Disturbances in the dental development and cranofacial growth in children treated with haematopoietic stem cell transplantation

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Abstract

Dental development and craniofacial growth were studied in 46 long term survivors of haematopoietic stem cell transplantation (SCT) performed in childhood, before the age of 12 (mean age 6.9 years). The aim of the study was to investigate the correlation between age at SCT, degree of disturbances in dental development and vertical growth of the face. A panoramic and a cephalometric radiograph were taken at a mean age of 15.9 years. For each patient two age and sex matched healthy controls were included. The area of three mandibular teeth was measured and a cephalometric analysis was performed. The results show that the mean area of the mandibular central incisor, first and second molar was significantly smaller in the SCT group and that the vertical growth of the face was significantly reduced, especially in the lower third, compared to healthy controls. We also found a statistically significant correlation between age at SCT, degree of disturbances in dental development and vertical growth of the face.

In conclusion: The younger the child is at SCT the greater the impairment in dental and vertical facial development. This supports the earlier suggestion that the reduction in lower facial height found in SCT children mainly is a result of impaired dental development, and that young age is a risk factor for more severe disturbances.

Keywords: stem cell transplantation, children, late adverse effects, dental development, craniofacial growth, irradiation, chemotherapy.
Introduction

Every year about 275 children in Sweden are diagnosed with cancer (Dreifaldt et al 2004) and it is estimated that 1 in every 900 young adults between the ages of 16 and 44 is a survivor of childhood cancer. The overall survival rate for all types of childhood cancer is now approaching 80%, due to improved diagnostic and therapeutic methods (Bleyer 1997).

The increased number of long term survivors has led to a heightened appreciation of the late complications of treatment and their quality of life. Important late effects include decreased growth, poor school performance, altered cardiac function, infertility and second malignant neoplasm (Shusterman and Meadows 2000). Late effects can be caused by the cancer itself; by the treatment, which may include chemotherapy, radiation, surgery; and supportive care such as transfusion antibiotics and immunosuppressive agents; or a combination of these factors (Leiper 2002a).

Approximately two thirds of survivors of childhood cancer experience at least one chronic or late-occurring complication of therapy (Oeffinger 2004) and for those treated with bone marrow transplantation for hematological malignances as many as 93% had one or more adverse sequelae reported (Leiper 2002b). Cancer treatment also affects the craniofacial and dental structures. Decreased salivary function, increased risk of caries, oral infections, disturbances in dental development and decreased craniofacial growth has been reported (Dahllöf 1998, Purdell-Lewis et al 1988, Dahllöf et al 1997a).
Childhood cancer

Childhood cancer is a relatively uncommon disease. The average annual incidence rate of childhood malignances reported in Sweden during 1990 to 1998 was 16.2 /100,000 per year or about 1 in 6200 (Dreifaldt et al 2004). The types of cancers that occur in children are different from those seen in adults. Most childhood cancers arise from the mesodermal germlayer which in embryonic development becomes connective tissues, bone, muscle, blood and lymphoid organs. Cancers in adults more frequently involves surfaces exposed to chronic environmental insults, such as skin, lung and gastrointestinal epithelia. Many pediatric cancers occur early in life, for example leukemia, neuroblastoma and Wilms’ tumour and the cause of most childhood cancers is not known (www.cancer.org). Ionising radiation contributes to certain types of cancer and epidemiological evidence suggests the presence of certain chemicals (eg. benzene), viruses (Epstein-Barr virus) and bacteria (Helicobacter pylori) in the development of leukemia and lymphoma in children and adults (Greaves 2002). Some children appear to be predisposed to cancer as a result of specific genetic disorders. Children with Down’s syndrome have a 10-20 fold increased risk of ALL (Berger 1997).

Today, most pediatric cancers are curable and the treatment of malignant diseases consists mainly of different combinations of cytostatic drugs. The cytostatic medication can be combined with irradiation and/or surgical treatment, depending on diagnosis (www.cancer.org). The most common malignancy among children younger than 15 years is leukemia, followed by central nervous system tumours and lymphomas, together constituting about 2/3 of all childhood malignancies (Dreifaldt et al 2004).
Leukemia

Acute lymphoblastic leukemia (ALL) accounts for 73-85% of all diagnosis of childhood leukemias being five times more common than acute myeloid leukemia (AML) (NOPHO 2000). In the Nordic countries the incidence rate of ALL has been stable during the last decades, with an overall incidence of about 4 cases per 100,000 children aged below 15. A significant peak in incidence of ALL is seen between the ages of 2 and 5 years and it is reported that ALL is almost twice as common in boys as in girls (Hjalgrim 2003).

The cure rate for both ALL and AML have improved dramatically during the last decades. The 5-year survival rate for children with ALL has increased from about 60% in the early 1970s to around 80% nowadays. For acute myeloid leukemia the survival rate has increased from 20% to about 50% during the last 30 years (Shusterman and Meadows 2000).

