are female patients, lower age (< 10 years of age), and a history of Hodgkins lymphoma. The incidence has been reported to be of 30% in a study population of 791 patients.[144]

#### *1.3.2.3 Skeletal complications*

The presence of skeletal problems in children after SCT is due to treatments used before and after SCT, such as TBI, intensive chemotherapy, CyA, and corticosteroids. Some of the most common manifestations are:

Non-traumatic avascular necrosis[145-147] is the result of a temporary or permanent cessation of blood flow to the bones and may develop after exposure to glucocorticoid therapy in male patients older than 16 years of age, with cGVHD, and diagnosed with SAA and ALL. The incidence in children is between 4–10%

Osteochondromas[148,149] are benign tumours composed of bone and cartilage. Multiple osteochondromas are common after SCT. Possible risk factors include male patients ≤ 3 years of age and TBI.

The development of osteoporosis (reduced bone mass)[149-151] may be due to corticosteroids, duration of exposure to CyA, previous cranial irradiation, and an untreated radiation-induced GH deficiency in childhood and adolescence.

Skeletal dysplasia[152] is defined as short stature with a height ≥ 3 SD below the mean height for age, may be a consequence of TBI and intensive chemotherapy. Final height depends on the total TBI doses, the volume irradiated and the age of the child.

#### *1.3.2.4 Orofacial*

Oral complications[153,154] are a major reason of morbidity and potential mortality for children undergoing SCT. This is because untreated dental caries and periodontal disease may cause severe oral infections and pathogens may spread producing a life-threatening condition. Children conditioned with TBI and Cy showed more disturbances in the dental development than those treated with several chemotherapeutic agents.

#### *1.3.2.5 Alopecia*

Temporary alopecia (abnormal hair loss)[152,155,156] is a frequent complication after myeloablative chemotherapeutic or radiation-based conditioning regimens. Other possible risk factors are higher age, and prior cranial irradiation.

#### *1.3.2.6 Pulmonary effects*

Late onset pulmonary infectious and non-infectious complications[157,158] contribute to early and late morbidity after SCT and develop in at least 15–25% of SCT patients. Patients receiving myeloablative regimens experienced a significantly higher rate of lung dysfunction after SCT than non-myeloablative patients. Pulmonary complications have been associatied with aGVHD, cGVHD, recipient's positive CMV serology, type of duration of immunologic defects and the conditioning regimens used, especially s-TBI.

#### *1.3.2.7 Cardiac effects*

Survivors of childhood malignancy represent one of the biggest risk groups for premature cardiovascular disease.[159-161] Radiation and Cy are the most cardiotoxic factors used as conditioning regimens. Radiation may directly affect all structures of the heart including the pericardium, myocardium, valves, conduction tissues, and coronary blood vessels causing functional abnormalities.

#### *1.3.2.8 Ocular effects*

The majority of the studies reporting ocular complications are undertaken with mixed groups of adults and children.[162-166] There are few studies performed only in children concentrating on ocular complications after SCT.[167-171] Ocular complications after SCT are common and can be classified into those affecting the anterior and posterior segments of the eye. The anterior segment complications[172] include keratoconjunctivitis due to GVHD, dry eye, anterior uveitis, and posterior subcapsular cataracts PSC). The posterior segment complications[173] more rarely investigated include microvascular retinopathies, optic disc oedema, and haemorrhagic and infectious complications. Anterior ocular complications occur 60–90% of patients with aGVHD and cGVHD. The more severe ocular complications are related to severe systemic cGVHD and poorer survival.[174,175]

##### 1.3.2.8.1 Dry eye syndrome

*"Dry eye syndrome (DES) is defined as a disorder of the tear film due to tear deficiency or excessive tear evaporation, which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort"*[176] DES is a frequent ocular complication after SCT but the majority of the studies had been made in adults and very few in children. However, De Marco and co-workers reported that 6% of the children developed some clinical manifestation of DES associated with GVHD.[168] DES has pathological and clinical similarities to Sjögren syndrome. It is

associated with tear hyposecretion, superficial epithelial thinning, and keratinisation of the cornea and conjunctiva that might progress to corneal ulceration, pannus, scarring, and eventually in extreme cases to perforation. The most common causes of DES after SCT in children are aGVHD, cGVHD, female gender, s-TBI, chemotherapy-induced ocular toxicity, immunosuppressive therapy, and infections.[107,166,174,177,178]

The presence of DES in children is less common than in adults because conjunctival epithelial cells which may be damaged in aGVHD and cGVHD can regenerate faster.[179] In mixed adult-children studies the development of dry eye or ocular sicca syndrome was about 76% of patients with aGVHC, between 62–82% with cGVHD, and about 10% in those without cGVHD.[165,166,180] There is a risk for females of developing late dry eye after SCT.[166] This might be the effect of hormonal changes due to conditioning levels. It can be compared with the hormonal changes menopausal and postmenopausal woman go through.

Methothrexate (MTX)[50] might be a drug that aggravates the clinical manifestations of dry eye since it seems to affect the function of the meibomian glands, manifesting as a severe blepharitis. It has been reported that at least 25% of patients on MTX may develop ocular irritation, periorbital oedema, blepharitis, conjunctival hyperemia, epiphora, and photophobia.

#### 1.3.2.8.2 Graft-versus-host-disease

The development and treatment of DES has been evaluated for several years and different studies have concluded that it is closely related to GVHD.[163,166,174,181-183] The course of ocular GVHD has been classified in four stages[174,181]:

Stage 1: This subclinical stage manifests with tearing, mild or no special discomfort or photophobia. The eyes are slightly red, and may present a mild chemosis and may or may not have positive Rose Bengal staining. The basal tear production can be normal. This stage can last from a few days to up to one month before systemic manifestations of GVHD appear or before it develops into a more severe form of ocular GVHD.

Stage 2: In the active stage of ocular GVHD, the patient also starts developing systemic manifestations of GVHD. The ocular manifestations vary from mucupurulent conjunctivitis, pseudomembranous conjunctivitis with or without epithelial slough, peripheral corneal neovascularisation, (Fig 2) calcium deposits in all corneal layers, keratinisation to punctuate keratitis, and corneal erosion.

Stage 3: In the convalescent stage the initial inflammatory process stops and the stage is characterized by secondary sicca syndrome. Examination under the biomicroscope shows irregular eyelid margins with obstructed meibomian gland orifices, tarsal and forniceal scarring and punctuated corneal epitheliopathy. Rose Bengal staining shows a dry eye pattern (staining of the interpalpebral bulbar conjunctiva). The eyes

are also heavily stained at the posterior lamellae of the eyelid margins and the superior and inferior limbus. (Fig. 2)

Stage 4: In the necrotising stage the cornea starts to melt and sometimes perforation can occur.

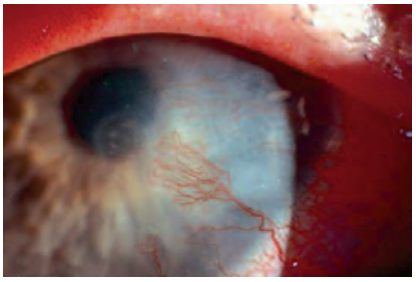

***Fig 2.*** *Ocular GVHD. Corneal Neovascularisation.*
*Courtesy Branca Samolov*

The presence of these ocular manifestations is mostly associated with severe systemic cGVHD and poor survival.[174]

In order to control and prevent permanent ocular damage a dual supportive care has been incorporated into systemic and ocular manifestations of GVHD. Depending on the severity of the condition different treatment alternatives are available, like preservative-free artificial tears, long-acting lubricants, systemic and/or topical CyA and/or other immunosuppressive medication, glucocorticoid, autologous serum eye drops, antibiotics, eye patching, bandage contact lenses, punctual occlusion, conjunctival patch, scleral flap and/or tarsorrhaphy.[174,181,184,185] One option for patients with severe corneal perforation may be amniotic membrane.[186]

Topical CyA helps to promote the healing process, decreases the immunology activity, increases the Schirmer scores, and decreases surface apoptosis. The use of topical CyA in the early period of ocular GVHD can minimize the development of DES. Initial studies [181] used 1% CyA but also CyA 0.05% and 0.1% are reported to be effective in leading to improvements in the objective and subjective findings.[187]

#### 1.3.2.8.3 Cataracts

Cataract is one of the first most common and most well reported of all late complications after SCT in children. (Table 4) Different risk factors for developing cataract have been reported including conditioning regimen (chemotherapy and/or TBI), type of fractionation, fractionation scheme, dose rate, type of transplant, age, aGVHD, cGVHD, systemic corticosteroids, and previous prophylactic irradiation (cranial or orbital irradiation).[188-190]

**Table 4. Previous studies on incidence of cataracts, and conditioning regimen effects after stem cell transplantation in childhood.**

| Reference | Number of patients (n) | Median age at SCT (years) | Follow-up time (years) | Chemo (n) | s-TBI (n) | f-TBI (n) | Incidence cataract (%) | Median time to cataract detection from SCT (years) | Cataract surgery (n) | Median time to cataract surgery (years) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lappi M, 1990 | 9 | 10.8 | 4.5 (3.3–7) | 9 | 9 | 0 | 100 | 3 | 2 | No info |
| Calissendorff BM, 1991 | 44 | 9.7 (1–17) | 4.9 (2–9) | 44 | 36 | 0 | 75 | 2.2 (1–3) | 20 | 4.7 (3–9) |
| Calissendorff BM,76 1993 | 61 | 9.5 (1–17) | 5 (1–10) | 18 | 43 | 0 | 72 | 3 | 17 | 5.1 (3–9) |
| Thuret, I 1995 | 13 | 9 (1–16) | 5 (2–10) | 13 | 0 | 8 | 15 | 2 | No info | No info |
| De Marco R, 1996 | 100 | 7.1 (3.8–10.4) | 4.2 (2–9) | 47 | 0 | 53 | 17 | 3 | No info | No info |
| Leahey, AM 1999 | 26 | 15.1 (3.3–25.9) | 5.6 (2–15.4) | 18 | 8 | 0 | 23 | No info | 1 | No info |
| Frisk 2000 | 29 | 9.7 (1.9–17.9) | 8 (4–10) | 8 | 20 | 1 | 76 | 3 | 6 | 5 (4–9) |
| Holmström G, 2002 | TBI: 21 Bu: 24 | TBI: 9.8 Bu: 5.9 | TBI: 5.1 Bu: 6.1 | 24 | 18 | 3 | TBI: 95 Bu: 21 | TBI: 2.5 (1–4) Bu: 5 (1–7) | TBI: 8 Bu: 1 | TBI: 3 Bu: 9 |
| Leung W, 2007 | 155 | 9.7 (0.5–21.4) | 9.0 3.1–15.9) | 155 | 123* | 0 | 33 | 4.5 (3.3–6) | No info | No info |

*Bu,busulfan; N,number of patients; f-TBI, fractionated TBI; SCT, stem cell transplantation; s-TBI, single dose total body irradiation; * not specified TBI;*

The administration of TBI has shown a higher incidence on cataract development than with chemotherapeutic agents.[169,191]

Long-term use of corticosteroids is related to the development of posterior subcapsular cataracts (PSCs)[192,193] and other side effects presented in Table 5. The incidence of drug-induced PSC might be related to the dosage and duration of the drug administration, susceptibility of the individual or genetic effects.[194-196] Corticosteroid induced-cataracts appears in both children and adults. The difference is that children tend to develop PSC at lower doses and within a shorter period of time than older patients.[192,197]

Cataract formation has also been associated with cGVHD.[190] This association is most probably due to the side effects of corticosteroids used in the treatment of cGVHD.

