

DEVELOPMENTAL ABERRATIONS OF PERMANENT

IN CHILDHOOD

A Study on Stem Cell Transplant Recipients

Päivi Hölttä



Helsinki 2005

**Institute of Dentistry,
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**DEVELOPMENTAL ABERRATIONS OF PERMANENT
TEETH AFTER HIGH-DOSE ANTICANCER THERAPY
IN CHILDHOOD**

A Study on Stem Cell Transplant Recipients

Päivi Hölttä

Academic Dissertation

**To be publicly discussed with the permission of the Medical Faculty of the University of
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Helsinki 2005

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Linnea Enholm (1996-2004)

She taught us that even in the bad days one can be happy.

**It is only with the heart that one can see rightly;
what is essential is invisible to the eye.**

Antoine de Saint-Exupery: The Little Prince

To children with cancer

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals. In addition, some unpublished results are presented.

- I Hölttä P, Alaluusua S, Saarinen-Pihkala UM, Wolf J, Nyström M and Hovi L. Long-term adverse effects on dentition in children with poor-risk neuroblastoma treated with high-dose chemotherapy and autologous stem cell transplantation with or without total body irradiation. *Bone Marrow Transplant* 2002; 29: 121-127.
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- II Hölttä P, Nyström M, Evälahti M, Alaluusua S. Root-crown ratios of permanent teeth in a healthy Finnish population assessed from panoramic radiographs. *Eur J Orthod* 2004; 26: 491-497.
© European Orthodontic Society
- III Hölttä P, Alaluusua S, Saarinen-Pihkala UM, Peltola J, Hovi L. Agenesis and microdontia of permanent teeth as late adverse effects after stem cell transplantation in young children. *Cancer* 2005; 103: 181-190. (Published online November 11, 2004.)
© American Cancer Society, Inc.
- IV Hölttä P, Hovi L, Saarinen-Pihkala UM, Peltola J, Alaluusua S. Disturbed root development of permanent teeth following pediatric stem cell transplantation. *Cancer* 2005; 103: 1484-1493. (Published online February 28, 2005.)
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ABBREVIATIONS

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
Ara-C	cytosine arabinoside, chemotherapeutic agent
BMT	bone marrow transplantation
CCT	conventional chemotherapy
CML	chronic myeloid leukemia
CNS	central nervous system
CR	complete remission
CRI	cranial/craniospinal irradiation
CT	(anticancer) chemotherapy
Cy	cyclophosphamide, chemotherapeutic agent
DeI	Defect Index (combines developmental injuries in the dentition)
Dlx	transcription factor, vertebrate homologue of the <i>Drosophila</i> distal-less gene
DNA	deoxyribonucleic acid
ECT	etoposide, carboplatin, thiotepa; chemotherapeutic agents used together in HDC
GVHD	graft-versus-host disease
Gy	Gray, unit of radiation absorbed dose = 1 joule (J)/kg of material; old unit, rad (radiation absorbed dose) = 0.01 J/kg; 1 Gy = 100 rad = 100 cGy (centigray)
HDC	high-dose chemotherapy
HERS	Hertwig's epithelial root sheath
HL	Hodgkin's lymphoma (Hodgkin's disease)
HSC(T)	hematopoietic stem cell (transplantation)
IDeI	Individual Defect Index (combines developmental injuries in the dentition)
M	"Middle" age group; patients 3.1 to 5.0 years at SCT
MDS	myelodysplastic syndrome
Mel	melphalan
Msx	vertebrate homologue of the <i>Drosophila</i> muscle segment gene
NBL	neuroblastoma
NHL	non-Hodgkin lymphoma
NOPHO	Nordic Society of Pediatric Hematology and Oncology
O	"Oldest" age group; patients ≥ 5.1 years at SCT
Pax	paired-like homeobox transcription factor
PRG(s)	panoramic radiograph(s); panoramic radiography
r	roentgen, unit for expressing exposure from X-ray or gamma radiation in terms of the number of ionizations produced in air; one roentgen delivers approximately an absorbed dose of one rad to soft tissues; see also Gy
R/C	root-crown
Rad	see Gy
RMS	rhabdomyosarcoma
RNA	ribonucleic acid
RT	radiation therapy
SAA	severe aplastic anemia
SCT	stem cell transplantation (transplant); in this thesis used as a synonym for BMT and HSCT
SCR	stem cell rescue
SDS	standard deviation score
TBI	total body irradiation
VCR	vincristine, chemotherapeutic agent
VMP	etoposide, melphalan, cisplatin; chemotherapeutic agents used together in HDC
Wnt	signaling molecule of the Wnt family
WT	Wilms' tumor
Y	"Youngest" age group; patients ≤ 3.0 years at SCT

