Effect of busulfan and total body irradiation on dental development in children treated with bone marrow transplantation

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Abstract
The cancer treatment of today saves many lives but not without consequences. Almost all patients exhibit some degree of side effects, also including the oral cavity. Today most of the preparative regimens before allogeneic bone marrow transplantation (BMT) include cyclophosphamide (CY) with either total body irradiation (TBI) or busulfan (BU). In the present study dental development in dental PRGs of 95 children conditioned with either TBI/CY or BU/CY before BMT were studied. The aim of this investigation was to study the effect of conditioning regimens used in BMT on dental development, comparing TBI to BU. The results of this study show that all developing teeth are irreversibly and equally by number affected by busulfan and radiation therapy. However, there seem to be a difference in the severity of disturbances in dental development between children conditioned with BU/CY and TBI/CY.

Introduction
Pediatric cancer
In Sweden about 275 children a year are diagnosed with cancer (1). Nowadays 2/3 of all children with cancer survives (2,3). This is because of improved diagnostic and therapeutic methods (2-9). Cancer is a generic term of a number of different types of malignant tumour diseases (10). Cancer develops when cells in a part of the body begin to grow out of control (10). Although there are many kinds of cancer, they all start because of out of control growth of abnormal cells (11). Pediatric cancers are very different from adult cancers with regard to physiology, sites of occurrence and response to therapy (11,12). Most childhood cancers arise from the mesodermal germ layer, which in embryonic development becomes connective tissue, bone, cartilage, muscle, blood, blood vessels, sex organs, kidneys, and lymphatic and lymphoid organs (12). Cancer in adults more frequently involves surfaces exposed to chronic environmental insults, such as skin, lung, gastrointestinal epithelia and mucous membranes.

Presenting symptoms are also different in children compared to adults. Typical symptoms in children include adenopathy, anemia, bruising, weight loss, abdominal mass, limp or persistent headache (13). Cancer is most often treated with surgery, radiation therapy, medicines, but foremost with chemotherapy and hormone affecting medication, or a combination of these treatments (2,4,10). Chemotherapy is a generic term of medicaments, which stops the growth of cancer cells, causing them to die (10,11). Although there are exceptions, childhood cancers tend to respond better to chemotherapy. Children also tolerate chemotherapy better than adults. Because chemotherapy can have long-term side effects, children who survive their cancer need careful attention for the rest of their lives (11).

The incidence of childhood cancers throughout the world is markedly by race and geographic location. The highest rates are seen in Israel and Nigeria and the lowest in India and Japan. The reasons for these differences in incidence are not fully understood but may be related to numerous influences, including racial, genetic and environmental factors (12).
Many cancers in children occur with high frequency at an early age, between ages 2-5, particularly acute lymphoblastic leukemia, neuroblastoma, Wilms’ tumour, hepatoblastoma, retinoblastoma and rhabdomyosarcoma. Some children appear to be predisposed to cancer as a result of specific genetic disorders. For example, children with Down’s syndrome have an increased incidence (1:100) of leukemia (13).

Leukemia is the most common cancer in children. It accounts for about one third of all cancers in children under 15 years of age and one fourth of cancers occurring before age 20 (1,11). Of about 80 children in Sweden who will develop leukemia this year about 90% will be diagnosed with acute lymphoblastic leukemia (ALL), (2,14,15). Most of the remaining children will be diagnosed with myelogenous leukemia (AML) (about 10%). Chronic leukemias are rare in children (14). ALL is most common in early childhood peaking between 2 and 3 years of age. AML is most common during the first 2 years of life and less common among older children. AML cases starts to increase again during the teenage years with AML becoming the most common acute leukemia in adults (11).

Central nervous system (CNS) tumours are the second most frequently occurring malignancy and the most common solid tumour in the pediatric population. Of the CNS malignancies, the most common is astrocytoma (12). Lymphomas are the third most common malignancy in the pediatric population. Non-Hodgkin’s lymphoma accounts for 60% of diagnosed lymphomas and Hodgkin’s disease accounts for the remaining 40%. Lymphomas are seen in both the pediatric and adult population; however, the subtypes are treated very differently (12).