**Table 5. Ocular side effects of corticosteroids***

| System affected | Adverse effects |
|---|---|
| Eyelids and periorbital tissue | Exophthalmos |
| Lens, iris, and ciliary body | Myopia, cataract, increase intraocular pressure |
| Ocular motor function | Cranial nerve palsy |
| Retina and optic nerve | Papilloedema, optic neuropathy, optic nerve atrophy |

** Abdollahi M, Shafiee A, Bathaiee FS, and co-workers.* [198]

1.3.2.8.4 Herpes zoster ophthalmicus

Herpes zoster ophthalmicus (HZO)[199,200] is very uncommon in the general paediatric population. The presence of HZO in children after SCT was first described in 1999. Ocular complications related to HZO are extraocular muscle palsies, cicatricial changes of the eyelid, conjunctivitis, episcleritis, scleritis, keratitis, iridocyclitis, sectoral iris atrophy, glaucoma, optic neuritis, retinitis, and retinal vasculitis. The visual acuity normally remains unaffected (> 0.65)

1.3.2.8.5 Posterior segment

The development of posterior segment complications[179,201] in children after SCT is lower when compared with the anterior segment complications. The incidence ranges from 7–13.5% and they occur within the first 2 years after SCT. The complications from the posterior segment seem primarily associated with TBI, high-dose chemotherapy, and CyA.

The most common clinical manifestations related to haemorrhagic complications are vitreous and/or intraretinal haemorrhages seen within the first six months after SCT and they resolved without long-term visual disturbances.[168,179] Cotton-wool spots have also

been reported in children.[179,201] Ischemic fundus lesions develop mainly in patients treated with TBI, high-dose chemotherapy and CyA.

Posterior segment infections[57,168,179] described in children are fungal retinitis and endophthalmitis, developing early (two months) after SCT. Viral and toxoplasmosis retinitis are late complications. CMV is not very common but is one of the most devastating complications with a risk of severe visual deprivation.

Optic disc oedema in children[168,179,202] has been associated with the administration of CyA and has been described as resolving after discontinuation or decrease of CyA dose. (Fig 3)

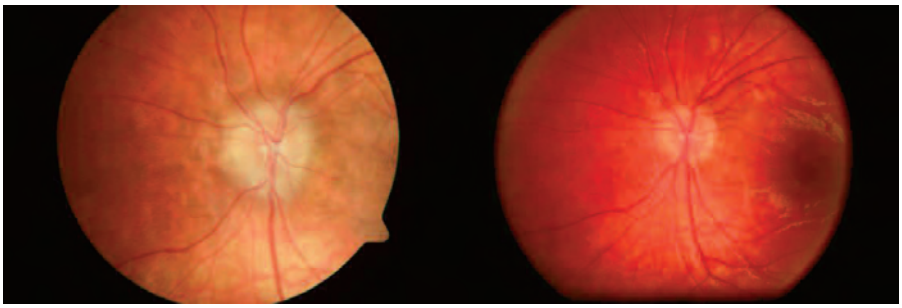

***Fig. 3.*** *Papilloedema related to cyclosporine A in a nine-year old girl. About one year time interval between the images.*

In summary, ocular involvement is very common in children after SCT. A great number of patients show evidence of cataract, dry eye syndrome to a less extent posterior segment changes, like ischemic retinopathy and papilloedema after SCT. These findings in most cases require pharmacological or surgical treatment. The ocular complications rate after SCT in adults is well documented but poorly reported in the paediatric group.

This study will describe the results of an analysis of the long-term visual and ocular outcomes in a large group of survivors of childhood cancer that went through SCT. The main goals of this study were (i) to describe visual outcomes and ocular features relevant to children undergoing SCT, like BCVA, rarebit (RB) perimetry, and disc morphology; (ii) to determine the effect of specific cancer treatment and underlying diseases on the risk of developing later ocular complications, like cataract formation, dry eye syndrome, and posterior segment changes; (iii) to analyse the timing of cataract formation and the relation to conditioning regimen.

#### *1.3.2.9 Neurological complications*

Neurologic complications are usually an early event after SCT and may be transient or result in a high mortality. There is an incidence of 59% of neurologic

complications in children.[203] They are classified as CNS infections, metabolic encephalopathies and haemorrhagic cerebrovascular events. They may be related to the underlying disease, the donor type, the drug neurotoxicity used during the conditioning regimen, especially from the use of Bu, immunosuppressive agents, like CyA, corticosteroids, or antimicrobials and antifungals used for the treatment of infections.[159,204] The most common clinical manifestations[159,205] are posterior leukoencephalopathy syndrome (multifocal demyelinating white and grey matter changes) related to headaches, confusion, cortical blindness, visual hallucinations, seizures, and motor deficits; vasculopathies, strokes and migraine-like episodes; CNS tumours, like meningiomas; and late CNS bacterial (Streptococcus pneumoniae, Haemophilus influenza, and Listera monocytogenes); fungal (Aspergillus fumigatus), parasitic (Toxoplasma gondii), and viral (CMV, Herpes simplex, Varicella zoster, and Epstein-Barr virus (EPV) infections.

#### *1.3.2.10 Neurocognitive and psychological effects*

Neurocognitive changes[46,206-208] are among the most incapacitating late effects experienced by survivors of childhood cancer, with developmental consequences in educational, social, and behavioural areas. There is a discrepancy in the results reported; some show no significant differences in cognitive function up to 5 years after SCT, while others show a deteriorating neurocognitive function already by 1 year after SCT. It seems that neurocognitive dysfunctions are more frequent in the younger age group (< 3 years of age) exposed to cranial irradiation in addition to TBI due to the higher sensitivity to the therapy induced-damage.[207-210] Delays in different cognitive areas are very frequent; the most commonly affected areas are fine motor skills, visual-spatial processing, verbal reasoning, memory, attention, and abstract thinking. The presence of any of these affected areas can lead to learning disabilities in the child that affect school performance.[159]

The underlying disease is another factor that has a major impact on the neurocognitive development of the children. The risk of neurocognitive deficits in patients with malignant diseases treated with SCT following relapse increases due to CNS prophylaxis.[211] In the non-malignant conditions,[212] neurodevelopmental results appear to depend to a larger extent to the functioning level at the time the child is transplanted. For example, early SCT seems to be related to better outcomes and children with cognitive impairment do not benefit to the same degree when compared with children normal cognitive development.

Psychological problems[213,214] in children receiving SCT are associated with factors, such as, chemotherapy, radiation therapy, treatment complications, prolonged isolation from home and school, feelings of guilt about consuming the family's resources and body image. These problems are present while the child is under treatment and follow throughout adulthood. The incidence of these manifestations is between 16–20% among young adult survivors of childhood cancer.

#### *1.3.2.11 Secondary malignancies*

Secondary malignancies after SCT have been well described.[215,216] The common complications in this group are:

Post transplant lymphoproliferative disorders and lymphomas[215,216] are more related to allogeneic SCT. The presentation occurs approximately 5–6 months after SCT. The incidence range is between 1–20%. Some risk factors are EBV, HLA disparity, use of ATG during conditioning regimen or GVHD prophylaxis, and GVHD.

Leukaemia and MDS are the most common haematological malignancies[216,217] that can develop after SCT. These malignancies occur more frequently in autologous recipients exposed to chemotherapeutic agents and TBI. They are very rare and have a very low incidence. It might be related more to the pre-transplant therapy than to the SCT itself.

The median age for developing solid tumours[215,218,219] after SCT is 3–8 years of age and it increases in children > 10 years of age. The major risk factors are radiation and chemotherapy prior to allogeneic SCT, immunodeficiency from incomplete recovery after allogeneic SCT, and immune stimulation and suppression from GVHD. Other associations are squamous cell carcinoma, GVHD, male gender, and leukaemia as an underlying disease.

#### *1.3.2.12 Quality of life*

"Quality of life (QOL)[220-225] is a dynamic concept related to physical, cognitive, emotional, and social functioning and well-being." Even though SCT is a life saving process, it also involves several acute complications and late effects creating a negative impact on QOL. There are several recovery phases the patient goes through after SCT. Physical functions rapidly deteriorate after SCT, and begin to recover during the first year and then continue to improve during the next four years. Emotional recovery begins very early in the process. It starts once the patient is discharge from the hospital and it continues to improve in the second to fourth year after SCT. In the social area no major changes are experienced and by the second year after SCT socializing is almost fully restored. The return to schoolwork and everyday life is difficult after SCT but there is a slow improvement over time. By one year after SCT most are back to normal activities. In general, survivors have reported good overall QOL as time increases from the SCT.

# 2 AIMS OF THE THESIS

The main aim of this thesis was to determine visual functions, ocular complications, visual evoked potentials, and visual processing skills in patients treated with allogeneic SCT in childhood. Further aims were to determine the possible impact of underlying diagnoses, the use and extent of irradiation, chemotherapy, immunosuppressive drugs, opportunistic infections and GVHD on ocular status and to update and improve clinical guidelines for ocular examination in patients treated with SCT in childhood. Further aims were to analyse groups of patients with rare metabolic or genetic disorders more in detail.

**Paper I**
To report visual functions and the prevalence of cataract in children and young adults after allogeneic SCT in childhood.

**Paper II**
To evaluate the incidence of DES with corneal complications, tear film abnormalities and insufficient tear production in children after allogeneic SCT.

**Paper III**
To investigate the prevalence of RB visual field defects and optic disc morphology in patients treated with allogeneic SCT in childhood.

**Paper IV**
To study visual outcomes, ocular findings and visual processing skills in patients with FA.

**Paper V**
To determine the effect of early treatment with SCT on visual function and ocular status in patients diagnosed with MPS I-H.

**Paper VI**
To determine the frequency of visual pathway disturbances detected by VEP after SCT in childhood

# 3 MATERIAL

The project was divided into two parts:

1. A cross sectional study with longitudinal follow-up of children and young adults who had undergone SCT at least 2 years earlier at Karolinska University Hospital, Huddinge and who came for annual follow-ups between June 2004 and September 2009.

2. A prospective/longitudinal study in which children were included just before a planned SCT, from June 2004 to May 2007, and will be examined annually for at least 10 years after SCT.

## 3.1 PATIENT POPULATION

**Paper I:**
The cross sectional study group consisted of 79 children and young adults (42 girls and 37 boys) who had undergone allogeneic SCT with HLA-matched donors between December 1986 and May 2004 at a median age of 6.5 (range 0.4–15.6 years). The follow-up time post SCT was 7.0 years (range 2–18 years). Median age at examination was 14.9 years (range 3.9–23.5 years). Ophthalmic medical records were retrieved in all patients, but two were excluded due to pre-existing lens opacities.

All eligible patients invited to participate accepted. No drop-outs occurred during the study period. Not all 79 children were included in all studies due to lack of cooperation and/or poor picture quality (papers II, III, and VI). In papers IV and V the patient population was grouped according to a common disease.

**Paper II:**
Sixty children and young adults (33 girls, 27 boys) from the cross sectional group with a median age of 7.6 years (range 0.4–15.5) were included. Age at latest ocular examination was median 15.5 years (range 5.5–23.5). DES was defined as corneal epithelial lesions occurring without any other evident explanation and in the presence of a pathological break-up time (BUT) and/or Schirmer. Only patients in whom it was possible to examine and grade the cornea with BUT and Schirmer values were included.