ABSTRACT

The effects of high-dose anticancer chemotherapy (CT), total body irradiation (TBI), and age at SCT were studied in relation to tooth agenesis, microdontia, and root-crown (R/C) ratios of permanent teeth in 56 stem cell transplant (SCT) recipients. Developing a simple method to describe in one figure the overall quantity of the developmental dental disturbances studied was another objective. The study patients were children and adolescents transplanted at the mean age of 4.3 (range 1-9.4) years mainly because of a malignant disease and followed up for a mean period of 7.3 (range 1-20.6) years after SCT.

Developmental dental defects were assessed from panoramic radiographs. Agenesis was not confirmed before the age of 5 years in the first premolars, 6 years in the second premolars and molars, and 12 years in the third molars. Teeth were considered microdontic if the mesiodistal crown size was about half or less than half the expected crown size. The method to assess R/C ratios was developed, and its reproducibility was determined in a healthy population. R/C ratios of the SCT recipients were compared to the reference values obtained from the healthy population, by calculating standard deviation scores (SDSs). Dental variables were studied in reference to TBI and age at SCT. Finally, a one-figure defect score was calculated for each subject by a novel Individual Defect Index (IDeI) developed during this work.

Prevalence of tooth agenesis in the SCT recipients was 31% (66% with third molars included). Young age at SCT was a stronger risk factor for tooth agenesis than was TBI. The risk for tooth agenesis was especially high in children ≤ 2 years at SCT, but very rare (excluding third molars) after age 3.5. After TBI, the number of missing teeth was higher, the maximum number being 11 (third molars excluded). Prevalence of microdontia was 44% in the SCT recipients, with at most 12 teeth affected. Risk for microdontia was minimal if the anticancer CT was initiated after the age of 4 years (excluding third molars). TBI had no significant effect on microdontia. Some disturbances in R/C ratios occurred in all SCT patients and in 77% of teeth. Root growth was most severely affected at the SCT age of 3.0 to 5.5 years, especially following TBI. When the defect points were added together with the IDeI (third molars excluded), the highest scores occurred in patients who were 2.0 to 4.5 years at SCT. TBI patients had higher mean IDeI scores, demonstrating worse tooth development compared to that of non-TBI patients.

In conclusion, in SCT recipients, the effects of high-dose anticancer CT and TBI on developing teeth were marked, since all patients and most teeth were affected. Young age (< 5 years) at SCT was a risk factor for high defect scores, when tooth agenesis, microdontia, and deficient root development were added together. High-dose anticancer CT alone caused considerable damage to developing teeth, but with TBI, its amount and severity increased. The clinical implications of these dental aberrations in long-term follow-up at the moment remain unknown. Dentists should be aware of the possible adverse dental effects of anticancer therapy and consider their implications for treatment planning.

INTRODUCTION

Numerous signal molecules that mediate communication between cells and tissues and determine the identity, size, and shape of teeth regulate tooth development from the oral ectoderm and the neural crest-derived ectomesenchyme (reviewed by Thesleff and Mikkola, 2002). Environmental factors may modify tooth development that is basically under strict genetic control (Grahnen, 1956; reviewed by Pindborg, 1982; reviewed by Thesleff, 2000; Alaluusua et al., 2004). In many organs, clinical consequences of short-lasting environmental disturbances may not appear later, but teeth have an extraordinary feature: They do not remodel. Therefore, developmental defects of teeth are permanent, which makes them good target organs in study of the impact of past events on development.

Cancer in children is an uncommon disease, and the likelihood of being diagnosed with cancer before adulthood is about 1:300 for males and 1:333 for females (Ries et al., 1999). The numbers of long-term survivors have increased; nearly 80% of children with cancer can be cured, since today extensive supportive care allows for very intensive anticancer treatment protocols. At the same time, the late effects of childhood cancer and its treatment are becoming more common and better known (Dreyer et al., 2002). Both radiotherapy (RT) and anticancer chemotherapy (CT) have unwanted side effects, and if injury occurs in tissues with a low repair potential or with no remodeling potential at all—like mineralized areas of teeth—permanent damage is possible.