The majority of children with ALL are treated according to systemic and intrathecal chemotherapy protocols, although some children also receive therapeutic craniospinal irradiation (18-24Gy) or as prophylaxis against CNS disease (Lehtinen 2003). In general, the treatment is separated into an induction phase, aiming at achieving remission (restoration of normal hematopoiesis), followed by intensification treatment and then prolonged maintenance therapy, to prevent relapse (Horton and Steuber 2005). The total duration of treatment in standard and intermediate therapy is 2.5 years in the Nordic protocol. In the Nordic countries, allogeneic stem cell transplantation is part of the extra intensive treatment protocol targeted to patients with leukocyte count >200*10^4/L or very low response, chromosomal translocation (4;11) or (9:22) or hypodiploidy <34 (Lehtinen 2003).
Bone marrow transplantation/Haematopoietic stem cell transplantation

Bone marrow transplantation, nowadays the term HSCT is used, has since the 1970s emerged as the treatment of choice for various haematological disorders (Tabbara et al 2002). Neoplasm derived from the bone marrow (leukemia, lymphoma and myeloma), congenital diseases (severe aplastic anemia, thalassemia and Fanconi’s anemia) and metabolic diseases (Mb Hurler and Mb Gaucher) are conditions where SCT is indicated (Bortin 1992). The goal with SCT is to eliminate the malignant cells or the cells with enzyme deficiency, by a complete replacement of the patient’s hematopoietic stem cells (Tabbara et al 2002). The source of hematopoietic stem cells may be bone marrow, peripheral blood or umbilical cord blood. If the source is the patient himself it is called an autologous SCT, but if the patient receives the graft from a donor it is called allogeneic.

Allogeneic transplantation procedure

The first important step is to find a suitable donor, preferably an HLA matched related or unrelated (Tabbara et al 2002). Bone marrow is aspirated from the donor’s pelvic bone and then mixed with some of the patient’s blood before being infused intravenously. The stem cells travel through the bloodstream to the bone marrow, where it after 2 to 4 weeks starts to produce new bloodcells, but a complete recovery of the immunfunction generally takes between 1-2 years (www.cancer.gov).
**Conditioning procedure**

Prior to engraftment the patient is conditioned with high-dose chemotherapy with or without irradiation. The purpose with this myeloablative conditioning regimen is to eliminate the malignant cells or the cells with immunodeficiency, suppress the immune system to decrease the risk of graft rejection and to create space in the bone marrow for successful engraftment of the new donor stem cells (Tabbara et al. 2002). The conditioning regimen varies according to the patient’s disease and medical condition. Most of the preparative protocols for leukemic patients include cyclophosphamide (Cy; 120mg/kg) together with 10-12 Gy total body irradiation (TBI) or busulfan (Bu) (Ferry and Socié 2003). Patients with severe aplastic anemia usually receive Cy without radiation and those with metabolic diseases have been treated with Cy in combination with low doses of TBI or Bu (Shaw 1986). TBI may be given in one dose or in multiple doses over the course of several days. Patients treated with allogeneic SCT are also given prophylaxis against graft-versus-host disease (GVHD) (Ringdén et al. 1993, Ringdén 2005).

**Acute treatment related complications**

The time following the SCT is a critical period for the patient and usually the patient stays at the hospital for 1 to 2 months, which make it possible for the medical staff to control engraftment or possible rejection and give the patient the supportive care needed (www.cancer.gov). One of the most serious acute side-effect of the conditioning treatment is the immuno-suppression, which might lead to serious and sometimes lethal infections. Veno-occlusive disease of the liver, acute graft-versus-host disease (Ferry and Socié 2003), nausea,
diarrhea, alopecia and cardiomyopathy are other acute general complications that may follow SCT (Tabbara et al 2002).

**Acute oral complications**

Mucositis is very common in the immediate post transplant period, it often appears about a week after the transplant. The mucosa becomes thin, fragile and sensitive due to the inhibiting effect of the chemo- and radiotherapy on cell turnover and renewal. In 4 to 24 hours after receiving TBI most patient exhibit parotitis, this is though transient and disappears with in a couple of days. Xerostomia, altered taste acuity and bleeding are other short term oral side effects after SCT (Berkowitz et al 1987). Lesions in the thin, fragile mucosa, xerostomia and a low number of leukocytes predispose to oral and systemic infections, which may be hazardous to the neutropenic patient (Dahllöf et al 1990). Oral lesions are often very painful and might cause malnutrition. With the use of cyclosporin A it is common to see gingival hyperplasia, further complicating oral hygiene measures (Daley et al 1986).

**Graft-versus-host disease**

Graft-versus-host disease (GVHD) remains one of the major complications after allogeneic SCT, being a reaction induced by the transplanted donor T-lymphocytes directed towards the recipient’s own cells. It is divided in two forms based on timing of occurrence and clinical manifestations. The acute form occurs in the first 2 to 3 months after transplantation and the chronic form manifests later, usually 3 to 6 months after engraftment (Tabbara et al 2002).
Chronic graft-versus-host disease, occurring in less than 50% of long term survivors, and its associated immunodeficiency state is the prime cause of transplant related mortality late after marrow grafting and contributes directly or indirectly to most non-malignant complications (Socié et al 2003). The clinical presentation of chronic GVHD is similar to that of autoimmune disorders, which may appear as skin lesions, kerato-conjunctivitis, oral mucositis, sicca syndrome, hepatic involvement, obliterative lung disease and suppressed haematopoietic reconstitution. Treatment of chronic GVHD consists of the use of immunosuppressive agents (i.e. cyclosporine or prednisolone) and in about half of the patients therapy may be discontinued after 9-12 months (Tabbara et al 2002).