**Paper III:**
Fifty-three patients (27 male, 26 female) with a median age of 15.4 years (7.4–20.9) underwent visual field examinations. Fundus photographs were obtained in 51 patients (27 males, 25 females) and photographs from 22 patients (44 right eyes and 42 left eyes) had adequate quality for digital analysis.

For this paper two reference groups were selected to enable inclusion of the data of the right eye of healthy children and young adults. The clinical data of the control children is presented in tables 6 and 7.

**Control group I:** The reference group for the RB perimetry analysis consisted of 51 subjects (26 male and 25 female) with a median age of 15 years (range 6.5–20). Only data from the right eye data was used.

**Control group II:** The reference group for the optic disc morphology analysis consisted of 49 healthy adolescents (24 boys and 25 girls) with a median age of 15 years.

**Table 6. Rarebit visual field test in the healthy control group***

| | **Right Eye**<br>**Median MHR % (range)** |
|---|---|
| SCT control ≤ 12 years | 93 (78–100) |
| SCT control > 12 years | 97.5 (89–100) |

*SCT = Stem cell transplantation; MHR = Mean hit rate. Martin L.*[226]

**Table 7. Optic disc parameters in reference group***

| | **Right Eye** |
|---|---|
| DA mean (mm²) | 2.13 ± 0.41 |
| CA mean (mm²) | 0.18 ± 0.18 |
| RA mean (mm²) | 1.95 ± 0.42 |

*DA = Disc area; CA = Cup area; RA = Rim area. Hellgren K, Hellström A, & Martin L.* [227]

**Paper IV:**
Ten unrelated FA patients (7 boys, 3 girls) with a median age of 11 years (range 9–16) were grouped together in part of the study. Six of the 10 children belonged to the cross sectional group and four to the prospective/longitudinal group. In all 10 patients the FA diagnosis was confirmed before SCT by a chromosomal fragility test after exposure to diepoxybutane or mitomicin. Disc parameters were compared with the reference data presented in table 7.

**Paper V:**
Four children (2 boys, 2 girls) diagnosed with MPS I-Hurler coming from the cross sectional study were included in this study. The median age at SCT was 16.5 months. Diagnosis of MPS I-H was based on clinical characteristics together with biochemical assessment of glucosaminoglycans (GAG) in urine and enzyme alpha-L-iduronidase in blood.

**Paper VI:**
Pattern VEPs were obtained post SCT in 47 patients (20 boys, 27 girls) with a median age of 15 years (range 7–20). The follow-up time was median 9 years (range 5–15).

The cross sectional study group was divided into four categories depending on the type of conditioning (groups I–III) and treatment after SCT (group IV). The numbers vary depending on the number of children included in each sub-study:

**Group I:** Thirty five patients diagnosed mainly with ALL were conditioned with TBI: 17 with s-TBI (10 Gy) and 18 with f-TBI (3 Gy/day x 4 days). Their eyes were not shielded during treatment. The median age at eye examination was 16.5 years (range 4–20.5). The median age when SCT was performed was 8 years (range 1–15 years). The median follow-up time post SCT was 8 years (range 2–17).

**Group II:** Instead of TBI, 33 patients diagnosed primarily with AML were conditioned with Bu. In most cases, Cy was also included in the conditioning regimen. The median age at eye examination was 14 years (range 3–20.5). The median age when SCT was performed was 3.5 years (range 6 months–13 years). The median follow-up time post SCT was 6 years (range 2–17).

**Group III:** Eleven patients diagnosed mainly with FA were conditioned with other chemotherapeutic drugs – mainly Cy (without Bu) or Flu together with ATG. The median age at eye examination was 13 years (range 9–20). The median age when SCT was performed was 7 years (range 3–11 years). The median follow-up time post SCT was 5 years (range 2–15)

**Group IV:** Of the 79 patients 27 patients were diagnosed with cGVHD of which 26 needed corticosteroid treatment at least 6 months after SCT, and 17 patients had CyA 250 ng/ml trough levels on more than 7 occasions.

## 3.1 ETHICS

The study was performed in accordance with the Helsinki Declaration and approved by the Human Research Ethics Committee of the Karolinska Institutet. Written informed consent was obtained from some children (> 15 years of age) and their parents after an oral presentation of the study.

# 4 METHODS

A comprehensive ocular assessment was performed. The examination lasted between 1 to 3 hours. All the tests were adapted to age and cooperability of each patient. The detailed examination is described below.

## 4.1 ANAMNESIS

A thorough somatic history including gestational age, obstetric history, prenatal and postnatal complications, family background and visual and ocular symptoms were obtained from patients, parents and previous medical records.

## 4.2 VISUAL FUNCTION TESTS

### 4.2.1 Visual acuity

Best corrected decimal visual acuity (BCVA) was measured at distance and near. For distance the Anders Hedin Chart and for near the "Stilskalor Svenska" chart (developed by Anders Hedin 1982) were used. When a patient could not respond to the Anders Hedin Chart or the "Stilskalor Svenska" chart, the Lea Hyvarinen symbol chart[228] was used. Distance and near visual acuity were tested monocularly and binocularly at a distance of 4 and 0.33 m respectively. The visual acuity measurement was the last full line read and it was expressed in decimal for distance and for near.

### 4.2.2 Ocular alignment and motility

Cover test was used to determine the presence and direction of ocular misalignment at a distance of 4 and 0.33 m and using the patient's habitual correction. In the presence of deviation, the magnitude was measured in prism dioptres (pD). The phoria was defined as a latent deviation of exophoria > 4pD or esophoria > 2 pD at near and/or distance.[229]

To evaluate motility a target placed at 40 cm was used. The patient was asked to follow the target in the nine positions of gaze. This test showed the presence of paralysis, paresia, or end-point nystagmus

### 4.2.3 Stereo acuity

This was performed using the Lang I stereo card.[230] The Lang I card was presented to the patient at 40 cm and the examinee will report a cat 1200", a star 600", and/or a car 550" seconds of arc. The responses were recorded as the smallest second of arc seen.

### 4.2.4 Sensory system

This was measured with the Worth 4-dot test. The test was made at 40 cm. During the examination, the patient wore red and green goggles and a modified flashlight with four coloured holes was presented. The holes are arranged with the top hole showing only red light, the left and right showing only green light and the bottom showing white light. Fusion was present when the patient reported all four holes, suppression when the

patient reported either two red or three green and diplopia when the patient reported five holes.

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### 4.2.5 Amplitude of accommodation

The amplitude of accommodation (AA) was measured with the RAF near-point rule (Royal Air Force)[231]. The rule consists of a 50 cm long square section rule with a plastic slider holding a rotating four side drum, showing four different types of targets. The measuring scales are centimeters and the equivalent in dioptres. The test was performed monocularly with the patient was wearing their habitual correction. Each patient was asked to maintain fixation at a selected accommodative target. The target was moved closer towards the patient until blur or diplopia was reported or until the slider came in contact with the cheek rest. The test was repeated three times; the values were averaged and then compared to reference material.[232]

### 4.2.6 Colour vision

The HRR (Hardy Rand Rittlers)[233] colour vision test was used. This is an easy test to administer and it can be used for verbal and non-verbal patients. It consists of 24 plates, each one presenting one or two symbols, a cross, a circle or a triangle. The symbols are composed of small coloured dots against a background of grey dots. The first four pages are seen by all patients, the following six are for screening and the subsequent 14 plates are to grade the severity of the deficit and to differentiate between the different colour deficiencies.

The patient was asked to name the shape of each symbol and its location on the page; if the patient missed any of the screening plates all 24 plates were presented. The criterion for failure is three or more errors.

### 4.2.7 Cone adaptation test

This was measured with the Lea Hyvarinen cone adaptation test.[234] This test consisted of 15 5 x 5 white, blue and red plastic squares. These were presented to the child under three different types of illumination, bright (540 lux), dim, (3 lux) and dark (0 lux). The different illuminations were measured by the Hagner Screen Master (Sweden) and are the results of five different measurements. Under each type of illumination, the patient was asked to sort the squares into three groups according to the colour and as soon as possible after the light have been dimmed. The responses were recorded as normal if the patient sorted the squares correctly in all three types of illumination and abnormal if the patient sorted the squares incorrectly in at least one type of illumination.

## 4.3 REFRACTION UNDER CYCLOPLEGIA

Cycloplegic refraction in both eyes was measured either by retinoscopy, auto refractor (Topcon RM-8000B) or both 40 minutes after a single instillation of a mixture of

cyclopentolate (0.85%) and phenylephrine (1.5%). Hyperopia was defined as a refractive error ≥ 2 dioptres (D); myopia ≥ 0.50 D; astigmatism ≥ 0.75 D, and anisometropia as ≥ 1 D difference between the eyes.[235]

## 4.4 OCULAR HEALTH

### 4.4.1 Biomicroscopy (Papers I, II, IV, and V)

The anterior segment of the eye was examined with a slit lamp. The cornea was evaluated after instillation of fluoresceine. The corneal staining was recorded in percentages of surface involved: 0 = normal; 1–10%; 10–25%; 25–50%, and > 50%.[178]

The lens was evaluated under dilation and the opacities were scored into grades 1–3: grade 1 was defined as minor/minimal opacities (including not only posterior subcapsular cataract but also other types if fine nuclear or cortical opacities); grade 2 as small cataract with slight impact on BCVA or normal BCVA but subjective symptoms; and grade 3 as pronounced impact on BCVA.

### 4.4.2 Tonometry

The intraocular pressure (IOP) was measured using a non-contact puff air tonometer (Computerized tonometer Topcon CT-80; Japan). Three measurements were taken and the average was put into the register.

### 4.4.3 Fundus evaluation

The fundus was evaluated after instillation of dilating eye drops. The retina, optic nerve head, and nerve fibre layer were observed first with indirect ophthalmoscopy and then using the biomicroscope.

## 4.5 ADDITIONAL PROCEDURES

### 4.5.1 Dry eye examination (Paper II)

#### *4.5.1.1 Dry eye syndrome questionnaire*

A questionnaire on dry eye symptoms adapted from Lemp[176] was used. This questionnaire asked about dry eye sensation, ocular irritation, red eyes, photophobia secretion, itchiness, burning sensation, and allergies.

#### *4.5.1.2 Break-up time test*

The break-up time test (BUT) was measured after instillation of fluoresceine to evaluate the tear film quality. The BUT was defined as the interval from the last blink to the first appearance of a dark spot. The test was repeated three times and the values were then averaged. This test was pathological if results were ≤ 10 seconds.[176]

#### *4.5.1.3 Schirmer test*

Schirmer I test[236] was performed without anaesthesia, thus measuring stimulated tear secretion. A 5 mm strip of filter paper was placed in the outer canthus and left for 5 minutes. The amount of tear film that covered the strip was measured in mm. The Schirmer test was considered positive if the line of imbibition was < 10 mm.