The effects of RT on teeth have been noticed long ago in animals (reviewed by Kimeldorf et al., 1963), and in children (Bruce and Stafne, 1950). Childhood anticancer therapy consisting of RT alone or combined with CT, but also CT alone, has disturbed tooth development (Jaffe et al., 1984). Since then, several studies have confirmed these findings (Section 5.2.). With the developing treatment regimens, new studies are necessary to verify their adverse sequelae. Transplantation of hematologic stem cells (SCT) has become an established mode of treatment also in children, but only a few studies report on the tooth development of the SCT recipients (Dahllöf et al., 1988; Näsman et al., 1994, 1997a; Uderzo et al., 1997).

The purpose of the present work was to study tooth agenesis, microdontia, and root-crown (R/C) ratios of permanent teeth in SCT transplant recipients. Other aims were to develop a more objective methodology and to find a means to express the total sum of selected developmental dental disturbances with one figure.

REVIEW OF THE LITERATURE

1. Tooth development

1.1. Formation of the tooth crown

Human dentition comprises two sets of teeth: 20 deciduous (primary) and 32 permanent (secondary) teeth. Four types of teeth, tooth families, form in the permanent dentition: eight incisors, four canines, eight premolars, and twelve molars. At the fusion of facial processes, epithelium of the first branchial arch and frontonasal process forms a continuous, primary epithelial band at the site of the future dental arches. Soon after this, it gives rise to the U-shaped *dental lamina*. Teeth develop from this oral epithelium (ectoderm), and from the underlying mesenchyme, populated by the ectomesenchymal cells having their origin in the cranial neural crest. Along the dental lamina, at the sites of individual teeth, condensation of the mesenchymal cells occurs around the *dental placode*, which directs the budding of the epithelium to the underlying mesenchyme. In tooth development this is known as the *bud stage* (Figure 1) (Ten Cate, 1998; reviewed by Pispa and Thesleff, 2003).

At the transition from bud to *cap stage*, the inductive potential of the mesenchyme brings about a new gathering of nonproliferative cells at the tip of the epithelial bud, forming the so-called *primary enamel knot* (Jernvall et al., 1994; reviewed by Jernvall and Thesleff, 2000). The enamel knot is a signaling center, believed to be involved in the regulation of tooth shape (reviewed by Thesleff and Nieminen, 1996; reviewed by Thesleff et al., 2001; reviewed by Pispa and Thesleff, 2003). This cap stage tooth germ consists of an *enamel organ* (also called dental organ) *dental papilla* and *dental follicle* (Figure 1). Later on, the cells of the enamel organ are to form the enamel of the tooth. The enamel organ consists of the *inner and outer enamel epithelia* (and stellate reticulum within the enamel organ), which meet at the rim of the enamel organ, forming the area known as a *cervical loop*. The condensed mesenchyme adjacent to the dental organ, the dental papilla, is the origin of dentin and dental pulp. The dense mesenchymal tissue surrounding the dental organ and the dental papilla, called the dental follicle, gives rise to the supporting tissues of the tooth (periodontium) (Ten Cate, 1998).

In the *bell stage* tooth germ, epithelial *secondary enamel knots* appear in the molars, at the sites of the future cusps. They express numerous signals guiding the crown shape. The enamel knots probably also guide the differentiation of the dental papilla cells, next to the inner enamel epithelium, into *odontoblasts*, starting from the cusp tips (Ten Cate, 1998; reviewed by Thesleff et al., 2001). Following the deposition of the organic dentin matrix by odontoblasts, *ameloblast* differentiation and enamel matrix secretion takes place at the interface of the epithelial and mesenchymal tissues. Mineralization of the dentine and enamel matrices follows almost immediately. The beginning of hard tissue formation fixes the final shape of the tooth crown (Ten Cate, 1998; Thesleff, 2003) (Figure 1).

Initiation of the permanent dentition is timed at the early bell stage. A new bud folds from the dental lamina towards the mesenchyme, lingual from the primary tooth bud. Permanent molars that have no primary predecessors develop from the distal extension of the dental lamina in the same way as do the deciduous teeth (Ten Cate, 1998).