**Bone marrow transplantation**

Bone marrow is the blood-producing organ of the human body. In new-borns all bone marrow is blood producing and therefore so called red type (16). Erythrocytes, trombocytes and leukocytes are produced in the red bone marrow (12,16,17). This red bone marrow is with time redrawn and in adults you can only find this type in specific parts of the body, such as the breastbone, pelvis and notches. The remaining is yellow and contains a large amount of fat (16). Bone marrow transplantation (BMT) has become a most useful approach to treatment of life-threatening human diseases (4,18,19). Indeed, it is now possible to cure more than 60 otherwise lethal diseases by treatments using BMT (18). ALL, AML, CML, severe aplastic anemia, severe combined immunodeficiency and thalassemia major are some of the established indications for allogeneic BMT (20). The most common treatment includes several types of chemotherapy alone or in combination with radiation therapy (4,21). The side effects determine how intense the therapy can be. First of all the patient’s own blood producing bone marrow is taken out of function with high dose chemotherapy and/or radiation. To survive such a therapy the patient must be saved with an infusion of new bone marrow (17). Three sources of hemapoietic cells can be used: allogeneic bone marrow from a related or unrelated donor, syngenic marrow from a genetically identical twin, or autologous bone marrow or peripheral blood stem cells (22). The majority of allogeneic marrow transplants are for the treatment of leukemia (16). To reduce the number of cancer cells chemotherapy is given to the patient before BMT. In nearly all cases the tumour disease can no longer be proven before BMT because of the chemotherapy treatment. Ten days before BMT the patient is hospitalised and pre-treated with chemotherapy and radiation therapy. The pre-treatment is performed with such high doses that the blood producing function of the bone marrow is ruled out. The type of chemotherapy and radiation therapy varies and depends on among others the pattern of side effects, the origin of the disease and the patients’ status of health (17).

The bone marrow is aspirated from the donor and during epidural or general anesthesia. The aspiration is performed from crista iliaca. The bone marrow is mixed with the patient’s
blood and then re-injected to the patient as an intravenous infusion. BMT may fail because of relapse, graft failure or transplantation-related complication (23). Patient can be affected by graft versus host disease (GvHD) an immunological reaction mediated by leukocytes in the graft (16).

There are two of principle important differences between autologous and allogeneic bone marrow. The autologous bone marrow can contain tumour cells even though the patient is in remission. The other difference is that immunological complications that can arise depending on incompatibility in HLA or in other immunological systems between the donor and the recipient of allogeneic transplantation do not arise when autologous transplantation is performed.

The methods to prevent relapse respective immunological complications therefore differ between the two methods (16). The treatment results after allogeneic and autologous BMT are practically the same. Lack of GvHD at autologous BMT makes early and late side effects less than those by allogeneic BMT. The absence of GvHD at autologous BMT leads to an increased risk of leukemia relapse. The limited access of bone marrow donors partly leads to a more common use of autologous BMT (17).

Side effects
The cancer treatment of today saves many lives but not without consequences. Almost all patients exhibit some degree of side effects. The side effects associated with BMT can be divided into acute and long-term complications (19).

Immediately following TBI, acute complications such as dizziness, nausea and vomiting develop in varying intensities. Parotid swellings and erythrodermia are also typical and can persist for several days. As a result of cell destruction and cytokine release chills, tachycardia and elevated body temperatures can also be seen (20).

Apart from acute toxicity conditioning regimens used in the setting of BMT also induce long-term oral side effects (5). The chemotherapy treatment can cause permanent damage in tissue and lead to delay in development. The most sensitive structures are organs with high cell turnover like the oral and intestinal mucosa, bone marrow, hair follicles, testicles and the liver (24,25). Other adverse effects include growth retardation, cardiomyopathy, skeletal changes and reduction of bone mass (2). Damages of this kind have especially deleterious effect on children because tissues during development are very sensitive to damage. The younger the patient is, the higher is the risk for late negative effects of the treatment. Side effects of the therapy can include everything from neuropsychological disturbances, heart problems and secondary malignancies to oral symptoms (24). The major risk factors for non-malignant complications after stem cell transplantation (SCT) are chronic GvHD and its associated immune-deficiency state. This is the prime cause of transplant related mortality late after marrow grafting and contributes directly or indirectly to most non-malignant complications (7).

