The effects of high-dose chemotherapy and total body irradiation on caries development one year after stem cell transplantation

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Summary
Due to advances in treatment more children become long-time survivors after SCT and effects of both the disease and the treatment need careful investigation. The aim of this study was to investigate dental caries and salivary function in children undergoing SCT conditioned with TBI and/or chemotherapy. The study group comprised 122 children with a mean age of 7.7±4.4 years (range 1-12), conditioned with TBI and/or chemotherapy. 73 were male and 49 were female. The patients were clinically examined and bite-wing radiographs were taken prior to SCT and after 3, 6 and 12 months at the Department of Pediatric Dentistry, Karolinska Institutet, Huddinge, Sweden. Patients conditioned with TBI showed a significantly higher DFS in permanent teeth 1 year after SCT compared to baseline (p=0.0032), as did the patients conditioned with chemotherapy (p=0.0132). Dental caries progression was also significant 1 year after SCT compared to baseline in the patients conditioned with TBI, both in those having caries lesions (p<0.05) and those caries free (p<0.001). When conditioned with TBI, the group diagnosed with low stimulated saliva secretion rate showed an increase with a significant difference (p<0.001) in DFS, when comparing baseline to one year after SCT. This was also evident for the group with stimulated saliva (p<0.05). In conclusion this study found an increase in dental caries one year after conditioning SCT children with total body irradiation and chemotherapy.

Introduction
History of stem cell transplantation-SCT
In 1901, by describing the ABO blood groups, Karl Landsteiner laid the foundation for transfusion medicine. The first attempt to treat aplastic anemia with intravenous (IV) injections in a person of the same blood group was published in 1939. After World War II it became evident that ionizing radiation caused bone marrow failure and death, this started new awareness in the field and lots of experiments on animals were made. Experiments on mice demonstrated that death after total body irradiation (TBI) could be prevented (1-3). By administration of spleen or marrow cells IV from syngeneic, but not genetically different mice, the animals could be rescued. With this, the potential value of marrow grafting in humans was recognized (4).

In 1957 and 1959, Thomas et al. reported the first cases of bone marrow transplantation (BMT) in man, but engraftment was only transient (5, 6). Mathé et al. confirmed this with their report about the first long term survivor after BMT, a patient with leukemia, treated with infusion of BM-cells from his syngeneic twin (7). The patient died due to relapse after 20 months. A lot of research was made in the field; both in animals and humans, and a breakthrough came with the discovery of the major histocompatibility complex (MHC) and the human leukocyte antigens (HLA). By discovering the importance of HLA compatibility...
between recipient and donor (8, 9), HLA identical siblings became the major donor source in a series of bone marrow transplants in the late 60-ies. The outcome further improved by the use of immunosuppressive drugs as prophylaxis for graft-versus-host-disease (GVHD), mainly methotrexate and cyclophosphamide.

Since only a few have access to a matched sibling donor, a search for alternative donors begun. In 1973, the first unrelated donor (URD) transplant was attempted (10) and the Anthony Nolan URD registry was initiated in 1974. In 1990, E. Donnall Thomas and Joseph E. Murray shared the Nobel Prize “for their discoveries concerning organ and cell transplantation in the treatment of human disease”. Further important advances have been made during the last 16 years to improve the stem cell transplantation (SCT) procedure, for example PCR diagnostic methods, genomic HLA-typing and recruitment of more than 7 million volunteers to the URD registries.

Over 6400 allogenic SCT were performed in the European BMT group (EBMT) area in 2001 (11). According to an earlier EBMT survey from 1997, 67% received a graft from a HLA-identical sibling, 8% from a non HLA-compatible family member, 1% from a twin and 24% from an unrelated donor. The first allogenic SCT at Huddinge University Hospital was performed in 1975 (12) and in 1984 the first unrelated donor SCT at HS was performed. During the last years about half of the children received their graft from an unrelated donor at Huddinge University Hospital.

Cancer in children
Every year approximately 300 children up to 18 years is diagnosed with cancer in Sweden. 30 years ago only 1 in 10 children could be saved, today we can cure about 75%. The last two decades research and development have showed great results for cancer treatment and thereby much better long-time-survival, especially for children.

Today leukemia is the most common cancer during childhood, with 70-80 affected children each year in Sweden. Out of these, 85% are acute lymphoblastic leukemia (ALL), the rest non-lymphoblastic leukemias, mainly acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). The most usual time for diagnosis is between 2-5 years of age, with no geographic difference in frequency.