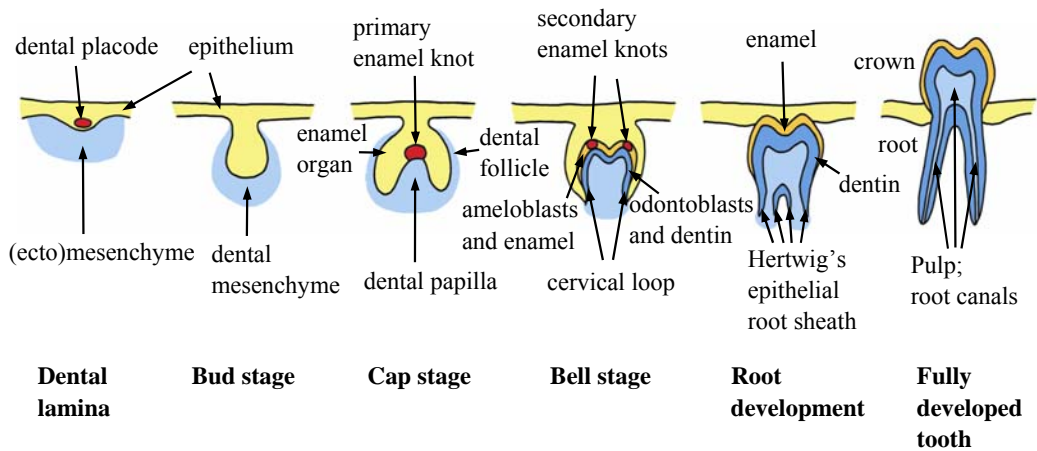


Figure 1. Schematic illustration of the main stages of tooth development. Oral epithelium (ectoderm) forms a thickened epithelial band, dental lamina. Budding of this epithelium towards the underlying cranial neural crest-derived ectomesenchyme starts at the sites of future primary teeth. Morphogenesis proceeds through the bud and cap stages to the bell stage where differentiation of odontoblasts (mesenchymal) and ameloblasts (epithelial) takes place. Secretion of the dentin and enamel matrices begins, followed by mineralization that fixes crown form and size. Hertwig's epithelial root sheath directs root development and induces cementoblast differentiation. Cementum (not shown) covers the roots of the teeth.

1.2. Formation of the tooth root

After the crown formation has been completed, a double-layered structure, *Hertwig's epithelial root sheath* (HERS), is formed as the cells of the inner and outer enamel epithelia start to proliferate apically at the cervical loop area. It directs root morphogenesis, including the size, shape and number of roots, and separates the cells of the pulpal ectomesenchyme (dental papilla) and the follicular ectomesenchyme (dental follicle) (reviewed by Ten Cate, 1996; reviewed by Cho and Garant, 2000; Yamashiro et al., 2003). The inner layer of HERS seems to induce the differentiation of odontoblasts from the mesenchymal cells and the formation of the first root dentin layer that is later covered with cementum (Thomas and Kollar, 1989; reviewed by Hammarström et al., 1996; reviewed by Cho and Garant, 2000). After the first radicular mantle dentin is deposited, HERS disintegrates, leaving *epithelial rests of Malassez* in the area of the future periodontal ligament (dental follicle). Several proposals exist as to cementoblast origin. The traditional belief was that following disintegration of HERS, the mesenchymal cells of the dental follicle come into contact with the root surface and differentiate into cementoblasts. Today, the role of HERS and the origin of the cementoblasts seem more complicated. For instance, HERS may possibly secrete on the root surface polypeptides, related to enamel proteins, which may be required in the formation of the acellular cementum covering the cervical two-thirds of the root surface (reviewed by Hammarström et al., 1996; reviewed by Ten Cate, 1996; reviewed by Cho and Garant, 2000). Furthermore, some evidence indicates that those cementoblasts forming acellular cementum may derive from HERS, and those forming cellular cementum and reparative cementum may

derive from the neural crest-derived cells (Lezot et al., 2000; Zeichner-David et al., 2003). In general, the formation mechanisms of cellular cementum in the apical third of the root are unclear (reviewed by Hammarström et al., 1996).