### 4.5.2 Visual fields (Paper III)

The computerized RB perimetry is designed to test the integrity of the neural architecture of the visual system. It depends on standard personal computer (PC) components. This test was used before dilation to measure the 30° x 20° central visual field. The test method is extensively described elsewhere.[237] The test was performed in a dark room at two distances 50 cm (tests more peripheral locations) and 1 m (tests more central locations, approximately 5–10° central visual field). No headrest was necessary since comfortably seated subjects will sit still enough. A fixation mark was presented on-screen allowing access to all 30 test locations in a pseudo-random order. This fixation mark changed dynamically to attract and hold attention. The fixation was controlled by the examiner who was sitting on the side. Each examination was performed with the patients' habitual correction. Each area was examined by presenting one or two bright microdots exposed for 200 ms against a dark background. The task of the examined patient was to respond to the single dot by one mouse click and to two dots by double click. Results are presented in a percentage format (the sum of the stimulus seen divided by the sum of the stimulus presented) and expressed as mean hit rate (MHR). A normal subject should obtain a MHR close to 100%. (Table 6)

### 4.5.3 Fundus photography and digital analysis (Paper III)

Digital fundus photographs were obtained in the majority of patients through a dilated pupil using one of the two fundus cameras: Canon EOS-1 Kodak Professional DCS 520C (Canon, Rochester, New York, USA) or Canon CRDG Non-mydriatic Retinal Camera (Canon, Tokyo, Japan). The ocular fundus photographs including the optic disc appearance and nerve fibre layer were subjectively evaluated.

The photographs were then analyzed digitally. Only correctly focused photographs from eyes with both the optic disc centred and the macula well-defined were accepted for analysis. In order to compensate for magnification due to camera optics and the refraction of the eye, the centre of the macula was marked. The disc area (DA), cup area (CA), and rim area (RA) were evaluated by marking the end-points of the long and short diameter of the optic disc and cup, assuming elliptical shape.[238,239] The distance between the centre of the macula and the optic disc centre was used as a reference measure when converting pixel units to metric distance.[113] The evaluations were made by an independent observer.

### 4.5.4 Visual evoked potentials (Paper VI)

Visual evoked potentials (VEPs) were recorded with a Nicolet Viking Select (Nicolet Biomedical Inc. Madison, WI, USA). The patients were comfortably seated in a darkened room and were instructed to fixate the centre of a reversing checkerboard pattern displayed on a monitor placed 1.3 meters in front of the eyes. Gold-pleated electrodes were attached over the scalp. The active electrode was placed at Oz (occipital midline), the reference electrode at Fz (frontal midline) and a vertex (Cz) electrode acted as ground. The impedance was maintained below 5 kΩ. Each eye was examined separately with the other eye covered with a black patch. Care was taken to keep the patients as relaxed as possible to minimize artefacts and cooperation of the subjects (fixation of the pattern) was monitored by the technician.

The field size of the checkerboard pattern was 12° x 16°, each individual square subtended 30 minutes of arc, the contrast of the pattern was around 87 % and the reversal rate around 1 Hz. Filter settings were 1 Hz and 100 Hz respectively. The average response to at least 100 reversals was recorded, the numbers of reversals were increased if the VEP was poorly defined and, in most cases, each eye was examined at least twice to confirm reproducibility.

The latency and amplitude of the P100 potential was determined with help of cursors and the values were compared with the reference values for children at the department. A latency > 2.5 SD of the normal mean, or an inter eye latency difference exceeding 7 ms, was regarded as an abnormal response. VEPs were rated as either normal or abnormal.

### 4.5.5 Corneal and periocular measurements (Paper IV)

Corneal diameter and periocular measurements (Fig 4) were obtained from photographs with a transparent ruler beneath the lower eyelid as reference. The mean corneal horizontal diameter has previously been reported to be 12.04 mm ± 0.42 in a normal population of children > 6 years of age.[240] Microcornea was defined as a horizontal diameter less than 2SD from the mean (< 11.23 mm)

Periocular measurements included outer canthal distance (OCD), inner canthal distance (ICD), palpebral fissure length (PFL), and palpebral fissure width (PFW). OCD, ICD, and PFL were compared to reference data,[241] in order to determine the presence of hypo- or hypertelorism. Ocular hypotelorism was defined as reduced OCD and ICD And ocular hypertelorism as an increased distance of the OCD and ICD.[241] The upper eye lid margin to the corneal reflex distance (UMRD) was measured to establish the presence of ptosis, defined as UMRD ≤ 2 mm, or if there was a ≥ 2 mm asymmetry between the two eyes[242] but also if the PFW was below reference values.[243]

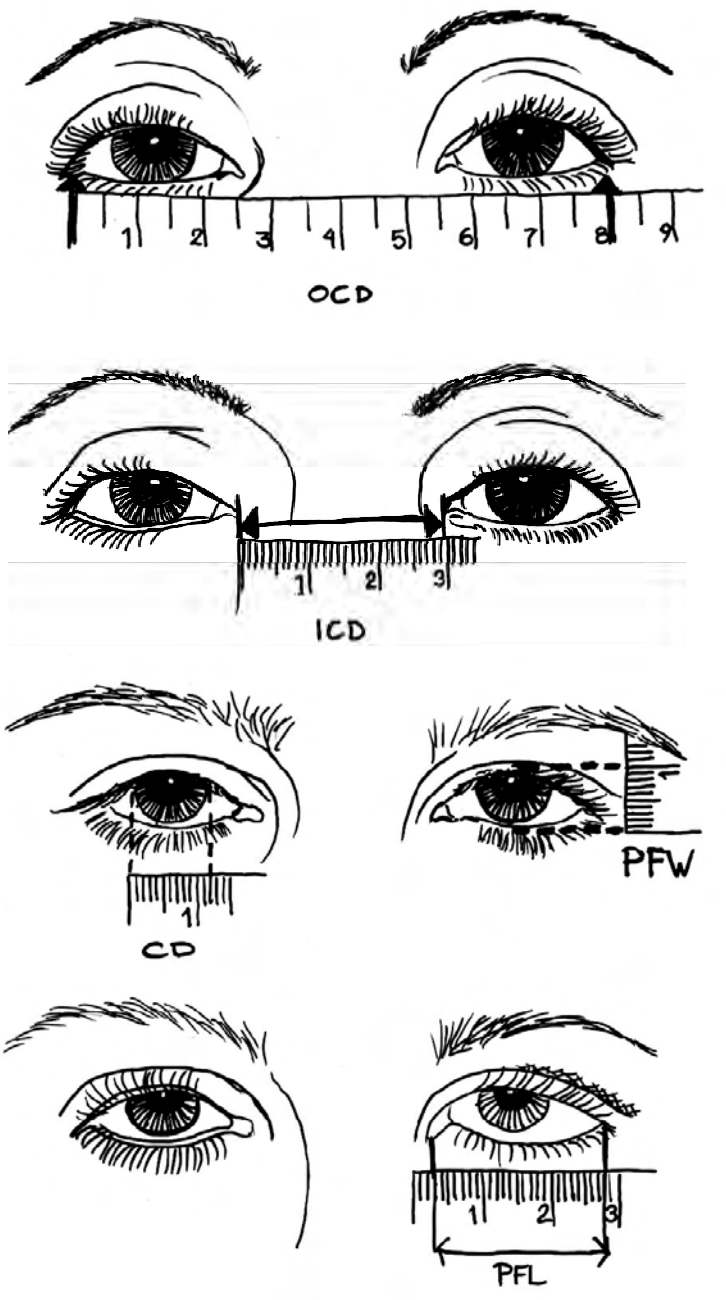


***Fig 4.*** *Measurements of outer canthal distance (OCD), inner canthal distance (ICD), corneal diameter (CD), palpebral fissure width (PFW), and palpebral fissure length (PFL). Drawings by Annika Botes.*

### 4.5.6 Developmental Eye Movement Test (Paper IV)

Saccadic eye movements and automaticity were tested with the developmental eye movement (DEM) test[244] which is a clinical ocular motor test of a visual-verbal format. The test was performed individually in a relative quiet room with good illumination and as free as possible from auditory and visual distractions. It was administered to children ≥ 5 years of age using the test instruction protocol described in the DEM Examiner's Booklet. Testing takes 5–10 minutes.

The DEM was given after full eye examination and before dilation. The pre-test, an abbreviated horizontal array of numbers, was used to assess number knowledge and articulation. The pre-test was given to all patients 6 years of age or to anyone when there was a concern about inadequate number knowledge or when difficulties in seeing printed numbers were suspected. The A (Fig 5) and B tests consisted of two vertical array of 40 numbers each. For these two tests the patient was instructed to read the numbers aloud down the columns as quickly as possible without stopping at the bottom of the first column. The patients were not allowed to use a finger to keep their place. The test C consisted of 16 horizontal rows with 80 numbers in total (Fig. 5). For this test the patient was instructed to read the numbers aloud across the rows as quickly as possible without stopping at the end of each line. The time and any errors were recorded and the results interpreted according to an age or grade dependent table supplied with the DEM test materials.

Scores from the horizontal and vertical subtests were computed into a ratio. The ratio of times indicates whether the patient's ability to make tracking movements appropriate for reading is well developed for his or her age.

An ocular motor deficiency was defined as an increase in time to complete the horizontal subtest together with a relative normal performance on the vertical subtest and a high ratio. Results were determined to be abnormal if the patient scored < 2 SD below the mean (< $20^{th}$ percentile).

### 4.5.7 Visual processing skills tests (Paper IV)

Visual perception strengths and weaknesses were evaluated. All tests were performed following the standard protocols from the test instruction manual. For each test and age percentile scores were given. The test results were classified as very low if the percentile rank was < 2SD below the mean (< $20^{th}$ percentile). These series of tests were performed individually in a relative quiet room with good illumination and as free as possible from auditory and visual distractions.

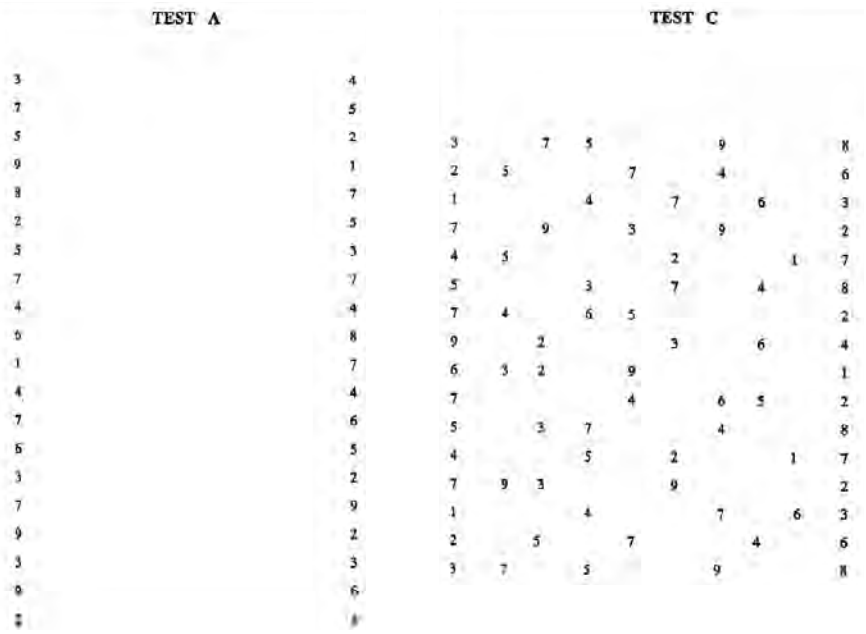


***Fig. 5.*** *Developmental eye movement test.*

#### *4.5.7.1 Gardner reversal frequency tests*

4.5.7.1.1 Recognition

This test evaluates the existence, nature and frequency of occurrence of receptive letter and number reversals. A test sheet was presented and the patient was asked to mark the letters and numbers which were written backwards, or reversed. (Fig. 6) Other observations were reported, like motor reinforcement (tracing over sample with finger or pencil or attempts to actually write the letter on paper or in space), impulsivity or loss of concentration. All the errors were then counted (raw score) and then converted to percentile rank. This test was administered following the test instruction manual.[245]

#### *4.5.7.2 Test of visual perceptual skills (TVPS)*

Depending on the age of the patient, two different tests were used. (Fig 7) For patients between 5 and 11 years 11 months the TVPS-R version was used and for patients above 12 years of age the TVPS-R upper level version was presented.[246,247] Each test consisted of 16 plates (TVPS-R) or 12 plates (TVPS-R upper level). Each plate was presented to the patient until the ceiling was reached. The ceiling was established when the patient failed three out of four consecutive items on those tests in which there were four choices, or four out of five failures on those subtests in which there were five choices. All patient responses were documented in the test recording sheet, both

successes and failures. Once the test was completed, the responses were matched with the correct answer. The number of correct answers were scored (raw score), then matched to age before being converted to percentile ranks.