Long term complications after SCT

Large numbers of patients now survive long term following SCT, rendering the late complications an important issue in the 21st century. Late death occurred in 11%, the leading cause of death was recurrence of disease (Pitcher et al 1999).

Secondary malignant neoplasms (SMN) post-transplant are of particular clinical concern and the most commonly reported are lymphoproliferative disorders (PTLD) and nonhematopoietic solid tumours (Leiper 2002b). Mortality from PTLD is high and although uncommon, it accounts for 40 to 50% of reported SMN. The majority appears in the first 6 to 12 months, thereafter the incidence declines rapidly (Bhatia et al 1996). In contrast, solid tumours have a later onset and latency period after SCT. The cumulative incidence rate of new solid tumours has been reported to be 11% at 15 years.
after SCT in a pediatric group. The most common malignancies reported were neoplasms of the skin, oral cavity, thyroid, CNS, bone and connective tissues (Socie et al 2000).

**Non-malignant late effects**

Non-malignant late effects are heterogenous and although not life threatening they significantly impaire quality of life. The major risk factors for non-malignant complications after SCT are chronic GVHD and/or its treatment and the use of irradiation in the pre-transplantation conditioning (Socié et al 2003).

A rather high incidence of subclinical cardiac, pulmonary and renal dysfunction late after SCT performed in childhood has frequently been reported. For late cardiac dysfunction, previous anthracycline usage seems to be the main causative factor (Leiper 2002a).

Eye lesions, as cataract formation and keratoconjunctivitis sicca are commonly reported side-effects after SCT (Tichelli et al 1993).

Persistent cognitive deficits, with decreased intelligence quotient (IQ) scores and lowered school achievement have been observed after hematopoietic SCT in children, but restricted to those previously treated with cranial irradiation in addition to TBI and to those treated at young age, especially before the age of three (Shusterman and Meadows 2000, Leung et al 2000).

Reduced bone mineral density and osteoporosis (Leiper 2002b), as well as avascular necrosis of bone (Socié et al 2003), are also frequently observed in long-term survivors of SCT.
Thyroid dysfunction is common late sequelae after SCT, most commonly presented as subclinical compensated hypothyroidism and less common as overt hypothyroidism. Single dose TBI is the major causative factor (Sanders 1991a).

Irradiation is also the major cause of direct gonadal failure, but similar damage can also be caused by busulphan. Most young girls treated with either busulphan or TBI need sex hormone replacement therapy to induce puberty and then to maintain menstrual cycles and bone turnover, while most boy spontaneously start and complete puberty (Socié et al 2003). The great majority of men and women treated with TBI or Bu/Cy become infertile, while those subjected to cyclophosphamide alone usually have normal puberty and reproductive development (Sanders et al 1996, Socié et al 2003). Decreased growth rate and lower than average final adult height is commonly seen in those treated with SCT in childhood, when TBI was included in the conditioning therapy. In contrast, children who only have been subjected to chemotherapy (eg Cy, Cy/Bu) usually grow normally. Growth deficiency is more pronounced in those treated at young age and in those previously exposed to cranial irradiation. It has been reported that the majority of children treated with cranial irradiation have a growth hormone (GH) deficiency and this has also been noted in about half of TBI recipients in some studies (Frisk et al 2004, Sanders 1991, Huma et al 1995). The role of GH deficiency as a cause of growth failure and its substitution in children after SCT is still controversial. Some studies have shown a beneficial effect from using GH replacement therapy, while others have not (Socié et al 2003). The positive effect thus seen often was less than the response usually
observed in non-irradiated growth-hormone deficient children (Frisk et al 2004, Sanders 1991). The reduction in growth seen in TBI treated children is influenced by a combination of factors, where GH deficiency might be one, but a direct effect on the bone epiphyses, the thyroid gland and the gonads may also contribute. Nutritional status, duration of chronic GVHD and its treatment can also have a negative effect on growth (Socié et al 2003).

Oral and dentofacial long term complications

Following SCT in childhood long term complications of the oral and craniofacial complex are also 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 (Dahllöf et al 1997, Pajari et al 1989). In the six first months following the transplantation, a decrease in salivary flow has been noted in both the TBI treated and in those solely subjected to chemotherapy. 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 (Dahllöf et al 1997, Chaushu et al 1995). But for those children subjected to 10 Gy TBI before 12 years of age, there seems to be a long term negative effect on the salivary secretion rate (Dahllöf et al 1997).

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 (Woo et al 1997). With a lowered salivary secretion rate, the risk for oral mucosal damage, through viral or fungal infections or trauma, may be increased. Taste acuity
and swallowing may be impaired and influence the quality of life (Bågesund 2000).

Lowered salivary secretion rate in combination with an elevated level of cariogenic bacteria (m.streptococci, lactobacillus) found in long term survivors of SCT, increases the risk for developing dental caries. But with a careful dental preventive care program (oral hygiene measures, flouride toothpaste/topical application, dietary counselling) the caries prevalence in these children can be held at a level similar to that found in healthy children (Dahllöf et al 1997).