Oral complications
Early effects of chemotherapy and radiation therapy on mucosa are well known as well as the long-term effects of different treatments on oral health and dental development (4). Normally the cell turnover of the oral mucosa is very high. This makes the cells highly sensitive to damage caused by chemotherapy or radiation therapy (26). Therefore the oral cavity is a frequent site of complications during BMT (18,27-34).

Mucositis
The most common oral complication in the immediate post transplant period is mucositis (27-34). Most, if not all patients experience some degree of mucositis (15,18). The mucosa
becomes thin, fragile and sensitive due to the inhibiting effect of chemotherapy on the cell turnover and renewal (12,26). This results in patient discomfort and pain and can make peroral nutrition impossible (15). About a week after start of treatment the risk of mucositis is the highest (26). Mucositis usually resolves when the absolute neutrophil count exceeds 500/mm³ (18,33). Mucositis is further specified as to its anatomic location: when it occurs in the oral cavity, it is called stomatitis; in the oesophagus, esophagitis; in the intestines, mucositis usually is manifested by diarrhea; and in the rectum, proctitis (12). The presence and frequency of these ulcers are depending on the dose, the combination of chemotherapy drugs, the treatment time and other individual factors (18,26).

**Infections**

Chemotherapy and radiation therapy also causes a decrease in the number of leukocytes (granulocytopenia). This increases the risk of infections in the oral cavity. The classical signs of inflammation (rubor, tumor, calor, dolor, functio laesa) are missing when the immune defence is decreased. The oral tissues are susceptible to a variety of fungal, bacterial and viral infections (6,12,18,26). Fungal infections are often caused by Candida albicans. Viruses like herpes simplex virus can be activated and are present during chemotherapy and radiation therapy (12,18,26). Bacterial and fungal infections in the oral cavity can be spread to the blood through ulcers in the oral mucosa. This can cause bacteremia and other complications. A healthy mouth prevents many complications during and after the treatment. The chemotherapy treatment also causes increased risk of bleeding (26). There is evidence of beginning tissue damage in 5-7 days after chemotherapy administration. Pale, dry mucous membranes, burning sensation, dry tongue with raised papillae and ridging of buccal mucosa can be seen (12).

**Other oral side effects**

The salivary glands are sensitive to chemotherapy and radiation therapy treatment and the patients often exhibit xerostomia, loss of taste, osteoradionecrosis and trismus (26,35,36). These sequelae may be dose limiting (36). A different bacteria composition in the saliva in combination with hyposalivation can cause dental caries (26). The prevalence of dental caries does not differ between children conditioned with TBI, chemotherapy and healthy controls (5). Faced with a diagnosis of malignancy, a patient and/or their parents may not see dental care as a high priority and may therefore have untreated caries (37).

Multiple dental disturbances are found in most patients conditioned with TBI and include the following: shortening of roots, agenesis of roots, enamel hypoplasia and microdontia (18,19,38). Enamel hypoplasia, insufficient mineralisation, ceased or inhibited root development, gracile and conical roots, premature closure of apices, microdontia and delayed or inhibited root development, hypodontia or failed dental development has been reported among children surviving longer treatments (6-30 months) of chemotherapy (25). A delayed eruption of permanent teeth also has been found in some patients (6). The injury in odontogenesis mainly involves the undifferentiated mesenchymal cells in the proliferating zone of the tooth pulp, resulting in acellularity of the basal part of the pulp, whereas the differentiated ameloblasts and odontoblasts appear to be unaffected (3). All children under the age of 12 at the start of chemotherapy and radiation therapy later get changes in tooth development. Those who are the most affected are children under the age of 5-6 years at treatment start (19,39).