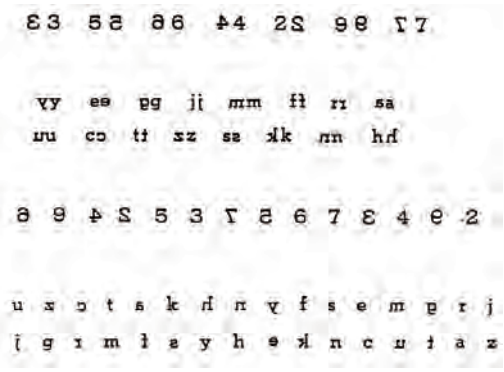

***Fig 6.*** *Gardner recognition test*

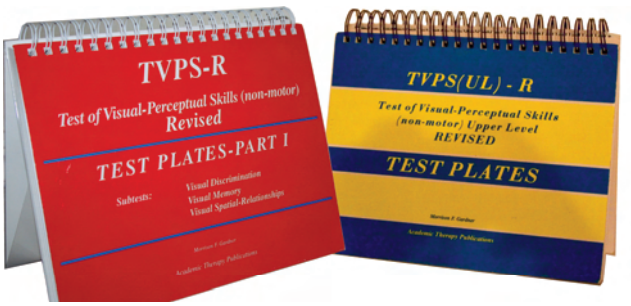


***Fig 7.*** *Test of visual perceptual test TVPS-R and TVPS-R upper level.*

#### 4.5.7.2.1 Visual memory test

Determines the ability to remember for immediate recall (after four or five seconds) all of the characteristics of a given form, and being able to find this form from an array of similar forms.

#### 4.5.7.2.2 Visual form constancy test

Determines the ability to see and find a form, even though the form may vary in size (larger or smaller) and location. Also, examines the ability to determine the form if rotated, reversed, or hidden among other forms.

#### 4.5.7.2.3 Visual sequential memory test

Assess the ability to remember for immediate recall (after four or five seconds) a series of forms among four separate series of forms.

### *4.5.7.3 Test of visual motor integration*

This test was designed to identify deficits in visual perception, fine motor skills and hand-eye coordination.[248] It was administered to patients from age five through young adulthood. The test consisted of 24 plates with geometrical figures that increased in complexity. The patient was asked to copy them without an erasure and without rotating the paper in any direction. Then the raw score was reported based on the number of correct figures copied and compared to norms for each age group. The raw score was afterwards converted to percentile. (Fig 8)

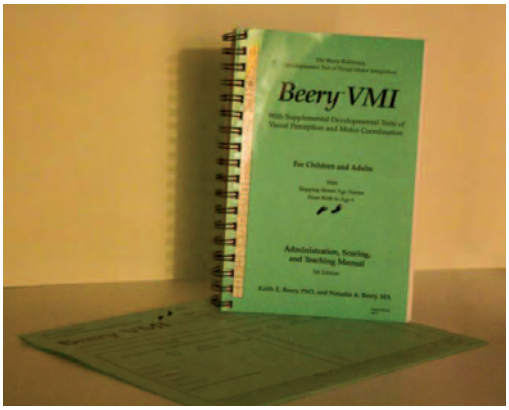


***Fig 8.*** *Test of visual motor integration.*

## 4.6 STATISTICS

The statistical program Statistica 8.0 (StatSoft®, Inc. Tulsa OK, USA) and Excel™ were used for calculations for papers I–IV and VI. Frequencies, means, medians, SD, and ranges were calculated for descriptive purposes in papers I–IV and VI. Different analysis methods were performed in the papers. A $p < 0.05$ was considered statistically significant. The statistical methods used in the different papers (I–IV and VI) are described below:

**Paper I:**
Kaplan-Meier life tables and survival curves were calculated for the time to cataract development in different groups. The graphs indicate the time of cataract by dropping down a fraction of its value. In comparison between independent groups of cataract-free times, the non-parametric log rank test was used. Exploring the effects of

several variables on “cataract-free”, Cox proportional hazards regression analysis was used. Association between severity of cataract and treatment groups was also analysed using Fisher’s exact test.

**Paper II:**
Chi square or Fisher’s exact test were used to determine the association between objective signs of dry eye syndrome and subjective symptoms and to establish the relationship between DES and factors like conditioning regimens, cataract, drugs, diseases, gender, and age. The kappa statistic (*k*) was used to measure the agreement between objective and subjective symptoms. Multiple logistic regression analysis was applied to investigate the multivariate structure in the data and the interactions between the factors.

**Paper III:**
The Spearman rank correlation coefficient was applied to determine the association between MHR and age, IOP and CyA trough levels in plasma. The relation between right and left MHR was determined by the Student’s *t*-test for dependent samples. The Student’s *t*-test together with the Mann-Whitney U-test were used to compare MHR with age, diagnosis, malignancy, gender, corticosteroid therapy, GVHD, BCVA, corneal status, presence of intraocular lens (IOL), intraocular pressure (IOP), and fundus pathology. The Kruskal-Wallis ANOVA by ranks was used to compare several independent groups, such as conditioning regimen and the presence of cataract. The p-values were adjusted according to the Bonferroni procedure. The multiple stepwise regression analysis was used to establish the multivariate structure in the data.

**Paper IV:**
The Mann-Whitney U-test was used to compare the CA, DA, and RA between FA patients and controls.

**Paper VI:**
The Fisher exact, one-tailed test was chosen for comparison between VEP results and BCVA, cataract, IOL, posterior pole abnormalities, malignancy, diagnosis, gender, prematurity, conditioning regimen, corticosteroid treatment > 6 months, chronic GVHD, CyA > 250 ng/ml. The Mann-Whitney U-test was used to compare the VEP results among patients who demonstrated > 7 with ≤ 7 occasions of > 250 ng/ml of CyA trough level with patients who demonstrated ≤ 7 occasions.

# 5 RESULTS

The results from the 79 children and young adults that underwent allogeneic SCT between December 1986 and May 2004 and belong to the cross-sectional part of the project will be presented. The most common indications for SCT in this study group were ALL (20 patients), AML (12 patients), and SAA (9 patients). Seven patients in the group were born prematurely (< 2500 g). Several ocular abnormalities were noted in 74 of the 79 patients.

## 5.1 VISUAL FUNCTION

**Visual acuity**

It was possible to assess the monocular BCVA with optotypes in all 79 patients. A total of 158 eyes were measured of which 152 (96%) had a BCVA of 0.5 or better. A BCVA of 0.8 or better was achieved by 127 eyes (80%). The BCVA among the 17 children treated with cataract surgery was $\geq 0.5$. Fifteen had binocular surgery and were corrected with glasses, while two had monocular surgery and were not wearing glasses. In two groups of specific underlying diseases, all FA patients reported a BCVA $\geq 0.65$ in both eyes whereas all four patients diagnosed with MPS I-Hurler reported a BCVA $\geq 0.63$ in the better eye.

**Visual field**

RB perimetry testing was performed in 53 (27 girls, 26 boys) of the 79 patients and compared to a healthy group of 51 subjects. The SCT patients had a lower MHR [median 91% (range 45–99) right eye and 91% (41–91) left eye] when compared to the controls ($p < 0.00005$). The MHR values significantly below reference values were found mainly in the group of patients diagnosed with ALL, the group which had TBI as conditioning regimen, the patients with IOLs, and the group over 12 years of age.

Boys showed a better MHR than girls ($p < 0.05$). Age did not significantly affect the MHR in the SCT group.

**Ocular alignment and motility**

The cover test showed that 13 of 76 patients presented heterophoria at distance (exophoria = 7; exotropia = 3; esophoria = 2; esotropia = 1) and 34 of 76 patients presented heterophoria at near (exophoria = 27; exotropia = 1; esophoria = 5; esotropia = 1). Ocular misalignment at near was more common among the patients diagnosed with ALL and in patients who received TBI. Nystagmus was present in three patients, one manifest and two with an end-point nystagmus of which one had a latent nystagmus. Muscle restriction was seen in four patients. Gross stereopsis was present in all patients.

**Amplitude of accommodation**
The AA could be measured in 82 eyes, of which 33 presented abnormal values when compared to the Duane table of accommodation. The missing data of 76 eyes was due to the presence of IOL in 30 eyes, age, and lack of cooperation in 46 eyes.

**Colour vision**
It was possible to administer the HRR colour vision test to 70 patients. Of the nine for which we could not get any final results, four were unable to respond due to age or mental status, two missed one plate and did not want to go through the entire test, and three did not take part in the test. All 70 patients reported normal colour vision.

**Cone adaptation test**
Seventy-one patients took part in the cone adaptation test (CAT). It was not possible to perform the CAT in eight patients due to age in one and lack of participation by seven patients. Only seven patients failed the test.

## 5.2 REFRACTION

Refraction could be tested in 146 eyes of 73 patients. Hyperopia ≥ 2.00 D were present in 22 eyes, myopia ≥ -0.50 D in 10 eyes, astigmatism ≥ -0.75 D in 17 eyes. Combined refractive errors, like hyperopic astigmatism were present in 9 eyes and myopic astigmatism in 13 eyes. It was not possible to measure the refractive error in 10 patients due to lack of cooperation or dull reflexes as a result of cataracts. All four patients diagnosed with MPS I-Hurler had high hyperopias with a spherical equivalent (SE) median +6.25 (range +3.75 to +7.75).

## 5.3 OCULAR COMPLICATIONS

A complete list of indications for SCT in this group and the different ocular complications found are presented in Table 8.

### 5.3.1 Ocular surface

The dry eye test with ocular surface evaluation, dry eye questionnaire, and Schirmer was performed in 60 children/young adults of the 79 originally included in the study at a median age of 7.6 years (range 0.4–15.5 years). Of these 60 patients, 35 reported subjective symptoms. The most common symptoms were ocular dryness, irritation, redness and secretion. The dry eye symptoms questionnaire showed a significant association to the objective findings ($p < 0.002$). Objective dry eye was defined as corneal staining without any other obvious reason and a short BUT and/or pathological Schirmer. Thirty-seven presented with objective signs of DES of which 29 had corneal erosions from mild to severe presentation (Fig 9). Three patients with GVHD had staining covering more than 50% of the corneal surface. No patient suffered corneal perforation. A short BUT ≤ 10 seconds was a common finding and occurred in 107

eyes (89%), even in eyes without any corneal staining. Pathological Schirmer (< 10 mm) was found in 13 of the 46 (28%) patients that it was possible to measure.