The function of the craniomandibular system may also be affected after SCT. Signs of craniomandibular dysfunction were recorded in 84% of SCT children subjected to TBI, compared to 58% in a healthy control group. Both irradiation and chemotherapy may induce long term alterations in connective and muscle tissues resulting in inflammation and eventually fibrosis, which may influence occlusion and mobility of the temporomandibular joint that might be a cause to muscle pain and headache (Dahllöf et al 1994a).

Several studies have also reported on the negative effects of the SCT preparative regimens on dental and craniofacial development (Dahllöf 1998, Purdell-Lewis et al 1988, Näsman et al 1997, Hölttä et al 2002). Disturbances in tooth formation and in the growth of the craniofacial skeleton, influences the development of the occlusion. Teeth and occlusion exert a life-long influence on a human’s well-being and negatively altered it may affect body image, self-esteem, nutritional habits, temporomandibular function and other factors leading to reduced quality of life.
Dental development is a unique process which, excluding third molars, takes 15-16 years to complete, starting in uterine life and ending with the apical closure of canine and second molar roots. During all this time the teeth are susceptible to environmental disturbances (Hölttä et al 2002). Both chemotherapy and radiation have been shown to have injurious effect on the developing teeth. The amelogenesis may be affected, resulting in aesthetically displeasing pits, grooves and discolorations. In a study by Purdell-Lewis et al (1988), 43 out of 45 examined children, previously subjected to chemotherapy, exhibited some type of opacity. Näsmann et al (1994) found that 4,1±5,0 teeth, in chemotherapy treated children, had signs of disturbed enamel mineralisation. For those receiving 10 Gy TBI before SCT the effect was even more pronounced, with 4,6±4,6 teeth affected. Agenesis, microodontic teeth, disturbances in root formation resulting in thin, short V-shaped roots with premature apical closure are commonly encountered in survivors of SCT performed in childhood (Jaffe et al 1984). Hölttä et al (2005a) recently reported the incidence of agenesis in SCT children to be 31%, with 1-12 missing teeth per patient. An even higher incidence (up to 80%) has been reported earlier (Hölttä et al 2002, Näsmann et al 1994). In the study by Hölttä et al (2005a) young age (<5y) was found to be a stronger risk factor for agenesis than TBI, although TBI caused additive impairment.

Young age at treatment as an important risk factor for dental developmental derangement has also been pointed-out in other studies (Näsmann et al 1997, Sonis et al 1990). This is closely related to the developmental stage of the tooth germ and the degree of mineralization. Prior to mineralization, chemo-
therapy and irradiation seem to be able to induce permanent destruction of the tooth germ. At the age of 5 years, most of the permanent teeth already are in the mineralization phase (Haavikko 1970), leading to a lower incidence of agenesis in those treated with SCT after the age of five (Hölttä et al 2005a). Tooth size reduction is evident in the majority of children treated with SCT, with the most pronounced effect seen in the formation of the roots (Näsman et al 1997), which may by indicated by a decrease in root/crown ratio (Hölttä et al 2005b). TBI seems to have a more detrimental effect on the tooth germ compared to chemotherapy alone. Näsman et al (1997) found short V-shaped roots in 94% of children treated with TBI/Cy, compared to 19% in the chemotherapy group. Reduction in tooth size varied between 19% in incisors to 39% in second molars in the TBI group and between 7% and 15% in the chemotherapy group. The corresponding value for root size reduction was 24% to 46% in the TBI group and 13% to 18% in the chemotherapy group. Other studies have also confirmed the more deleterious effect on tooth development of TBI compared to chemotherapy protocols. Duggal (2003) measured root area of mandibular teeth and found statistically significantly smaller root areas in TBI treated children in comparision with both a healthy control group and a chemotherapy group. Those treated with chemotherapy alone or in conjunction with cranial irradiation also had significantly smaller root areas than the control group. Previous cranial irradiation, e.g. for treatment or prophylaxis of CNS leukemic involvement, do not seem to cause any additive negative effect on mandibular tooth development (Duggal 2003, Näsman et al 1997), although some studies have shown an effect on developing maxillary teeth if treated at
a young age. Those maxillary tooth germs affected are at young age placed high up in the maxillary bone and may therefore be found in the irradiated field (Sonis et al 1990).

Young age at SCT seems to be a risk factor for reduction in tooth size and severe dental developmental disturbances. Näslöf (1997) saw a significant correlation between age at TBI and crown/root ratio. Earlier studies have also reported an increased detrimental effect on dental development in younger children, especially for those 5-6 years or younger (Sonis et al 1990, Jaffe et al 1984, Dahllöf et al 1988).

Delayed eruption of teeth in children treated for cancer has been reported in some studies (Purdell-Lewis et al 1988, Jaffe et al 1984), but Dahllöf and co-workers (1989a) did not find any significant difference in dental maturity and number of erupted teeth in 44 chemotherapy treated children compared to healthy controls and this was in agreement with a study by Pajari et al (1988).

**Craniofacial growth and development**

As for the growth of the body, multiagent chemotherapy does not seem to inhibit the growth of the craniofacial skeleton. In contrast, several studies have reported that radiation therapy may induce deficient growth of the craniofacial complex (Sonis et al 1990, Dahllöf 1998, Dahllöf et al 1989b). High dose irradiation (40-60 Gy) locally administered to the head and neck has been shown to induce facial deformities and abnormal occlusal relationship (Guyuron et al 1983). It has been reported that the threshold for direct harmful effect of irradiation to the soft tissues is 4 Gy and 30 Gy for the craniofacial skeleton, in growing individuals (Guyuron et al 1983). Sonis
et al (1990) found craniofacial abnormalities in 90% of patients treated with chemotherapy plus 24 Gy cranial irradiation (CI) before 5 years of age. Mean cephalometric values showed significant deficient mandibular development, but normal values in other cephalometric measures. In those treated at an older age and in those subjected to lower doses of CI, no effect on craniofacial growth was noted.