Conventional chemotherapy will today cure up to 70% of children affected by leukemia, with best prognosis noted for children with low risk ALL (13, 14). SCT is increasingly used as therapy for children with malignant and non-malignant disorders (15-17).

SCT-procedure
Stem cells have the potential to develop into all the different cell types in the human body. When a stem cell divides, each new cell can remain a stem cell, or become another type of cell with a more specialized function, such as a muscle cell, a nerve cell or a blood cell. Once determined for a blood-forming or hematopoietic stem cell, the cell can mature into three types of blood cells: white blood cells, which fight infection; platelets, which help the blood to clot; and red blood cells, which carry oxygen. Most hematopoietic stem cells are found in the bone marrow, but some cells, called peripheral blood stem cells (PBSCs), are found in the bloodstream. Blood in the umbilical cord also contains hematopoietic stem cells. Cells from any of these three sources can be used in transplants. BMT is the original terminology for stem cell transplantation (SCT), which now is commonly used to incorporate all of the above described techniques.

The stem cells used in SCT are obtained from the donor in a procedure called “harvesting”. If the stem cells are harvested from the bone marrow, the donor is given general anesthesia or regional anesthesia, needles are inserted in the pelvic bone, or in rare cases the sternum, and into the marrow to draw marrow out of the bone. Harvesting bone marrow takes
about an hour. When PBSCs are harvested, the blood is collected from a larger vein in the arm, stem cells are obtained in a process called apheresis, the blood runs through a machine that removes the stem cells and returns the blood to the donor. This procedure takes 4 to 6 hours. The harvested cells are frozen and stored, up to several months, until the transplantation. In cases where stem cells from the umbilical cord are harvested, it is done right after the moment of birth and the umbilical cord is cut. Blood is then collected from the umbilical cord and the placenta. Then the stem cells are separated from the blood, frozen and stored. This is a minimal risk process for both the mother and the child, mostly used if the child or any other family member should need it later. Since only a small amount of stem cells can be harvested with this technique, it is typically used for children or small adults.

As in all transplantation procedures it is important to find a matching donor. In contrast to solid organ transplantation, in SCT, a bilateral immunological reaction occurs. The immune system of the SCT recipient may reject the graft, as in organ transplants, but the SCT graft itself can also reject the new host. This is called the graft-versus-host disease (GVHD). GVHD occurs when white blood cells from the graft identify cells in the patients body (the host) and attack them. The most commonly damaged organs are the skin, liver and intestines. This complication can develop within a few weeks of the transplant (acute GVHD) or much later (cronic GVHD). To prevent this problem as far as possible it is of most importance to carefully HLA-match the donor with the recipient and use prophylactic immunosuppressive agents to increase the chance for engraftment. HLA-mismatching between the donor and the recipient was shown to increase the risk of graft failure and GVHD after BMT (18-20).

Before SCT, the patient is admitted 2 weeks before transplantation for medical evaluation, assessment of vital functions, ascertaining complete remission status in leukemia and for indwelling of a central venous line. Approximately one week before the stem cell donation the patient’s conditioning starts. Several high dose protocols can be employed to attain marrow ablation, disease eradication and a sufficient immunosuppressive effect to enable engraftment and avoid rejection (21). The standard protocol at Huddinge University Hospital was until the early nineties based on single fraction TBI, preceded or followed by cyclophosphamide (Cy). Since then, the single fraction TBI has been replaced by a fractioned TBI (fTBI), given as 3 Gy / day for four days. This protocol shows an equivalent antileukemic and immunosuppressive effect, with fTBI less toxic than TBI (21).

SCT and peripheral blood stem cell transplantation (PBSCT) are procedures used for restoring stem cells that have been destroyed by high doses of chemotherapy and/or radiation therapy. There are three types of transplants;

**Allogeneric SCT**

The graft consisting of hematopoietic stem cells, is donated by one individual to the patient, the recipient. The donor can be a relative (RD), a sibling or a family member, or an unrelated donor (URD).

**Syngenic SCT**

The graft is received from a genetically identical twin, the donor and recipient are completely HLA-compatible. In this case the immunological rejection called graft-versus-host will not occur. Syngenic SCT is not advised in leukemia, due to the lack of graft-versus-leukemia effect.