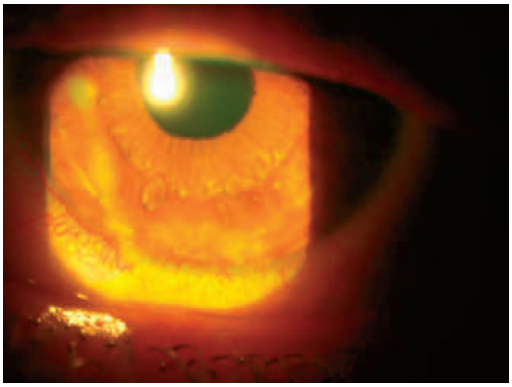

***Fig 9.*** *Nineteen year-old boy with acute lymphoblastic leukemia who underwent stem cell transplantation at 9 years of age after conditioning with single dose total body irradiation (10 Gy), developed acute graft-versus-host-disease in the skin and a chronic intestinal graft-versus-host-disease. At the 10-year follow-up he demonstrated dry eye syndrome with inferior corneal punctuate and pannus in the left eye; the break-up time was below 5 seconds, and the Schirmer was pathological (7 mm).*

**Table 8. Ocular complications related to diagnosis in the present study.**

| **Number of patients (girls/boys)** | **79 (42/37)** | **s-TBI** | **f-TBI** | **Bu / Other** | **Ocular surface** | **Cataract I–III** | **Posterior Segment** |
|---|---|---|---|---|---|---|---|
| All diagnoses | 79 | 17 | 18 | 33 / 11 | 37 | 29 | 8 |
| ALL | 20 | 10 | 10 | | 15 | 8 | 2 |
| AML | 12 | 1 | 1 | 10 Bu | 8 | 3 | 0 |
| CML, MDS, and JMML | 7 | 3 | 2 | 2 Bu | 2 | 2 | 0 |
| MPS I-Hurler, Gaucher, AGU, ALD, and LCH | 9 | | 2 | 7 Bu | 1 | 3 | 1 |
| SCA and Thalassaemia major | 6 | | | 6 Bu | 4 | 3 | 0 |
| SAA, FA, Amega | 17 | 2 | 3 | 1 / 11 | 4 | 7 | 4 |
| SCID, WAS, CGD, HLH, and Wegener + NHL | 8 | 1 | | 7 Bu | 3 | 3 | 1 |

*AGU, aspartyl glucose aminuria; ALD, adrenoleukodystrophy; ALL, acute lymphoblastic leukaemia; Amega, amegakaryocytic thrombocytopenia; AML, acute myelogenous leukaemia; Bu, busulfan; CGD, chronic granulomatous disease; CML, chronic myeloid leukaemia; FA, fanconi anaemia; f-TBI, fractionated total body irradiation; HLH, haemophagocytic lymphohistiocytosis; JMML, juvenile myelomonocytic leukaemia; LCH, Langerhans cell histiocytosis; MDS, myelodysplastic syndrome; MPS-I-H, mucopolysaccharidosis I-Hurler; NHL, non-Hodgkins lymphoma; SAA, severe aplastic anaemia; SCA, sickle cell anaemia; SCID, severe combined immunodeficiency; s-TBI, single-TBI; WAS, Wiskott–Aldrich syndrome.*

Twenty-six of 35 patients diagnosed with malignant disorders and 11 of 25 diagnosed with non-malignant disorder presented with DES. The risk increased in female patients with malignant disease ($p = 0.01$). Statistically, there was no significant association between DES and the different conditioning regimens ($p = 0.19$). On the other had, DES had a significant association with patients who had repeatedly high monitored CyA trough levels. The median number of occasions per patient that measured high levels of CyA (> 250 ng/ml) was seven. Those who had a number of occasions above median ($\geq 7$) had a higher prevalence of DES than those below seven ($p = 0.002$). DES was also present in 16 of the 25 patients who received a prolonged corticosteroid treatment (> 6 months) and in 21 of the 35 patients that used corticosteroids for a shorter period of time or not at all.

Among the 60 patients in whom it was possible to evaluate the cornea, and measure Schirmer and BUT, 20 had a history of GVHD, of which 14 had DES. Of the 40 patients without cGVHD 23 had DES. There was no significant influence of cGVHD on the prevalence of DES.

A total of 30 patients had had some type of ocular treatment to control the DES symptoms. The majority were prescribed with lubricants. Three with severe DES and a history of severe cGVHD had received topical corticosteroids with subjective and objective improvement and two were treated with topical CyA 1% with positive corneal results.

Other ocular changes besides DES were present among the 79 patients, such as ptosis present in eight patients, poliosis (grey eyelashes) in three patients, and blepharitis also in three patients.

In the FA group not all the corneal diameter and periocular measurements could be analyze in all patients mainly due to poor quality of the pictures. The most common findings were microcornea, ptosis, abnormal PFL, and hypotelorism.

In the MPS I-Hurler group all four patients presented corneal opacities which improved after early SCT. All were conditioned with Bu and Cy and received corticosteroids for GVHD treatment.

### 5.3.2 Crystalline lens

Posterior subcapsular cataract together with DES was the most common ocular abnormality. (Fig 10) Out of the 79 patients 46 developed some degree of cataract, varying from clinical insignificant opacities without any visual limitations to dense cataract causing severe visual impairment and needing surgery. The development of cataract occurred more frequently in patients diagnosed with malignant diseases and who had TBI early in life. The risk of developing cataract was higher in s-TBI compared to f-TBI ($p < 0.01$). The mean time to cataract detection was dependent on the type of conditioning regimen the patient was exposed. PSC appeared approximately 5 years (range 1–6) after SCT in patients exposed to TBI. The formation was earlier if

the patient received s-TBI compared to f-TBI and even earlier when compared to Bu and other chemotherapeutic drugs. Table 9 presents the development of cataract based on the conditioning regimen.

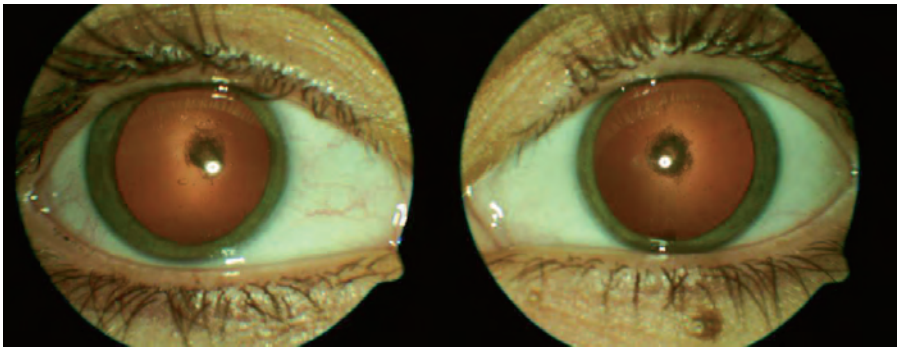

***Fig 10.*** *Eighteen-year old girl with amegakaryocytic thrombocytopenia who underwent stem cell transplantation at 11 years of age preceded by single total body irradiation (10 Gy) and received corticosteroids for more than six months. Cataract was diagnosed one year after transplantation. At the 8-year follow-up she presented with bilateral cataract grade III and visual acuity 0.65 bilaterally.*

**Table 9. Cataract development and conditioning regimen in the present study.**

| | s-TBI | f-TBI | Bu / Cy | Others |
|---|---|---|---|---|
| N | 17 | 18 | 33 | 11 |
| Clear lenses, n | 0 | 3 | 21 | 8 |
| Children with cataract, n | 17 | 15 | 12 | 3 |
| Time to detection after SCT, years (range) | 2 (1–4) | 3 (2–6) | 5 (1–17.3) | 5.4 (2–15.1) |
| Cataract surgery, n | 13 | 4 | 0 | 0 |
| Time to cataract surgery after SCT, years (range) | 3.8 (2.6–9.2) | 3 (2.2–5.3) | | |
| No cataract surgery, n | 0 | 11 | 12 | 3 |

*Bu/Cy, Busulfan and Cyclophosphamide; f-TBI, fractionated total body irradiation; N/n, number of patients; SCT, stem cell transplantation; s-TBI, single-TBI.*

In 17 of 45 patients with cataract, the BCVA decreased to a level where cataract surgery was recommended. Cataract surgery was performed between 2.2 and 9.2 years after transplantation. Final visual outcome after surgery was good, 73.5% had a BCVA > 0.8. Only one had a BCVA of 0.3 at examination due to posterior capsule opacity.

Twenty-seven patients developed cGVHD after SCT and all but one received corticosteroids for more than 6 months. Statistically neither the prevalence of cGVHD nor its treatment (corticosteroids for more than 6 months and > 250 ng/ml of CyA more than seven times) affected the cataract formation in this group.

Secondary cataract was found in 7 patients and 10 eyes needed treatment. It appeared at a median age of 35 months (1.1–54.4 months).

### 5.3.3 Intraocular pressure

Intraocular pressure (IOP) measured with pneumotonometry was documented bilaterally in 63 patients, all of whom were older than 3 years of age. All but one eye had IOP measurements $\leq 20$ mm Hg and no patient had glaucomatous optic neuropathy. The median IOP was 13.5 mm HG (range 8–22).

### 5.3.4 Ocular fundus

The posterior segment evaluation of the 79 patients showed no signs of active chorioretinal disease, other progressive disease, or papilloedema. Posterior segment findings found in this group were (1) thin retina with a thin nerve fibre layer and visible choroidal vessels in three patients (2) vessel stretching in a treated retinopathy of prematurity in one patient (3) optic nerve hypoplasia (ONH) in three FA patients (4) a larger CA/DA ration (0.5). (Fig 11)

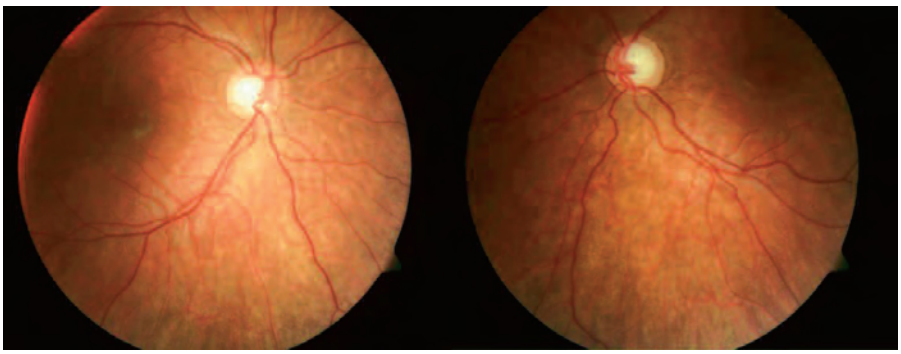

***Fig 11.*** *Eleven year-old boy with acute lymphoblastic leukaemia who underwent stem cell transplantation at 8 years of age preceded by fractionated total body irradiation (3 Gyx4). Three years after transplantation the visual acuity was 1.0 bilaterally, the intraocular pressure was 20 mmHg in the right eye and 17 mmHg in the left eye, a borderline MHR of 89% in the right eye (no data on the left eye), and a normal MRI with no signs of periventricular leukomalacia.*

The digital analysis of the optic disc in 22 patients showed that the SCT patients had a significantly larger CA ($p < 0.0005$: right eye and $p < 0.0001$: left eye) smaller RA ($p < 0.0005$: right eye and $p < 0.005$: left eye) and no significant difference in the DA than normal controls.