SCT in childhood after conditioning with TBI has been shown to impair linear growth, by inducing neuroendocrine deficiency and by direct toxicity to the bone epiphysis. TBI preparative regimens have also been shown to impair the growth of the craniofacial complex (Dahllöf et al 1989b, Forsberg et al 2002). When studying 14 children, subjected to Cy/TBI conditioning treatment, Dahllöf et al (1989b) found, that all cephalometric values were significantly diminished, compared to a healthy control group. Different parts of the craniofacial skeleton grow at different times and with different growth rates and are thus sensitive at different points in development. The least effect on growth was found in the dimension of the anterior cranial base, probably due to the early completion of growth of this structure (Dahllöf et al 1989b). The adult dimension of the middle (ethmoidal) part of the cranial base is reached at about 7 years of age (Proffit 2000). The reduction in length of the mandible was reported to be four times as great as for the maxillary length, probably owing to the fact that the maxilla lacks endochondral bone formation (Proffit 2000). A significant reduction of the vertical growth of the face was also found. And for the vertical dimensions, the degree of reduction appeared to be associated with the patient’s age at the time of SCT. This correlation was most pronounced
for the values expressing the development of the alveolar height, with the greatest reduction seen in those treated at a young age (Dahllöf et al 1989b). The effect of growth hormone (GH) treatment on craniofacial growth, in children exhibiting growth retardation has also been studied by Dahllöf and co-workers (Dahllöf et al 1994b, Forsberg et al 2002, Dahllöf et al 1991). A group of nine children, who commenced GH treatment at a mean age of 12,1 years were followed up after a median time of 3,5 years. The GH treatment resulted in a mean growth rate of the craniofacial structures similar to that found in healthy controls. The GH treatment did not seem to induce a catch-up growth, but rather prevented further loss in growth potential, which also was evidenced by the SDS (standard deviation score) for standing height, which was –2,18 at start of GH therapy and -1,87 after a mean of 3,5 years. In SCT children not treated with GH, significantly reduced growth increments were recorded in the variables mandibular length and mandibular molar height. The mean growth increment of the mandibular length was only 30% of that found in healthy controls (Dahllöf et al 1994b). With regard to the effect on mandibular length (cd-pgn), GH therapy resulted in an average increment exceeding that of the controls and this was in agreement with an earlier study (Dahllöf et al 1991). This finding indicate that the condylar cartilage is the most likely site for growth activity stimulated by exogenous GH and this is in agreement with the hypothesis that GH encourage longitudinal bone growth both directly, by stimulation differentiation of epiphyseal growth plate precursor cells and indirectly by increasing the responsiveness to insulin-like growth factor-1 (Green et al 1985).
In a recent study, Forsberg et al (2002) studied the growth pattern of the mandible in GH treated children, as neither the deficient mandibular growth in SCT recipients nor the increased mandibular growth in GH treated SCT patients was reflected in the anterio-posterior position of the mandible (ANB, SNB) in earlier studies (Dahllöf et al 1989b, 1991, 1994b). They noted an obvious effect of GH on the growth of the mandible, with the condyle displaced in an backward-upward direction in relation to basion. In the healthy control group the condyle was displaced in an opposite direction. They concluded that this might reflect a normalization of the condyle-fossa relationship, which before GH treatment seemed to be altered with the condyle in an anterior position in the fossa, which also could be advantageous for the function of the craniofacial complex.

SCT, particularly after TBI, thus seems to result in impaired facial growth and disturbed dental development. Treatment at a young age (<5-6 years) seems to result in the most extensive disturbances in dental development and also in the greatest reduction in the vertical dimensions of the face, especially noted in the growth of the lower face and in the alveolar processes. Vertical development of the face and in particular in the lower third is intimately associated with the growth of the alveolar bone. The alveolar bone grows in response to the formation and eruption of teeth (Proffit 2000) and an impaired dental development will thus result in a reduction in alveolar and dental height (Forsberg et al 2002).

It has been suggested that the reduction in facial height seen in SCT children, is mainly a result of impaired dental development, particularly in the younger groups.
The hypothesis to be tested in this study is that the younger the age at SCT, the more severe dental developmental disturbances and the more deficient vertical growth of the lower face will be found.
Subjects and methods