**Autologous SCT**

Here the hematopoietic stem cells are taken from one individual and later, after high-dose chemotherapy given back to that same individual again.
After transplantation, the stem cells travels to the bone marrow, where they begin to produce new white blood cells, red blood cells and platelets in the process called “engraftment”. This usually takes 2 to 4 weeks after transplantation. Before the “new” immunocells have grown in sufficient amount, the patients are very immunosuppressed and sensitive for infections. Regular evaluation of the blood counts are being maid to confirm that new blood cells are being produced and control of eventual relapse. The immune system usually takes several months to recover for autologous patients and 1 to 2 years for patients receiving allogenic or syngenic transplants.

Complications of SCT treatment

**General complications**
The most common short-term side-effects of the treatment are nausea, vomiting, fatigue, mouth sores, hair loss and skin reactions. Potential long-term effects are infertility, cataracts, relapse of cancer and damage to the liver, kidneys, lungs and/or heart.

**Oral complications**
The major risk with SCT treatment is an increased susceptibility to infection and bleeding as a result of the immunosuppression due to the high-dose cancer treatment. Antibiotics are given to the patient to prevent and/or treat infection and sometimes transfusions of platelets and red blood cells are given to prevent bleeding and anemia. An immunosuppressed patient has always an increased risk for oral complications because of the low defense against microorganisms. Oral candidosis is therefore a common an unpleasant side-effect. Damaged salivary function and negative effects on dental and craniofacial development are other long-term effects studied after TBI and chemotherapy conditioning (22-25).

Following SCT in childhood, long term complications of the oral complex are frequently encountered. For the salivary glands, both chemotherapy and irradiation may be damaging, resulting in decreased salivary secretion rate and change in the composition of the saliva (26). In the six first months following the transplantation, a drop in salivary flow has been noted in both the TBI treated and in those solely subjected to chemotherapy (26). The decrease in secretion rate is less for the chemotherapy treated patients and for these patients the salivary glands usually have recovered after 6-12 months (26, 27). For those children subjected to 10Gy TBI before 12 years of age, there seems to be a long term negative effect on the salivary secretion rate (26). In 1997, Dahllöf et al have reported that TBI, female sex and seropositivity for three to four herpes viruses are significant risk factors associated with decreased salivary secretion rate 1 year after SCT in children (24). Age at SCT also seems to influence the recovery of the salivary glands, with those treated at an older age having a better prognosis (25).

Chronic GVHD of the oral cavity is common and may be the only affected site. During active disease a thin, fragile mucosa might be seen in addition to xerostomia (28). After recovery of GVHD, a negative long term effect on salivary flow has been suggested (26). Due to a lowered salivary secretion rate, the risk for oral mucosal damage, through viral or fungal infections or trauma, may be increased. Swallowing may be impaired and influence the quality of life (29).

Lowered salivary secretion rate in combination with an elevated level of cariogenic bacteria (mutans streptococci, lactobacilli), found in long term survivors of SCT increases the risk for developing dental caries (25, 26). But with a careful dental preventive care program such as oral hygiene measures, fluoride toothpaste/topical application, dietary counseling, the caries prevalence in these children can be held at a level similar to that found in healthy children (26). Patients who receive therapy for hematological malignancies are at increased
risk of developing oral complications, where severe mucositis, gingivitis and ulcerations of
the oral mucosa are common findings among these patients.

In children undergoing SCT, chronically infected teeth, untreated or inadequately
restored teeth can lead to serious systemic sequelae during the period of profound
immunosuppression following the conditioning regimen (30, 31). Therefore it is essential to
remove all possible foci of infection in the mouth before the conditioning regimen and to start
preventive dental care. This includes effective tooth brushing to reduce the oral bacterial
loading and fluoride supplements to reduce the incidence of dental caries. The patient and the
parents is given a thorough instruction in how to manage with the dental health in best
possible way before and during therapy and this is only one of many things to which the
patient must adapt. A change of habits, which this often brings, can be hard and compliance
may not always be adequate. SCT patients conditioned with TBI and chemotherapy show an
increased caries experience (32-36).

The aim of this study was to assess the progression of dental caries in children one year
after treatment with SCT. My hypothesis is divided into three parts; 1) Patients treated with
TBI has more caries than those treated with chemotherapy only, 2) Patients that already have
caries before TBI increases in DMFT after TBI, 3) There is a correlation between low saliva
secretion rates and caries progression in children conditioned with TBI.