Studies of busulfan (BU) and cyclophosphamide (CY) regimen in the early 1980s were motivated by a desire to reduce toxicity and improve the probability of long-term remission (23). BU also offered the advantage of an easier administration than TBI, particularly in small children (21). BU belongs to the general group of chemotherapy drugs known as alkylating...
agents. In combination with CY, BU is an important preparative regimen for patients undergoing stem cell transplantation (SCT) for both malignant and non-malignant disorders (40, 41). BU stops the growth of cancer cells. It is used to treat chronic myelogenous leukemia but BU can also be used to treat other cancers in much higher doses (11, 40, 41). A low BU concentration might lead to a leukemic relapse, and a high BU concentration can result in severe toxicity such as veno-occlusive disease, interstitial pneumonia, or multi-organ failure, which are life threatening complications. Studies have reported that children have a higher clearance than adults and the dosage should be adjusted according to the body surface area. The bioavailability in children can differ between 22-100%, and in adults 47-100%. Since BU has a small therapeutic window, the dose adjustment is an important part of the treatment (40). Today there are few studies on BU and dental development and, therefore more studies concerning this subject are needed.

Table 1. Eruption order and time of permanent teeth:

<table>
<thead>
<tr>
<th>Boys</th>
<th></th>
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<th></th>
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<td>7</td>
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<td>6</td>
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<td>2</td>
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<td>4</td>
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<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
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<table>
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<th>Girls</th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Upper jaw</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Lower jaw</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

Hypothesis
The aim of the present investigation was to study the effect of conditioning regimens used in bone marrow transplantation on dental development, comparing TBI/CY to BU/CY. Our hypothesis is that children conditioned with BU/CY show fewer disturbances in dental development than children conditioned with single dose TBI/CY.

Patients and methods
Patients
The study included 95 recipients of allogeneic BMT grafted between 1980 and 2001. Of these recipients 56 were boys and 39 were girls, grafted between ages 12.0 months to 195.2 months. The children were divided into two groups according to conditioning therapy: TBI/CY group (n=66) and BU/CY group (n=29). Fourteen of these patients could not participate in this study because of unsatisfactory information about these children. Four patients died before any panoramic radiograph (PRG) could be taken. Three patients moved to another city/country and the remaining seven children who did not participate in the study did not collaborate when radiographic examinations were performed. Of the remaining 81 patients, 57 were
conditioned with TBI/CY, 34 male and 24 female, and 24 were conditioned with BU/CY, 14 were male and 9 were female. In the TBI/CY-group the mean age at PRG examination was 178.6 months and in the BU/CY-group the mean age was 156.5 months. Baseline characteristics of the children are presented in table 1.

Table 2. Baseline characteristics of BMT recipients conditioned with TBI/CY or BU/CY

<table>
<thead>
<tr>
<th>Variable</th>
<th>TBI/CY-group (n = 66)</th>
<th>BU/CY-group (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± s.d. (months)</td>
<td>102.6 ± 49.8</td>
<td>73.5 ± 78.5</td>
</tr>
<tr>
<td>Male/ female</td>
<td>39/27</td>
<td>17/12</td>
</tr>
<tr>
<td><strong>Underlying malignancies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>B-cell lymphoblastic leukemia</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>T-cell lymphoblastic leukemia</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Acute erythroblast leukemia</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Oligoblast leukemia</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Severe aplastic anemia</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Aspartylglucosaminuria</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Chronic granulomatous disorder</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Trombocytopenia</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mb Gaucher</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Mb Sandhof</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>San Philippe anemia</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>GvHD prophylaxis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYA</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>CsA</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>MTX + CsA</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>MTX</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>MTX + low</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>CsA + pred</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
Methods
The PRG of 81 BMT recipients were examined for aplasia, microdontia (reduction in crown size) and disturbances in root development of permanent teeth. The most recent PRG was selected from each patient. The measurements from the PRGs were performed with the investigators blinded to which group the child belonged. Each PRG was analysed by the investigators individually, in case of disagreement consensus was reached by discussion. The examination included aplasia of teeth (absents of signs on radiographs > 9 years of age), microdontia (crown size less or equal to half of the expected size) and arrested root development including short v-shaped roots, gracile roots and/or premature apical closure. Recordings were performed taking into account the difference calcification schedules of teeth. Because calcification in permanent incisors, canines and first molars begins at birth or soon after birth, these teeth always were recorded. Because the third molars develop late, and the mineralization schedule shows wide variation, their agenesis was not recorded until age 12 years. Aplasia and microdontia were therefore each divided into two subgroups, one including third molars and one excluding third molars. (When analysing aplasia including third molars only patients twelve years and older were included). Commonly used criteria for microdontia assessment of PRGs are not available; therefore, the recording was based on subjective visual judgement measured with a ruler to the nearest millimetre. When the size of a tooth crown was $\leq 50\%$ of the size considered “normal”, it was diagnosed as microdontia. Teeth with arrested root development were also divided into two subgroups, one including all teeth and one excluding second and third molars. The reason for also excluding second molars is that the roots of the teeth are mineralized later than the crowns and can therefore not be recorded. The number of permanent teeth with closed apices, permanent teeth with at least half the crown mineralised and primary teeth were also registered.