SUBJECTS
This study included 46 recipients of allogeneic SCT, performed at Huddinge Hospital between 1980 and 1998. The majority was treated for a hematological malignancy, but non-malignant diagnosis, such as severe aplastic anaemia (SAA) and metabolic diseases were also found. The conditioning treatment differed with diagnosis. Children treated for a malignancy usually received high-dose cyclophosphamide (total dose of 120 mg/kg) in combination with 10 Gy TBI in a single dose or combined with busulphan (4 mg/kg p.o in divided dosed daily for 4 days). Patients with SAA commonly received Cy (200mg/kg), but in some cases, as for those with a metabolic disease, combined with lower doses of TBI, busulphan or TAI (total abdominal irradiation) (Ringdén et al 1989). As prophylaxis against GVHD, methotrexate, cyclosporine A or a combination of these were given to all children (Ringdén et al 1993). Baseline characteristics for the patient group are outlined in table 1.
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<th>Value</th>
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Cy, cyclophosphamide; TBI, total body irradiation; Bu, busulphan; VP16, etopside; TAI, total abdominal irradiation; Mtx, methotrexate; CsA, cyclosporine A.
These 46 subjects included had been treated with SCT at an age of 12 years or younger, had survived for at least 3 years, had a cephalometric and a panoramic radiograph (PRG) of good quality taken when the patient was at least 12 years of age and taken at least 3 years after the transplantation.

For each patient, two healthy controls matched for age and gender, were selected from the patient files at the Department of Orthodontics. Only those with a moderate degree of malocclusion and those having a good quality PRG and cephalogram taken before orthodontic treatment were selected.

For characteristics concerning age and gender for the two groups, see table 2.

<table>
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<td>Number</td>
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<td>92</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (54 %)</td>
<td>50 (54 %)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (46%)</td>
<td>42 (46%)</td>
</tr>
<tr>
<td>Age at X-ray (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>15.918</td>
<td>15.894</td>
</tr>
<tr>
<td>S D</td>
<td>2.448</td>
<td>2.415</td>
</tr>
</tbody>
</table>
METHODS
A panoramic radiograph of the teeth and a lateral cephalometric radiograph were taken of all children in connection with dental examination (patients) or orthodontic treatment planning (controls), at the Department of Odontology at the Karolinska Institute in Huddinge.

Area measurements
The degree of dental developmental disturbances was evaluated by measuring the area of the mandibular left central incisor, first and second molar on the PGR. If the outline of any of the teeth was difficult to trace, the contralateral tooth was measured instead. The measuring of the tooth area was performed by the author (MJ), by using a manual planimetric instrument (OTT-Planimeter/ A.Ott Kempten Bayern), with an accuracy of 0.005 cm² (Linder-Aronson 1970). Each tooth was measured twice and the mean was registered.

The radiographic procedure produces an enlarged image of the teeth. The enlargement factor is difficult to control as it varies in different areas of the picture and between individuals. It is reasonable to assume, however, that these variations are randomly distributed, and under such circumstances the enlargement error should not affect comparisons of mean values to any mentionable degree. This assumption has been tested and confirmed by Näsman et al (1997) in an earlier study.

Cephalometric analysis
A digitized cephalometric analysis was performed of the lateral head films, which were taken according to a standard method. All cephalometric registrations were performed by the author (MJ). The craniofacial variables evaluated were based on the cephalometric reference points and lines shown.
in Figure 1. All measurements were made with an electronic digitizer on-line with a microcomputer. The resolution of the instrument was 0.1 mm and 0.1 degree. Since different cephalostats were used, all linear measurements were corrected for magnification. The cephalometric variables of interest are those describing the vertical dimension of the face and of the alveolar processes and these are outlined in Table 3.

Reference points and lines

Figure 2  The cephalometric points and lines. For definitoin of points and lines not fully defined below, see Björk (1960). A, subspinale; ans, anterior nasal spine; B, supramentale; cd, condylion; gn, gnathion; id, infradentale; lj6, the mesial cusp tip of the mandibular first molar; n, nasion; pg, pogonion; pgn, prognathion; pns, posterior nasal spine; pr, prosthion; s, sella; se, ethmoid registration point, intersection of the sphenoid plate with the average greater sphenoid wing; sn, subnasale, a point on the anterior contour of the maxilla where the vertical dimension of the anterior nasal spine is 3mm; uj6, the mesial cusp tip of the maxillary first molar.
**Statistical analysis**

To test the reliability of the method, duplicate measurements were made on the cephalograms of 19 randomly selected subjects. The error of the method ($S_i$) was calculated using the formula:

$$S_i = \sqrt{\frac{\sum d^2}{2N}}$$

Where $d$ is the difference between two measurements and $N$ is the number of double determinations. The error was found to vary between 0.07 mm (id-ML) and 1.76 mm (cd-pgn) for the linear measurements, and between 0.11° (ML/NL) and 0.39° (SNA) for the angular measures.

For comparison of mean values and standard deviations, unpaired t-tests were used. Linear regression analyses were performed for the correlation of the variables age at SCT, mean tooth area and alveolar height.

Level of significance, $P < 0.05$.

The protocol was approved by the local Ethical Committee at Huddinge hospital.
Results

The results of this study show that both dental development and craniofacial growth are impaired in children treated with hematopoietic SCT before the age of 12 years and that there exists a correlation between age at SCT, degree of dental developmental disturbances, growth of the alveolar processes and the vertical growth of the face.

Area measurements

For all three teeth analysed (the mandibular left central incisor, first and second molar), the mean area was significantly reduced in the SCT group compared to the control group. The mean difference in size of the first molar, between the groups, was about 0.5 cm$^2$ and even larger for the second molar (Table 2).