Materials and methods

Patients
The study included 122 recipients of allogenic stem cell transplantation, grafted at Huddinge
hospital between January 1982 and December 2004. The diagnoses were acute lymphoblastic
leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), other
hematological malignancies, severe aplastic anemia (SAA) and metabolic disorders. The
mean age was 7.7±4.4 years (range 1-12 years), 73 were male and 49 were female.

Conditioning and treatment
Patients with hematological malignancies were treated with cyclophosphamide (CY),
120mg/kg combined with TBI in a median dose of 10Gy, delivered by a linear accelerator at a
mean dose rate of 0.04 Gy/min with the lungs shielded to receive no more than 9Gy, or
busulfan (Bu), 4mg/kg on each of 4 consecutive days in combination with CY (37, 38).
Patients with SAA received CY (200mg/kg) and since 1988 in combination with
antithymocyte globulin (ATG), 3-5mg/kg/day for 5 days. Patients receiving marrow from
unrelated donors were also given ATG (38). The patients were kept in reverse isolation until
the numbers of neutrophils were above 0.5x10⁹/l on 2 consecutive days. In general the
patients were conditioned according to the Seattle protocols (30, 31, 37, 38).

As prophylaxis against GVHD, either methotrexate was given once weekly for 3
months, cyclosporine for 1 year, four doses of methotrexate combined with cyclosporine for 2
months up to 1 year, or not given depending on diagnosis of the patient and status. GVHD
was treated with steroids, cyclosporine and in a few cases with ATG. The protocol was
approved by the local Ethical Committee at Huddinge hospital.

Methods
During the aplastic period the children rinsed the oral cavity twice daily with a 0.1%
chlorhexidine solution, nystatin 4 x 100,000 IU/ml and 2 x 0.025% sodium fluoride solution.
The children and their parents received instructions in preventive dental care, individualized
fluoride prophylaxis based on assessment of caries risk factors. The program consisted of
tooth brushing with fluoride tooth paste twice daily, fluoride tablets and fluoride varnish
application every third month. Children with low salivary rates were given treatment with sodium fluoride gel 0.1% combined with chlorhexidine gel 0.1% in customized trays. All 122 children were examined clinically and two bite-wing radiographs were taken at baseline, 3, 6 and 12 months after SCT at the Department of Pediatric Dentistry. The number of permanent and deciduous teeth was registered. Caries lesions (DMFT and DS) were registered according to WHO (1978). Approximal dental caries lesions and initial caries were controlled on the bite-wing radiographs. Paraffin-stimulated whole saliva was collected during 5 minutes and the salivary secretion rate was determined to the nearest 0.1 ml. The number of mutans streptococci per ml saliva was estimated according to Gold et al. (39) using a selective MSB-agar and the number of lactobacilli per ml saliva were determined using Rogosa SL-agar (40) at the Department of Oral Microbiology, Karolinska Institutet.

**Statistical Analysis**
Comparisons between children with different conditioning regimens were performed using an unpaired t-test.

**Results**
The mean DFS at baseline examination was 1.4±2.5 for both the non-irradiated group and the patients conditioned with TBI/fTBI, a non-significant difference. As can be seen in Table 1, DFS increases in each group after SCT, significant compared to baseline for all groups except the group of patients with DFS for deciduous teeth conditioned with chemotherapy only.

Table 1. Mean DFS and significance when one-year examination is compared to baseline.

<table>
<thead>
<tr>
<th>Conditioning for SCT</th>
<th>Baseline DFS</th>
<th>1 year DFS</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Primary teeth</td>
<td></td>
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<tr>
<td>TBI/fTBI</td>
<td>3.404</td>
<td>4.995</td>
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<tr>
<td>Chemotherapy</td>
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<td>3.575</td>
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<tr>
<td>Permanent teeth</td>
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<tr>
<td>TBI/fTBI</td>
<td>1.443</td>
<td>2.487</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1.444</td>
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The children conditioned with fTBI/TBI showed an increasing caries progression in their permanent teeth from baseline examination till the one-year examination (Fig. 1). This was significant for both the group starting SCT conditioning without any caries lesions (p<0.001) and the group with pre-existing caries lesions (p<0.05). Both these groups were significantly different compared to baseline, p<0.001. The children who were caries active at baseline had a more significant caries progression during the first year after SCT compared to those caries free at baseline.
Fig.1: Caries progression, permanent teeth, in SCT-children conditioned with fTBI/TBI.