Statistical analyses
When comparing the number of teeth with disturbances in dental development between the two groups Students t-test was use. When analysing the proportion of patients exhibiting disturbances the chi-square test was used.

Results
The children in the TBI/CY-group had a mean age of $102.6 \pm 49.8$ months at treatment. The BU/CY-group were significantly younger at treatment, mean age of $73.5 \pm 48.5$ months at treatment ($p<0.01$). As can be seen in table 3 there was no statistical significant difference in age at follow up. Both groups had few remaining primary teeth.
Table 3. Variables studied in 66 children conditioned with TBI/CY and 29 children with BU/CY.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TBI/CY group</th>
<th>BU/CY group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
</tr>
<tr>
<td>Age at BMT</td>
<td>102.6</td>
<td>49.8</td>
<td>73.5</td>
</tr>
<tr>
<td>TBI=66, BU=29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at OPG</td>
<td>178.6</td>
<td>49.6</td>
<td>156.5</td>
</tr>
<tr>
<td>TBI=57, BU=24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of permanent teeth</td>
<td>22.6</td>
<td>8.6</td>
<td>22.1</td>
</tr>
<tr>
<td>TBI=57, BU=23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of permanent teeth with ½ the crown mineralized</td>
<td>28.5</td>
<td>4.7</td>
<td>28.4</td>
</tr>
<tr>
<td>TBI=57, BU=23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of primary teeth</td>
<td>2.9</td>
<td>5.0</td>
<td>3.7</td>
</tr>
<tr>
<td>TBI=57, BU=23</td>
<td></td>
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</tbody>
</table>

* Students t-test

Aplasia

When comparing the prevalence of aplasia including third molars in the TBI/CY-group and the BU/CY-group there was no statistically significant difference between the two groups (p=0.5711). In the TBI/CY-group 67% (28/42) of the children and in the BU/CY-group 59% (10/17) of the children exhibited one or more missing teeth.

When excluding third molars 19% (11/57) in the TBI/CY-group and 22% (5/23) in the BU/CY-group exhibited aplasia (p=0.8061).

The mean number of teeth missing was 2.5 ± 2.9 in the TBI/CY-group and 1.5 ± 1.6 in the BU/CY-group, a non-significant difference (Table 4). When excluding third molars the mean number was 0.8 ± 1.9 in the TBI/CY-group and 0.3 ± 0.9 in the BU/CY-group.

![Fig.1. Aplasia including third molars in BMT children conditioned with TBI/CY.](image)
Fig. 2. Aplasia including third molars in BMT children conditioned with BU/CY.

Fig. 3. Aplasia excluding third molars in BMT children conditioned with TBI/CY.