Table 2  Mean tooth areas of mandibular teeth obtained from panoramic radiographs in SCT group and control group: comparison of mean values.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>SCT group (n=46)</th>
<th>Control group (n=92)</th>
<th>Significance p-value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower central incisor</td>
<td>0.762 (0.152)</td>
<td>0.973 (0.135)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Lower first molar</td>
<td>2.349 (0.346)</td>
<td>2.895 (0.352)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Lower second molar$^2$</td>
<td>1.959 (0.507)</td>
<td>2.839 (0.330)</td>
<td>&lt;0.001***</td>
</tr>
</tbody>
</table>

$^1$ Levels of significance. * P<0.05, ** P<0.01, *** P<0.001

$^2$ BMT n = 43, due to aplasia
A significant correlation between age at SCT and mean tooth area was found (p<0.001) as can be seen in figure 3 for the lower first permanent molar. The same correlation was found for the other teeth examined as well. The older the child was at SCT less disturbances in tooth development were seen.

![Regression Plot](image)

**Figure 3** Correlation between age at SCT and mean area of the lower left first molar.

**Cephalometric analysis**

When analysing the vertical growth of the face, all linear vertical measures, except for upper anterior facial height (n-ans), were significantly diminished in the SCT group compared to the control group (Table 3). The mean difference in total facial height (n-gn), between the groups, was more that 4 mm and this difference was mainly due to a reduction in the lower facial height (mean diff; 4.33 mm, p <0.001). A statistically significant difference, between the groups, was seen in the height of the alveolar
processes, ranging from 1.3 mm (p=0.0032) in the upper anterior alveolar height to 2.1 mm (p<0.001) for the lower posterior alveolar height (Lj7-ML).

When considering the length of the anterior cranial base and the maxillary length no significant difference was seen between the groups. The length of the mandible was significantly diminished (mean difference; 5.1 mm, p<0.001) in the SCT group.

The anterio-posterior position (SNA,SNB) and the vertical inclination (ML/NSL, ML/NL) of the mandible and maxilla were not influenced by SCT, with the exception of the inclination of the maxilla (NL/NSL) that was slightly increased in the SCT children (mean diff; 1.2º, p<0.05)

Table 3  Comparison of vertical cephalometric measures, obtained from analysis of lateral radiographs, between SCT group and control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SCT group (n=46)</th>
<th>Control group (n=92)</th>
<th>Significance p-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-gn</td>
<td>107.784 (7.409)</td>
<td>112.100 (6.480)</td>
<td>0.0006 ***</td>
</tr>
<tr>
<td>n-ans</td>
<td>48.076 (3.175)</td>
<td>48.531 (2.663)</td>
<td>0.376 ns</td>
</tr>
<tr>
<td>se-pns</td>
<td>42.968 (3.444)</td>
<td>45.377 (3.576)</td>
<td>0.0002 ***</td>
</tr>
<tr>
<td>ans-gn</td>
<td>57.551 (5.142)</td>
<td>61.882 (4.990)</td>
<td>&lt;0.0001 ***</td>
</tr>
<tr>
<td>pr-NL</td>
<td>13.997 (2.548)</td>
<td>15.276 (2.261)</td>
<td>0.0032 **</td>
</tr>
<tr>
<td>Uj6-NL</td>
<td>20.616 (2.467)</td>
<td>22.462 (2.539)</td>
<td>&lt;0.0001 ***</td>
</tr>
<tr>
<td>Ulj7-NL²</td>
<td>18.258 (2.315)</td>
<td>19.967 (2.589)</td>
<td>0.0005 ***</td>
</tr>
<tr>
<td>id-ML</td>
<td>26.673 (2.554)</td>
<td>28.452 (2.591)</td>
<td>0.0002 ***</td>
</tr>
<tr>
<td>Lj6-ML</td>
<td>29.072 (2.555)</td>
<td>30.525 (2.814)</td>
<td>0.0038 **</td>
</tr>
<tr>
<td>Llj7-ML³</td>
<td>27.340 (2.159)</td>
<td>29.403 (2.723)</td>
<td>&lt;0.001 ***</td>
</tr>
</tbody>
</table>

¹ Levels of significance. * P<0.05, ** P<0.01, *** P<0.001, ns non-significant
² BMT n=41. Control n=79. Due to aplasia or not fully erupted teeth
³ BMT n=42. Control n=83. Due to aplasia or not fully erupted teeth
A statistically significant correlation between age and the measures of alveolar height, Uj6-NL (p <0.01) and Lj6-ML (p <0.05) was found, with more pronounced disturbances in alveolar bone growth in those treated with SCT at a young age (Figure 4).

For the other values describing alveolar height, an association between age and growth was also seen, but without reaching the level of significance.

**Figure 4** Correlation between age at SCT and mean alveolar height in regio 36 (p-value <0.05)
Correlation between tooth area and vertical growth

The correlation between dental disturbances and vertical growth of the face was also analysed by plotting the area of the three mandibular teeth against the alveolar height in the corresponding region. A statistically significant correlation (p<0.001) was found between tooth area of the first lower molar and the alveolar height in that region, shown in Figure 5.

![Regression Plot](image)

**Figure 5** Correlation between mean area of the first lower molar and the mean alveolar height in SCT children.
Correlation between age at SCT, tooth area and vertical alveolar height

Finally, three multiple regression analyses were performed, to test the correlation between the age at SCT, the degree of dental developmental disturbances and the vertical growth of the alveolar processes. The results are shown in figures 6 to 8, and these shows that there are statistically significant correlations between the age, tooth area and alveolar height. The younger the child is at SCT, the greater the impairment in dental and then also in vertical facial development.