When comparing the group conditioned with fTBI/TBI diagnosed with low saliva secretion (<0.5ml/min) at the 1 year follow-up to the group with normal secretion (>0.5ml/min), there was no difference in DFS at baseline or at the one-year examination. The group with low stimulated saliva secretion rate showed an increase with a significant difference (p<0.001) in DFS, when comparing baseline to one year after SCT. This was also evident for the group with stimulated saliva, with a p<0.05.

**Discussion**

An important finding in the SCT children was a significantly higher DFS 1 year after SCT compared to baseline. For this, there were a number of reasons. All the children had received regular dental care until the intensive treatment necessary for their medical condition became priority. This treatment may extend over several months and becomes long and protracted, particularly if there is a poor response. The chemotherapeutic regimens cause side-effects such as gastro-intestinal disturbances and loss of appetite. This combined with ingestion of refined carbohydrates to increase the calorie intake and little or no preventive care, may be contributory factors in the development of dental caries.

In agreement with the hypothesis this study found a significant increase in dental caries in SCT children conditioned with fTBI/TBI in permanent teeth (p=0.0032) and primary teeth (p=0.0489). Chemotherapy also showed to increase the DFS in the permanent teeth group one year after SCT (p<0.05). These findings are supported by earlier studies, which also showed an increase in dental caries in SCT children (33-36, 41).

This study also showed that SCT children with caries at baseline had a significant increase in DFS in permanent teeth after conditioning with fTBI/TBI, p<0.05, this was also for patients who were caries free at baseline (p<0.001), but to a lesser degree.

An increase of dental caries can also serve as origin for infection in immunocompromised patients (42-44). Their increased caries situation is likely multifactorial and resulting from poor oral hygiene, altered salivary flow rates, dietary factors and higher counts of cariogenic microorganisms. In 1997, Dahllöf et al. showed that lowered salivary secretion rate in combination with an elevated level of mutans streptococci and lactobacilli in long term survivors of SCT increases the risk for developing dental caries (25). A high-carbohydrate diet and difficulty with oral hygiene because of mucositis, may also contribute to the risk of caries (34, 41). In this discussion we must not forget that cancer is not only a
trauma to the patient, but also to the parents. When having to deal with all factors involved with the disease and the treatment, oral hygiene may not always be the main priority.

When investigating stimulated salivary flow rates, this study showed higher DFS in both the groups with low and normal stimulated saliva secretion. Other studies have showed that radiation of 1000 cGy or more may result in reduced salivary flow and a resultant is altered pH balance in the oral cavity, causing a shift in oral flora and an increase in dental caries (16, 34). TBI and chemotherapy is widely studied and it was found that TBI gives permanent damage to the salivary glands and that chemotherapy also have a damaging effect, though sometimes reversible (25). Xerostomia after SCT has also been reported in several studies (16, 41, 45, 46).

Due to the treatment the patients often obtain oral ulcers, which are a great problem for this group, especially in the early stages of treatment, when the immunosystem practically is non-existing. For these immunosuppressed patients, an important part of the mouth care regimen is rinsing with 0.2% chlorhexidine, which if used effectively, reduces plaque accumulation and bacterial loading (47, 48). Using this can often be problematic, first because of the unpleasant taste, but mainly because of the burning sensation it causes in the frequent mouth sores. The mouth sores also makes it harder to brush the teeth effectively and this can be one explanation to the higher DFS after SCT in these children.

The children in this study received an extended oral examination and treatment both prior to and after SCT, with prophylaxis cleanings, fluoride varnishes, mouth rinses and instructions in changing their dietary habits during regular check-ups. Still many of the children increase in DFS, which implies that maybe compliance is not always in the best position, which is understandable during this hard period in the patient’s life. The patients are often very young when getting treatment and may of course not always handle their own oral hygiene, but even for the parent it can be difficult to keep the child’s teeth clean.

Due to advances in treatment more children become long-time survivors after SCT, and effects of both the disease and the treatment need careful investigation and usually life-long medical and dental support. Comprehensive preventive care and examination before stem cell transplantation and follow-up during the period after SCT as clinically indicated can not only reduce increasing dental caries, but more important stop potential sources of infection in these immunosuppressed patients.

In conclusion this study found an increase in dental caries one year after conditioning SCT children with total body irradiation and chemotherapy.

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**References**