### 5.3.5 Visual evoked potentials

Pattern VEPs were obtained after SCT in 47 of the 79 patients, median age after SCT of 6 years (range 1–17). Eleven eyes in eight patients demonstrated pathological VEPs (3 binocularly and 5 monocularly), of these, eight had a BCVA ≤ 0.65. (Fig. 12) Three of the 11 eyes showed retinal changes, like thinning of the nerve fibre layer and residual effects of cryotherapy. Pathological VEPs were equally common in boys as in girls and were significantly associated with decreased BCVA in the left eye. Although the presence of pathological VEPs was higher in the patient group with malignant diseases, in the group conditioned with TBI, in patients with cGVHD, and with an IOL, the differences were not statistically significant.

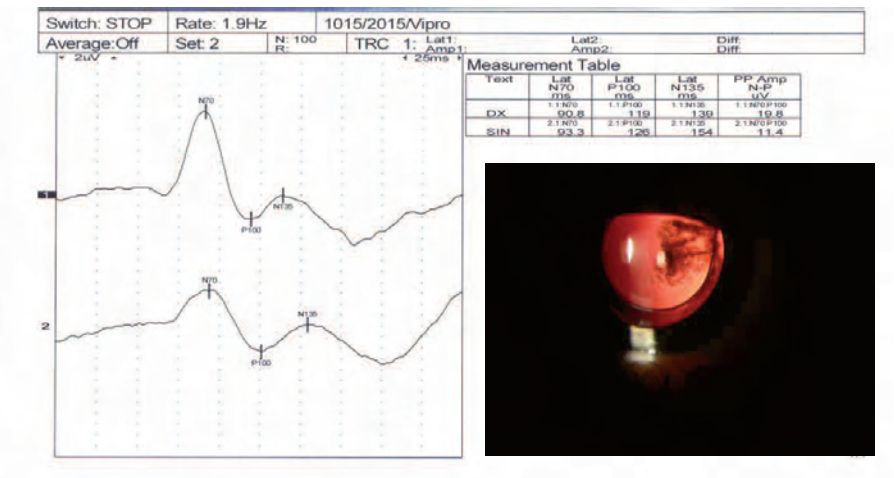


***Fig 12.*** *Ten year-old boy with chronic myeloid leukaemia who underwent stem cell transplantation at three years of age preceded by fractionated total body irradiation (3Gyx4), developed acute and chronic graft-versus-host-disease. Cataract surgery was performed three years after SCT and secondary cataract developed on the left eye. At the 7-year follow-up the visual acuity was 1.0 in the right eye and 0.65 in the left eye and visual evoked potentials were normal in the right eye (upper trace) and abnormal in the left eye (lower trace).*

### 5.3.6 Developmental Eye Movement test and Visual processing skills

These tests were performed in nine of the ten FA patients. Eight patients had an ocular motor deficiency due to inaccurate saccadic movements. Four of them had additional difficulty in automaticity in number calling skills, (low speed and low accuracy of response to the stimulus).

The nine patients that participated in the visual processing skill evaluation displayed a general delay in all areas examined. Out of 45 results, 20 were < 2 SD from the mean, 6 corresponded to Gardner Reversal Frequency test – recognition, 2 to TVPS-Memory, 6 to TVPS- Form constancy, 3 to TVPS-Visual sequential memory and 3 to VMI Beery Buktenika.

# 6 DISCUSSION

Stem cell transplantation is the treatment of choice for many patients with severe, malignant and non-malignant disorders in children and young adults. The number of transplants and survivors has steadily increased mainly due to better donor availability, a more precise donor selection through genomic HLA-typing, and by improved supportive care. Thus, more patients become long-term survivors who will need special management for the different post-SCT ocular complications.

The first study of ocular complications after SCT dates from 1981[117] in which the main area of study was related to ocular surface changes. Nowadays, the incidence of ocular complications after SCT in adults and in mixed adult-children groups has been well described,[162-166] as well as in the paediatric population.[167-171,191,249]

**Visual function and visual fields**

In general, the visual outcomes in the present study group were good regardless of the different ocular manifestations. The BCVA was > 0.8 in the majority of the patients (80%) including those treated with cataract surgery. Only one patient treated with cataract surgery had a BCVA of ≤ 0.5 due to a dense untreated secondary cataract. Suh and co-workers[179] reported that 96% of the patients had a BCVA ≥ 0.5 including those treated surgically due to cataract. Ng and co-workers[201] also reported that the BCVA ranged from 0.5 to 1.0 including those with cataract and fundus abnormalities.

Regarding visual field outcome, the patients in the present study showed a significantly lower MHR than controls. These visual field defects were found to be related to the presence of IOLs, gender, and laterality (boys and left eye). The reason why boys and left eye were related with low MHR values is not clear.

The results of colour vision did not differ from reference material.[233] The presence of strabismus was slightly higher (7.6%) in our study than the one reported by Grönlund and co-workers[235] in which 3.5% of 143 normal subjects between 4 and 15 year old showed some type of strabismus. The majority of refractive errors were similar to those found in normal population.[235]

**Dry eye syndrome**

In the present study 58% of the patients presented subjective symptoms of dry eye, such as ocular dryness, irritation, redness, and secretion while objective findings were found in 62% of the patients. The most common objective findings in 89% of the eyes was abnormal BUT including eyes without any corneal staining; while corneal staining graded from mild to severe was found in 78% of the eyes. Pathological Schirmer was found in 28% of the patients who allowed measurements. These results can be compared with those previously reported in adults and mixed groups.[163,178]

Calissendorff and co-workers[163] reported that 43% of 77 in a mixed group developed subjective symptoms of dry eye during long-term follow-up, while Mencucci and co-workers[178] described the incidence of 20% of corneal staining, 37% of abnormal tear BUT and 34% of pathological Schirmer values in 35 adult patients.

The prevalence of DES in children has previously been reported to be slightly lower when compared to the present results. Out of a population of 29 patients Ng and co-workers[201] found a reduced Schirmer test production in 36%, a subnormal tear BUT in 33%, and corneal staining in 33% of the patients. The difference when compared to our results might be due to a smaller sample size, the shorter follow-up time from SCT (range < 1–10 years), and the fact that the patients received a different type of conditioning regimen (only f-TBI and chemotherapeutic agents). In a larger study, Suh[179] described that ocular surface abnormalities were seen in 22% of 104 patients with a follow-up time of < 1–15 years, which is within the range of the present study. The conditioning regimen was based on Cy and f-TBI. Even though the present study shows a slightly higher prevalence of DES it is not possible to determine if this was related to the different conditioning regimens.

In the present study female patients with malignant disorders were at higher risk for developing DES. In the Ng study there was no specification on the gender of the participants. Frequent occasions of high CyA trough levels, which we found to be a risk factor, has not been described in previous studies.

Even though the present study did not show any association between DES and conditioning treatment or DES and cGVHD, previous reports in mixed adults and paediatric groups have noted the important role GVHD and TBI have played in the development of DES.[164,175,180,181,249] According to these studies cGVHD and TBI induces changes in the lachrymal gland and conjunctiva with destruction of meibomian glands, the surface epithelium of the conjunctiva and cornea, the goblet cells, and the lachrymal glands. An animal study showed an evident reduction of Schirmer-test values 72-hours and one month after s-TBI.[250] Other studies in human subjects after TBI have shown a reduction in the number and size of acinar cells and pathological changes in the lachrymal system and meibomian glands affecting the production of the aqueous component of the tear film predisposing the eye to changes in the anterior ocular surface.[251,252] Other less often reported associations with DES are the prolonged administration of Bu and Cy.[50,253]

Chronic GVHD was not very common in the present study, it developed in 33% of the 60 possibly examined patients of which 70% developed DES but statistically DES and cGVHD did not correlate. This might be due to the relatively small number of patients with cGVHD in the paediatric group. In the present study, the presence of DES was similar in patients treated with corticosteroids for > 6 months (64%) with corticosteroids < 6 months or not at all (60%) which was somewhat surprising.

Regarding treatment of ocular surface pathology, lubricants, topical corticosteroids, and topical CyA were the medications of choice to manage DES. Topical CyA was

recommended when conventional lubricants failed to achieve adequate relief. It is a safe drug since it does not inhibit wound healing or produce lens changes as corticosteroids do. It has been reported that topical CyA might be an effective and safe treatment option in dry eye related to cGVHD, especially in patients with severe signs of DES.[187,254]

**Cataract**

The most well described ocular complication after SCT in children is the development of cataract.[167-170,179,191,201,249] In the present study, 92% of the children treated with TBI developed some type of PSC, from mild to severe and 34% of the children exposed to chemotherapeutic agents developed cataract. This is similar to previous studies in which the incidence for those receiving TBI ranged from 72–100% [167,169,170,191,255] and for those conditioned with Bu or other chemotherapeutic agents the range was between 2–21%.[168,169,201]

In the group who received s-TBI all developed some type of moderate to severe PSC and the need for cataract surgery was 82% while in the group who received f-TBI 83% developed PSC and 28% were surgically removed. This is line with other studies. Lappi and co-workers[170] reported the incidence of cataracts in a group who received s-TBI to be 100% compared to none in the f-TBI group. Calissendorff and co-workers[191] compared children who received s-TBI for malignant haematological diseases with children with SAA who received no TBI or minor dose plus eye shielding. In the s-TBI group, all developed PSC three years after SCT and none developed PSC in the group of no TBI plus eye shielding. Another aspect found in this study was the presence of PSC was detected five years after SCT; this is slightly later than the time reported by Lappi and Calissendorff.[170,191] There was also an increased risk of developing PSC when conditioned with s-TBI or f-TBI compared with Bu (36%) or other chemotherapeutic agent (27%). This is in alignment with Holmström and co-workers[169] in which 21% of patients who received Bu developed PSC compared with 95% who were conditioned with TBI. The latest study in children[249] presented the risk of cataract in a population of 438 survivors after five or more years after SCT. The incidence presented in this study was of 6%. This was significantly associated with the radiation dose and the use of prednisone.

Cataracts after s-TBI developed earlier after transplantation and almost half required cataract surgery due to severe visual impairment in the present study. Patients receiving f-TBI developed cataract later, the severity of visual impairment was moderate and the need of cataract surgery also decreased. In order to reduce the incidence of cataract formation after TBI protective eye shield have been used and this does not increase the incidence of relapse in the eye.[256,257] This is controversial and other authors suggest that this procedure should not be used due to the concern for leukemic relapse within the orbit.[258] Also, a large part of the bone marrow containing cranium is shielded when shielding of the eyes is applied, which may increase leukemic relapse risk. It has been stated that ocular shielding is common when irradiating the head and neck but is not practical during TBI. The leukemic

relapse has more severe consequences than the development of cataracts, which when necessary can be removed with excellent results.