1.3. Regulation of tooth development

Epithelial-mesenchymal interactions are mediated by numerous simultaneous and repeatedly utilized signal substances or growth factors, and these signaling networks are regulated by genes that determine the location, type, size, and shape of a tooth. Signals may be inductive or inhibitory. Messages can be mediated through receptor molecules at the cell membrane: Ligands of the signaling cells bind to specific receptors, activating the intracellular cascade leading to the regulation of the target genes via transcription factors. Due to the sequential nature of molecular signaling, an activated gene has the ability to activate the next target gene (reviewed by Thesleff and Nieminen, 1996; reviewed by Thesleff, 2000; reviewed by Thesleff and Mikkola, 2002).

Signals and growth factors fall into several families, four of which have been the most intensively studied: the transforming growth factor β (TGF β) including bone morphogenetic proteins (BMPs), the fibroblast growth factors (FGFs), the Sonic hedgehog (Shh), and the Wnt families. More recently, two members of the tumor necrosis factor (TNF) family have been linked to tooth development, along with some other growth factors (reviewed by Thesleff and Mikkola, 2002). Early signals in oral ectoderm, belonging to the BMP and FGF families, shift the odontogenic potential from the epithelium to the mesenchyme, where it thereafter remains. It is noteworthy that these signals are shared not only between organs but also between species; i.e., they have been conserved for a long time in evolution, and results of tooth development studies conducted on animals are considered highly relevant also in humans (reviewed by Thesleff, 2000). Sequential and reciprocal signaling events during tooth morphogenesis are schematically presented in Figure 2. A detailed list of the molecules known to act during the stages of tooth development is presented by the Tooth and Craniofacial Development Group of the Developmental Biology Program, Institute of Biotechnology, University of Helsinki, Finland (Gene expression in tooth, <http://bite-it.helsinki.fi>).

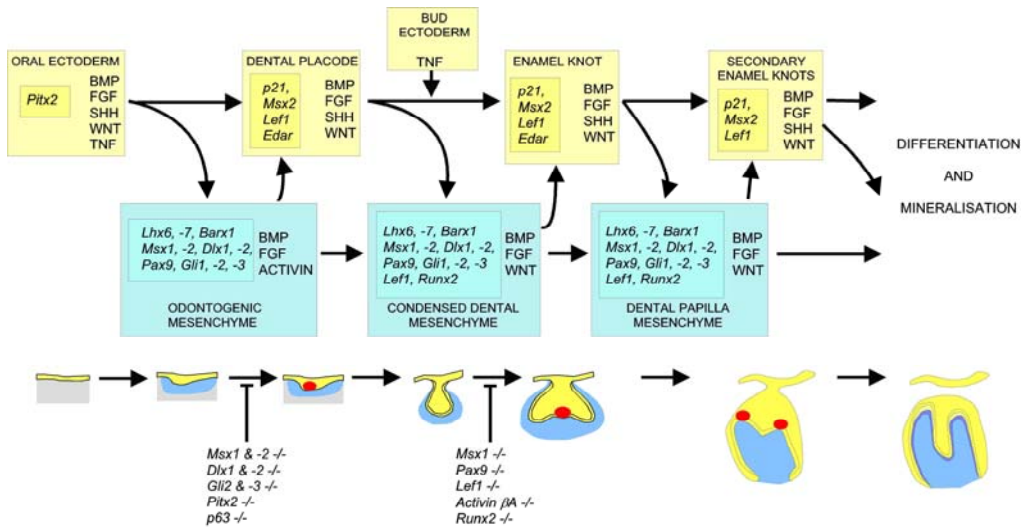


Figure 2. Schematic presentation of sequential and reciprocal signaling events during tooth morphogenesis. Growth factors and signals (upper case in boxes) and their target genes (italics in small boxes) expressed in epithelial (yellow) and mesenchymal (blue) tissues mediate cellular communication between or within epithelium and mesenchyme (arrows) in a developing tooth germ. In epithelium, signals are expressed in signaling centers (dental placode and primary and secondary enamel knots; in red). Knockout of several genes in mice (below diagrams) results in the arrest of tooth morphogenesis at the initiation or bud stages. (By courtesy of Irma Thesleff and Thomas Åberg.)