Figure 6  Linear regression analysis correlating age at SCT with mean area of the lower central incisor and the alveolar height in that region.
Figure 7  Linear regression analysis correlating age at SCT with mean area of the first lower molar and alveolar height in that region

Figure 8  Linear regression analysis correlating age at SCT with mean area of the second lower molar and the alveolar height in that region.
Discussion

The results of this study show that there is a correlation between age at SCT, degree of dental developmental disturbances and vertical growth of the face. In this study, 46 children treated with hematopoietic stem cell transplantation before the age of 12 (mean age 6.9 y), were followed-up after a mean of 9 years, at a mean age of 15.9 years at a time when all permanent teeth (except third molars) should be erupted and in occlusion. The majority had received total body irradiation in the conditioning regimes, but there also were some who only received high-dose chemotherapy before transplantation. As earlier studies have shown that chemotherapy alone has less detrimental effect on tooth development than TBI (Näsman et al 1997, Duggal 2003) and that chemotherapy alone does not seem to influence craniofacial growth (Sonis et al 1990, Dahllöf 1998, Dahllöf et al 1989b), it is possible that the results and correlations we have found could have been more pronounced if those subjected to chemotherapy alone had been excluded.

Concerning the effect of SCT on tooth development, we found significantly reduced tooth areas in the SCT group compared to the control group and this was in agreement with several earlier studies (Duggal 2003, Jaffe et al 1984). A significant correlation between age at SCT and tooth size was also seen, indicating a more detrimental effect on tooth development in those treated with SCT at a young age. Other studies have also reported the negative influence of young age (Näsman et al 1997) and recently Hölttä et al (2005a) suggested that young age at SCT was a stronger risk factor than TBI for severe dental impairment.
We also found significantly diminished linear craniofacial measures in the transplanted individuals in accordance with earlier studies (Dahllöf et al 1989b, 1991). For the length of the anterior cranial base (n-s) and the maxilla (ans-pns) no significant differences compared to the controls were seen, probably reflecting the early completion of growth of the anterior cranial base and the lack of endochondral bone formation of the maxilla (Proffit 2000). In comparison to the maxilla the length of the mandible was significantly reduced in the treated group. This is in accordance with earlier reports; Dahllöf et al (1989b) reported the negative effect on mandibular growth to be four times as great as for the maxilla, probably reflecting the radiosensitivity of dividing cells in the condylar cartilage, resulting in decreased growth of the condyle. They also found a significant difference in the length of the maxilla between the groups, among the youngest individuals, which was not seen in this study. This might be explained by a higher mean age at SCT in our group and that we did not analyse the effect of age at SCT on the maxillary length. Another factor might be that we did not exclude those subjected to chemotherapy alone, which was done in the study by Dahllöf et al (1989b).

The vertical growth of the face was significantly reduced in those treated with SCT. The only vertical variable not statistically different between the groups was the upper anterior facial height (n-ans). As the upper posterior facial height (se-pns) was reduced in the SCT group, a slight posterior inclination of the maxilla was found, evidenced by a minor increase in the angle NL/NSL compared to controls. This difference in maxillary inclination was also reported by Dahllöf et al (1989b). This increase in the inclination of
the maxilla was the only positional change found of the jaws, as neither the anterio-posterior position of the jaws nor the vertical inclination of the mandible seemed to be influenced by the SCT, and this was also in accordance with earlier findings (Dahllöf et al 1989b, Forsberg et al 2002). The reduction in total facial height was almost totally a result of the decrease in growth of the lower third of the face, with significantly reduced height of the alveolar processes, implicating an association between tooth eruption and alveolar bone growth (Dahllöf et al 1989b). When correlating the age at SCT with growth of the alveolar processes, statistically significant correlations was seen in the upper and lower first molar regions, with less disturbances in vertical growth found in children treated at an older age. In the anterior alveolar regions and in the second molar regions, an association between age and alveolar height was also seen, but without reaching the level of significance. As discussed earlier, both TBI and non-TBI preparative regimens were used in this study which could have influenced the results, as chemotherapy alone does not seem to cause the same degree of disturbances in tooth development or craniofacial growth. It is possible that a stronger correlation had been found if only patients subjected to TBI had been included.

Finally multiple regression analyses were performed for the variables age at SCT, tooth area and alveolar height. The results of these analyses did show a significant correlation between these factors, supporting the hypothesis that the younger the age at SCT, the more severe dental developmental disturbances and the more deficient vertical growth of the lower face will be found.
It is known that eruption of teeth is important for the development of the alveolar processes and vertical growth of the face (Proffit) and this study supports the earlier suggestion (Dahllöf et al 1989b) that the reduction in lower facial height found in SCT children mainly is a result of impaired dental development, and that young age is a risk factor for more severe dental disturbances.

This study further underlines the negative effects on dental and craniofacial development of treatment seen in long term survivors of SCT, effects that might influence present and future occlusion, temporomandibular function, dental and periodontal health and in that way the quality of life. As more and more survivors now are entering adulthood more studies with longer follow up should and could be performed.
Acknowledgement

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