Fig. 4. Aplasia excluding third molars in BMT children conditioned with BU/CY.
Reduction in crown size (microdontia)
Fifty-eight percent of the children in the TBI/CY-group exhibited microdontia (33/57) compared to 52% in the BU/CY-group (12/23). There was no statistically significant difference between the two groups (p= 0.6412). When excluding third molars in the TBI/CY-group 23% (13/57) compared to 52% (12/23) in the BU/CY-group exhibited microdontia (p=0.0119) (Table 3).

The mean number of teeth exhibiting microdontia was 1.3 ± 3.0 in the TBI/CY-group and in the BU/CY-group, 1.0 ± 1.3, a non-significant difference (Table 4).

Fig. 5. Microdontia in BMT children conditioned with TBI/CY.

Fig. 6. Microdontia in BMT children conditioned with BU/CY.
Fig. 7. Microdontia excluding third molars in BMT children conditioned with TBI/CY.

Fig. 8. Microdontia excluding third molars in BMT children conditioned with BU/CY.
Table 4. Variables studied in PRG from 66 children conditioned with TBI/CY and 29 children with BU/CY.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>BU</th>
<th>Significance</th>
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</thead>
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<tr>
<td>Aplasia including third molars</td>
<td>X=2.5 s.d. 2.9</td>
<td>X=1.5 s.d. 1.6</td>
<td>0.1995</td>
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<tr>
<td>TBI=42, BU=17</td>
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<tr>
<td>Aplasia excluding third molars</td>
<td>0.8 1.9</td>
<td>0.3 0.9</td>
<td>0.3232</td>
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<td>TBI=57, BU=23</td>
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<tr>
<td>Microdontia including third molars</td>
<td>2.2 3.2</td>
<td>1.2 1.6</td>
<td>0.1333</td>
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<tr>
<td>TBI=57, BU=23</td>
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<tr>
<td>Microdontia excluding third molars</td>
<td>1.3 3.0</td>
<td>1.0 1.3</td>
<td>0.6358</td>
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<tr>
<td>Disturbances in root development 16-46</td>
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<td>8.4 7.4</td>
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<td>*Students t-test</td>
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Disturbances in root development

When comparing the prevalence of disturbances in root development there was no statistically significant differences between the two groups (p=0.0943). In the TBI/CY-group 79% (44/56) compared to 96% (22/23) in the BU/CY-group had disturbances in dental development. When excluding second and third molars in the analysis no significant difference could be seen (p=0.1685).

Seventy-three percent (41/56) in the TBI/CY-group compared to 87% (20/23) in the BU/CY-group exhibited disturbances. The mean number of teeth exhibiting disturbances in root development was 12.2 ± 9.6 in the TBI/CY-group and 10.3 ± 8.6 in the BU/CY-group. When excluding second and third molars the mean number in the TBI/CY-group was 9.5 ± 8.5 and 8.4 ± 7.4 in the BU/CY-group (p=0.5726).
Fig. 9. Disturbances in root development in BMT children conditioned with TBI/CY.

Fig. 10. Disturbances in root development in BMT children conditioned with BU/CY.
Fig. 11. Disturbances in root development excluding second and third molars in BMT children conditioned with TBI/CY.

Fig. 12. Disturbances in root development excluding second and third molars in BMT children conditioned with BU/CY.
Fig 13. Panoramic radiograph of a 4.5-year-old girl diagnosed with ALL, grafted at 4.3 years of age.

Fig. 14. Panoramic radiograph of the girl in figure 13, at 16.3 years of age, showing disturbances in root development of all teeth and aplasia of the third molars.
Fig. 15. Panoramic radiograph of a 7.2-year-old boy diagnosed with Thalassemia, grafted at 7.5 years of age.

Fig. 16. Panoramic radiograph of the boy in figure 15, at 13.4 years of age showing disturbances in root development in all teeth excluding second and third molars. Microdontia can be seen in the upper right third molar.