1.4. Chronology of development of permanent teeth

Initiation of permanent dentition occurs between the 20th week of fetal life (permanent central incisors and first molars) and the first year of life (permanent second molars). Initiation of the third molars occurs in the fifth year (reviewed by Ten Cate, 1996). The timing of onset and the sequence of mineralization in permanent teeth have been controversial subjects. Early reports were reviewed and sternly criticized by Logan and Kronfeld (1933), who wrote: "Still more surprising, to our minds, is the fact that the old table of Legros and Magitot,¹ according to which all permanent teeth from central incisor to first molar begin to calcify at the same time, was copied over and over again, and apparently no one found it worth while to see whether the statements contained in the table were correct before commending it to dental students and to the profession." Their own histologic study of tooth development was based on 25 human jaws ranging in age from birth to 15 years (Logan and Kronfeldt, 1933). The schedule they constructed was slightly modified by Schour and Massler, and it constitutes the basis for today's concept (Figure 3) (Schour and Massler, 1940).

In histologic studies, calcification of the permanent first molars has been reported to start around 32 weeks of fetal life (Ten Cate, 1998), at birth (Schour and Massler, 1940), or one month after birth (Logan and Kronfeldt, 1933). In radiographs, the earliest calcification could be seen only at the age of 6 months (Hess et al., 1932). Assessed from 1162 panoramic

¹ According to Logan and Kronfeld, the table of Legros and Magitot stated that all permanent teeth appear one month after birth. The table was introduced in 1893 as "Chronologie des follicles dentaires chez l'homme," Congrès de Lyon.

radiographs (PRGs) of Finnish children, crowns of the permanent teeth were completed between 3 and 7 years (third molar crowns at 13-14 years) (Haavikko, 1970). From this stage forward, it still took a minimum of 5 to 6 years and a maximum of 8 to 9 years before the roots had reached their full length and apices had closed. At the age of 15 to 16 years, the permanent dentition was fully developed, except for the third molars that matured only at about 20 years. Dispersion between individuals was wide, especially for the third molars. Tooth development in girls was, on average, 0.6 years ahead of the boys. A slight difference of 0.2 years occurred between the maxillary and mandibular teeth, the latter being earlier (Haavikko, 1970).

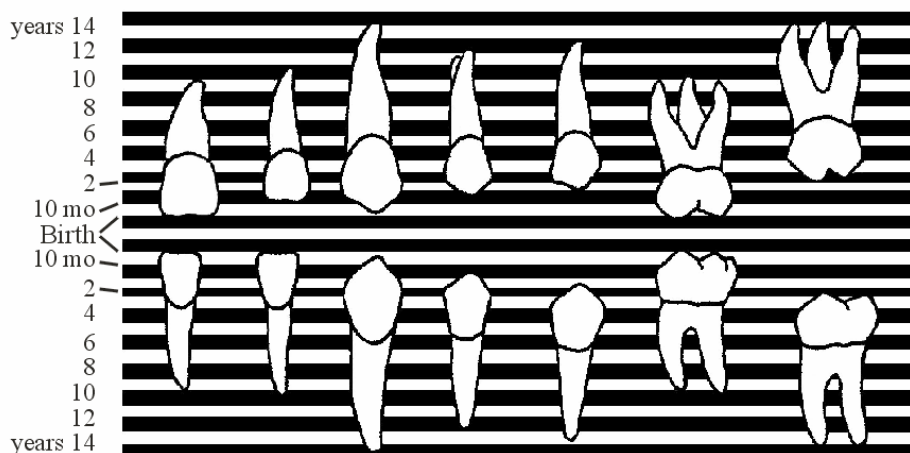


Figure 3. Chronology of mineralization of permanent teeth (Koch and Thesleff, 2001). (Reprinted with permission from the Rights Department of Blackwell Publishing.)

2. Disturbances in tooth development

2.1. Etiology

Disturbances in tooth development may involve the number, size, and shape of teeth but also aberrations in the matrix formation and mineralization of enamel or dentin or both. The etiology of disturbed tooth development can be genetic or acquired (environmental).

2.1.1. Genetic factors—from mice and men

Developmental disturbances of the teeth may be one clinical feature in several syndromes, such as Down's (Gorlin et al., 2001), Rieger (Semina et al., 1996), Kabuki (Matsune et al., 2001), and Wolf-Hirschhorn syndromes (Nieminen et al., 2003), and recessive incisor hypodontia (Pirinen et al., 2001), to mention a few. The OMIM database (Online Mendelian Inheritance in Man, OMIM; TM) lists about 60 syndromes associated with hypodontia. Genetically determined dental defects also involve such conditions as amelogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasias not to be discussed further here.