Another factor that can increase cataract formation is the administration of chemotherapeutic agents. The development of cataract in patients taking Bu was first described in 1969.[259] Bu is thought to induce PSC formation by inhibiting nucleic acid formation of the lens epithelium interfering with the differentiation of fibre cells resulting in severe cataracts or slight irregularities in the posterior subcapsular region.[260,261] Histologically the major changes induced by Bu are located beneath the posterior capsule although the appearance is not pathognomonic for Bu cataracts but similar to cataracts after irradiation.[262]

In the present study 34% of 44 patients who had received Bu or other chemotherapeutic agents had developed cataract 5 years after SCT but none had had cataract surgery at time of follow-up. This is in line with the study reported by Holmström and co-workers[169] in which 21% of 24 patients conditioned with Bu developed cataract 5 years after SCT but only one had surgical treatment. Thus chemotherapeutic agents result in a lower incidence of cataract development when compared with TBI.

The dose-dependent relationship between systemic corticosteroids and PSC formation has been well documented, but may be influenced by individual susceptibility and duration of treatment after SCT.[193,197] In the present study there was no correlation between PSC development and prolongued corticosteroid treatments for > 6 months. De Marco and co-workers[168] showed comparable results. It is possible that the time of exposure to corticosteroid therapy in our patients ultimately was not long enough to produce any lens changes. According to Black and co-workers[194] it is necessary to have at least one year of moderate/high dosage of systemic corticosteroids exposure for PSC to develop. Dunn and co-workers[263] reported that the total dose and duration of steroid therapy were strongly associated in the PSC development while f-TBI was not a statistically significant risk factor.

Another possible side-effect of prolonged used of corticosteroid therapy is the transient increase of the IOP, which returns to baseline once the steroid is withdrawn.[264] In the present study the IOP measurements were < 20 mmHG in all but one patient, and none reported glaucomatous optic discs. By the time we examined these patients the corticosteroid treatment was completed, therefore we can assume that the IOP values have normalized leaving no trace of glaucomatous damage.

Patients developing cataract early in life are at high risk of developing amblyopia. The visual system has developed completely by 8 to 10 years of age. If cataract appears before this the risk of amblyopia is higher. A prompt removal is suggested to decrease the risk of amblyopia. In some cases despite an early surgical treatment mild amblyopia might be present.

Secondary cataract developed in 42% between 1 and 4.5 years after cataract surgery in the present study. This is different from previous studies[167,179] where a majority of the

patients who underwent cataract surgery needed a laser capsulotomy within two years after cataract extraction.

Chronic GVHD was present in 20 of the 79 patients, six out of these 20 developed cataract. These six patients were also treated with TBI. Due to this and the small sample of patients with cataract it was impossible to establish a relation between cataract and cGVHD. Suh and co-workers[179] presented a weak association between severe cataract and cGVHD.

**Posterior segment**

The prevalence of posterior segment complications in the present study was lower when compared with the prevalence of anterior segment complications. Minor changes were a thin retina with a thin nerve fibre layer (NFL) and visible choroidal vessels present in 10%. In another study, Coskuncan and co-workers[173] reported that almost 13% of 397 adult patients presented posterior segment complications, like vitreous hemorrhages, cotton-wool spots, optic disc oedema, and infectious retinitis. The possible reason for not having a considerable amount of posterior segment complications in our study group might be that they are often described as occurring within the first two years after SCT and the median follow-up in the present group was seven years.[179,201,265] The other posterior segment findings were related to specific ocular conditions, vessel stretching in a treated ROP, and ONH in FA patients.

In the present study group, the patients showed a normal DA, a larger CA, and a smaller RA when compared to the controls. A similar finding has been reported in children with periventricular leukomalacia.[266] In the present study there was no association with glaucoma since all except one patient had an IOP < 20 mmHg and none of the discs had a glaucomatous appearance.

**Visual evoked potentials**

The conditioning regimen and treatment subsequent to SCT is known to adversely affect different parts of the brain, causing central white and/or grey abnormalities.[267] In the present study 12% of 94 eyes demonstrated pathological VEP responses at a median of 6 years after SCT and half of these had a BCVA of ≤ 0.65. TBI, IOL, and cGVHD were associated with pathological VEPs. Results on VEPs *after* SCT in childhood have not been reported before. There is only one single study on VEPs in children *before* SCT where Kaleita and co-workers[268] reported pathological VEPs in 50% of 14 children with ALL and AML. This study stated that the exposure to previous radiation and/or the underlying disease might be possible causes of a bilateral slowing of conduction in the visual pathways. On the other hand, the presence of IOL may cause a delay in the conduction of the stimulus probably due to poor light transmission through the optic media, especially the intact posterior capsule or the implant itself.[269]

**Fanconi anaemia**

The most significant ocular manifestations not previously reported in the FA patients were ONH and microcornea. Other previously described findings that were common among these 10 patients were microphthalmia and ptosis.[94] Microphthalmia was suspected based on the low values of the periocular measurements in the present study but were not confirmed with bulb length measurements. A possible cause for the facial and optic disc features might be a disturbance during the embryonic development of the eye, particularly in the cranial neural crest cells. These cells give rise to the meningeal sheath of the optic nerve, corneal stroma and endothelium, ciliary muscles, ciliary ganglion, sclera and extrinsic muscles and could thus theoretically have an implication on the development of the cornea, the optic nerve sheath and motility.[270]

In a recently published report ocular features in 22 FA patients were described.[88] Eighty-two percent had small palpebral fissures, 69% had microphthalmia, 64% had small OCD, 55% had microcornea, 28% had ptosis, and 6% had epicanthal folds. These findings are in agreement with the findings of our study.

The 10 FA patients in the present study showed developmental delays in several areas of the visual processing skill functions, like visual memory, visual motor integration, and directionality. Other studies have reported the incidence of developmental delays in this group of patients, but the areas examined have not been mentioned.[271,272]

**Mucopolysaccharidosis I-Hurler**

All four patients with MPS I-Hurler developed bilateral corneal opacities early in life. A partial resolution of these corneal opacities was clinically observed after SCT. Some studies have reported a complete resolution of the corneal opacities while others have also reported worsening of the corneal condition. Summers and co-workers[273] described a clearing of the corneal clouding, resolution of the optic nerve oedema, and improvement of the retinal function in 11 patients 3 years after SCT while Gullingsrud and co-workers[274] reported that in 30% of 20 patients improved clinically the corneal clouding between 7–24 months after SCT while 25% of the patients experienced deterioration of corneal clouding.

High hyperopia (> 3.75 D) was found in all four MPS I-Hurler patients. This is in line with a recent report.[275] These high hyperopic refractive errors might be due to the storage of GAGs which increases the rigidity of the cornea and sclera,[276,277] thus making the corneal surface straighter and reducing its refractive power. [76] The BCVA found among MPS I-H patients was better than other reports. Ashworth and co-workers[278] reported 15 of 19 (79%) patients had a BCVA < 0.5 in their better eye and Connell and co-workers[275] presented a BCVA < 0.5 found in 60% of 23 patients despite an early SCT.

# 7 CONCLUSIONS

Paediatric patients are at risk of developing severe and potentially visual threatening complications after SCT. Both anterior and posterior segment complications can occur. Regardless of the occurrence of these ocular complications, the overall visual prognosis in this group was excellent, probably at least partly due to the fact that all patients were regularly followed at the eye clinic and medical and surgical management was provided at an appropriate time.

Cataract was a frequent finding and occurred in 59% of the patients. Patients conditioned with TBI were at higher risk of developing cataract which eventually needed surgery compared to patients who received Bu or other chemotherapeutic agent and there was no need of surgery in any Bu patient during follow-up. Patients conditioned with f-TBI developed cataract later than those exposed to s-TBI. Long term corticosteroids or GVHD did not influence the development of cataract in this study. Dry eye symptoms and findings were common in the whole group. Corneal epithelial lesions and tear film abnormalities were found mainly in girls diagnosed with malignant diseases, boys who underwent SCT at older ages and children exposed to high trough levels of CyA.

Visual field defects were present in half of the examined patients with lower MHR values than controls. A possible risk factor was the presence of an IOL. Additionally, SCT patients had a significantly larger non-glaucomatous CA and a smaller RA than normal controls. Abnormal VEPs were highly associated with decrease BCVA in the left eye. VEP might be of some clinical use in patients diagnosed with malignant diseases and those who received TBI as conditioning regimen. However, the clinical use seems limited in this group of patients.

Patients diagnosed with FA presented several ocular manifestations, including small optic discs or ONH, microcornea, and ptosis. Despite these ocular manifestations the visual outcome was very good. Visual processing skills were affected showing delays in the areas of visual memory and visual motor integration. Early SCT might be beneficial in reducing but not eliminating corneal clouding in children with MPS I-Hurler. The risk of high hyperopia might be due to GAG storage, causing corneal and scleral rigidity and decreasing the refractive corneal power and reducing bulb length.

Based on these and previous findings regular periodic visual and ocular health assessments are highly recommended for early detection and treatment of these potentially visual threatening conditions. It is important for the examiner becomes familiar with the diagnoses that benefit from SCT and recognizes the disease specific ocular features of the underlying disease, like MPS I-Hurler and FA. The side effects of the different medical options that these patients are exposed to and the various potential ocular complications in order to implement a successful treatment plan. Suggested guidelines for the ocular assessment of SCT patients before and after transplantation are described in the appendix.

Stem cell transplantation is an increasingly successful treatment for an expanding list of diseases and the group of long-term survivors increases rapidly. Thus, more attention is required to implement comprehensive follow-up programmes in order to assure adequate health and good quality of life for these children.

# 8 APPENDIX

Suggested guidelines for the ocular assessment of SCT patients before and after transplantation is described below:

SCT patients should be scheduled for ocular examination immediately before SCT and annually post SCT, unless more frequent visits are needed. Reasons for more frequent examination are the development of aGVHD, rapid progression of cataract in a young patient, the presence of papilloedema, and/or posterior retinal complications.

**Patient history (before and after SCT)**

A comprehensive patient history for this group of patients may include:

- General health
- Visual and ocular history (including dry eye symptoms, light sensitivity, near correction in case of IOL)
- Primary diagnosis and any secondary disease, e.g. diabetes, hypertonia, etc
- Conditioning protocol before SCT
- Development of aGVHD or cGVHD
- Family history
- School performance
- Ongoing medication

**Clinical assessment**

- Visual acuity at distance and near
- Ocular motility
- Assessment of the refractive error under cycloplegia
    - In MPS I-Hurler patients retinoscopy is difficult to perform due to dull reflexes
- Anterior segment evaluation
    - Adnexa (poliosis, madarosis, ptosis, microcornea)
    - Corneal status (clouding, staining, BUT)
    - Lens (PSC after TBI often present TBI 4–5 years post SCT)
- Schirmer I test
- Posterior segment (papilloedema, chorioretinitis, hemorrhages, ONH)
- IOP
- Additional tests
    - Periocular measurements (suspecting of microphthalmia in FA patients)
    - Visual field evaluation (RB perimetry)
    - VEP in specific cases, and repeated if pathological.

**Management**

- Best optical prescription
- Early detection and surgical intervention of cataract, especially in the very young population, in order to avoid amblyopia.
- Children with moderate to severe ocular GVHD are in the need of frequent ocular health care. Treatment compliance is difficult to attain. The treatment

plan for ocular GVHD is to begin with lubricants. Some cases will benefit for short periods of topical corticosteroids. Punctum plugs can also be considered. In severe cases CyA 0.05% eye drops can be added for 6–12 months.

- In cases of suspected developmental delays, referral to a cognitive psychologist is indicated. Subtle learning problems can remain undiagnosed throughout the entire school years.

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