Discussion
Both TBI and BU cause long-term side effects. Some complications, such as secondary malignancies, appear late after transplantation, and may therefore be difficult to trace. Interstitial pneumonitis, cataract, and growth retardation are some of the potential toxic effects of TBI/CY. When using BU/CY these side effects can possibly be reduced, but on the other hand, BU/CY has some disadvantages. It has some toxic effects that are rare using TBI/CY, such as permanent alopecia, hemorrhagic cystitis, and veno-occlusive disease of the liver.

According to Ferry et al. (21) endocrinological disturbances are frequent after TBI/CY, but results are less definitive after a BU/CY-based conditioning regimen. Their result also shows
that growth impairment is well established after a TBI including regimens. This is confirmed in the analysis by Cohen et al. (8), which also suggest that BU/CY conditioning regimen have less interference with the growth process than irradiation.

The aim of the present investigation was to study the effect of the conditioning regimens used in bone marrow transplantation on dental development, comparing TBI/CY to BU/CY. Our hypothesis was that children conditioned with BU/CY show fewer disturbances in dental development than children conditioned with single dose TBI/CY. Our current results reject the hypothesis. Our results show the same level of disturbances in dental development in children conditioned with BU/CY as in those conditioned with TBI/CY.

Concerning disturbances in dental development all developing teeth are irreversibly affected by multiagent chemotherapy and radiation therapy. In the current study aplasia, microdontia, and disturbances in dental development where analysed.

When studying aplasia including third molars no statistically significant difference was found between the TBI/CY and BU/CY groups. Hölttä et al. (19 found that age is a stronger risk factor than TBI, although TBI cause additive impairment. This could also be seen in our study, when analysing aplasia including third molars. The younger the patient at treatment, the more aplasia could be seen.

Näsman et al. (4) reported a microdontia prevalence of 68% in SCT recipients (n=19 patients) conditioned with TBI and Hölttä et al. (19) found a prevalence of 44% in SCT recipients (n=55 patients). These percentages are in the same magnitude as found in this study in children conditioned with TBI/CY (58%). Fifty-two percent of the patients in the BU/CY-group exhibited microdontia in this study. Few studies have previously documented microdontia in children conditioned with BU/CY and therefore it might be difficult to compare this result to previous analyses.

Generally there are more disturbances in root development than in the crown, which can be seen both in the current study and in earlier reports such as in Näsman et al. (42). This depends on that the roots are completed a few years later than the crown, and the roots are therefore more sensitive to radiation therapy and chemotherapy treatment. The present results show that there is no statistically difference between the number of teeth with root disturbances in the TBI/CY-group and in the BU/CY-group, which again rejects our hypothesis and earlier studies regarding the difference in prevalence between the two groups.

Näsman et al (4, 42) reported that children who were treated with TBI prior to BMT exhibited more severe and extensive disturbances in dental development than children treated with chemotherapy only. This is in conflict with the present results that there is no difference in prevalence regarding disturbances in dental development between TBI/CY and BU/CY. Despite the similarity in prevalence there seem to be a difference in severity between the two groups, where TBI/CY is the most effected.

In this study we found that the younger the patient is at start of the treatment, the more severe the disturbances in dental development will be. Dahllöf et al. (43) and Bågesund et al. (24) reported a similar correlation between age and developmental disturbances, but here concerning facial development. They reported a reduction in the growth of the facial skeleton, which was observed in BMT children conditioned with TBI/CY.

Disturbed dental development is a consequence of TBI and may be partly responsible for the vertical reduction in the growth of the facial skeleton.

In conclusion, the results of this study show that all developing teeth are irreversibly and equally by number affected by busulfan and radiation therapy. However, there seem to be a difference in the severity of disturbances in dental development between children conditioned with BU/CY and TBI/CY. The results indicate that irradiation produces more severe effects than chemotherapeutic agents.
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References


22. Champlin R. Bone Marrow Transplantation. (Boston); 1990


25. Cole BOI, Welbury RR, Bond E, Abinun M. Dental manifestations in severe combined immunoodeficiency following bone marrow transplantation. Bone Marrow Transplantat 2000;25:1007-1009


35. Friedlander AH. A new responsibility for dentists managing the irradiated patient, SCD Spec Care Dent. 1998;18:100-1