Mutations in many different genes are able to halt the development of all or some teeth (or other organs) or just disturb it (Figure 2). The same gene defects that are recognized in mice are often responsible also for aberrations in humans. The first gene shown to be essential in tooth development was *Msx1*: In homozygotic mice, no incisors were detectable, and the development of the first and second molars was arrested at the bud stage (Satokata and Maas, 1994). Soon, mutations of the human *MSX1* gene were found to cause oligodontia (absence of > 6 teeth) (Vastardis et al., 1996; van den Boogaard et al., 2000; Jumlongras et al., 2001). Teeth of the *pax9* homozygous null mice failed to develop beyond the bud stage (Peters et al., 1998), and families with *PAX9* mutations presented with molars missing along with some other teeth (Stockton et al., 2000; Nieminen et al., 2001; Lammi et al., 2003). Moreover, corresponding gene defects of mice (*Tabby*, *downless*, and *crinkled*) and men (*EDA*, *EDAR*, and *EDARADD*, respectively) are known in several ectodermal dysplasias that primarily involve ectodermal organs such as teeth, hair, nails, and salivary and sweat glands. Their disturbances result in both missing teeth and alterations in tooth size and shape (reviewed by Mikkola and Thesleff, 2003; reviewed by Pispá and Thesleff, 2003; Pispá et al., 2004; Tucker et al., 2004).

2.1.2. Environmental factors (“acquired disturbances”)

Developmental dental disturbances may result from trauma, nutritional deficiencies, some diseases, drugs, environmental pollutants, and other factors. The etiology of many dental aberrations remains idiopathic, however. Treatment of cancer in childhood, both irradiation and anticancer chemotherapy, may compromise future tooth development. This topic is covered in Section 5.2.

Local tooth injuries, directed either to primary or permanent teeth, may disturb tooth development or destroy the tooth. Accidental removal of a permanent tooth germ may occur during primary tooth extraction. Following traumas to primary teeth (especially intrusion and exarticulation in patients < 5 years at the time of injury), alterations from minor enamel defects to severe distortion, may be seen in about 50 to 95% of their permanent successors (Andreasen and Ravn, 1971; Ravn, 1975). Injury of a permanent tooth may arrest its development or result in tooth loss due to inflammatory resorption. However, although up to 30% of 12-year-old children have suffered dental injuries, severe developmental consequences are rare (Andreasen and Ravn, 1971; Andreasen et al., 1999).

Many systemic causes may affect tooth development. For instance, in patients who have suffered from rickets in early childhood, the cusp tips of permanent first molars and incisal edges of incisors are typically misshapen. Other diseases affecting calcium-phosphate metabolism, such as kidney and liver dysfunction, may have dental consequences that are mostly characterized by hypoplastic and/or hypomineralized enamel and/or discoloration of teeth (reviewed by Pindborg, 1982; Funakoshi et al., 1992; Nunn et al., 2000; Wondimu et al., 2001). Congenital heart disease, severe malnutrition, gastrointestinal disorders including celiac disease, otitis media, high fever, pneumonia, infections of the upper respiratory tract, and asthma, among others, are possible etiological factors for hypomineralization disturbances of enamel (reviewed by Pindborg, 1982; Aine, 1986; Jälevik et al., 2001; Beentjes et al., 2002). No increased prevalence of hypodontia or microdontia has been reported in these conditions.

Dioxins, environmental pollutants such as polychlorinated dibenzo-*p*-dioxines (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have shown developmental toxicity, especially in those organs whose formation is dependent on epithelial-mesenchymal interactions. The most toxic man-made chemical, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known to have several adverse effects on organogenesis, including that of teeth. Following environmental accidents in Taiwan and Japan, children born to mothers exposed to PCDFs/PCBs showed (among other defects) an increased number of natal teeth and poor tooth mineralization (Yamashita and Hayashi, 1985; Rogan et al., 1988). After the Seveso accident in Italy, developmental defects in teeth increased and the prevalence of missing permanent teeth was higher in children with heavy TCDD exposure than in those with low exposure (Alaluusua et al., 2004). Even at the prevailing levels of PCDFs/PCBs, hypomineralization (demarcated enamel defects) in the permanent first molars have increased in children exposed to dioxins via mother's milk, especially following prolonged breast-feeding (Alaluusua et al., 1996; Alaluusua et al., 1999).

Dioxin toxicity to teeth throughout their development has been well elucidated in rodents and in studies on cultured embryonic mouse teeth. In addition to the dose of dioxin, the stage of tooth development determines the clinical outcome. Following exposure of pregnant dams to TCDD, early exposure between the initiation and early bud stages of the pup's third molars leads to frequent agenesis of these teeth (Miettinen et al., 2002). Higher doses may halt third molar development somewhat later—at the late bud stage (Lukinmaa et al., 2001). It has been concluded that the target tissue of TCDD is the presumptive dental epithelium that governs tooth development during the early stages. Thereafter, dental mesenchyme directs tooth development, and the first and second molars, developed beyond the bud stage at the time of the TCDD exposure, were present. However, their mesiodistal size was reduced; the earlier was the exposure, the greater the reduction (Miettinen et al., 2002). The reduction in pups' tooth size was also dose-dependent (Kattainen et al., 2001).

When lactating dams were exposed to high doses of TCDD one day after delivery, some of the pups' third molars were missing and development was retarded in the ones present; root formation was also arrested in more advanced first and second molars, and apices were prematurely closed (Lukinmaa et al., 2001). In vitro studies showed that a very high TCDD concentration also affected the bell-stage first molars, where cusp morphology was disturbed. In the cap stage second molars, morphogenesis was totally inhibited or severely disturbed (Partanen et al., 1998). Furthermore, TCDD arrested tooth development in vitro when exposure began at the initiation stage, whereas later exposure reduced tooth size and disturbed cusp morphology. The mode of TCDD action was to induce early apoptosis in the cells of the dental epithelium, which would normally undergo apoptosis later (Partanen et al., 2004). In a recent study, pregnant rhesus monkeys received TCDD starting on day 20 of gestation and continued until day 90 after delivery. Of the offspring exposed to the highest dose of TCDD in utero and via milk most (10 of 17) presented with precocious eruption of primary teeth, or with missing or misshapen permanent teeth (Yasuda et al., 2005). Some dental adverse effects following TCDD exposure closely resemble the dental aberrations that are clinically and radiographically seen after childhood anticancer therapy.

2.2. Agenesis of permanent teeth

2.2.1. Terminology and diagnosis

The term *tooth agenesis* refers to the “endpoint,” a missing tooth, not taking into account the timing of the developmental disturbance or the underlying reason, which can be genetic or environmental. The commonly used term “*congenitally missing teeth*” has been called a misnomer, since permanent teeth are not present in the mouth at birth. This term also usually refers to genetic tooth agenesis and rules out environmental insults that may result in tooth agenesis after birth, following a normal start of tooth development. *Hypodontia*, perhaps the most widely used term, is used when 1 to 6 teeth (third molars excluded) are missing. Occasionally hypodontia is classified as mild (1 or 2 missing teeth), moderate (3 to 5 missing teeth), or severe (6 or more missing teeth) (Brook et al., 2002). *Oligodontia* mostly refers to situations with more than 6 teeth missing (third molars excluded), although the definition “6 or more missing teeth” also exists (Schalk-van der Weide et al., 1992). *Anodontia* means the complete absence of teeth. This literature review prefers the term originally used in the papers. Hypodontia and oligodontia have often been classified as *isolated (nonsyndromic)* or *syndromic*. The former shows no other aberrant developmental features, but in the latter, tooth agenesis is only one finding associated with syndromes. Syndromic hypodontia or oligodontia are not further discussed here.

The schedule of tooth development and variation between individuals must be considered before tooth agenesis is assessed. Although calcification usually begins at the age of 2 to 3 years in premolars and permanent second molars (Logan and Kronfeldt, 1933; Schour and Massler, 1940), mineralization of the second premolars in particular may show late onset. Agenesis should thus not be diagnosed before the age of 6 years in the permanent dentition, if third molars are excluded. Calcification of the third molars begins at a median age of 8 to 10 years but their very late appearance (14 to 18 years) is possible every now and then (Haavikko, 1970; Pirinen and Thesleff, 1995).

2.2.2. Etiology and prevalence of isolated tooth agenesis

Although environmental factors during tooth development may result in tooth agenesis, in the majority of cases the etiology of isolated permanent tooth agenesis is genetic. The best-known environmental factors are probably anticancer CT and irradiation, discussed in Section 5.2.1. Isolated tooth agenesis has also occurred in a group of people heavily exposed to dioxins due to an environmental accident (Alaluusua et al., 2004) (Section 2.1.2.).

A genetic basis for tooth agenesis has emerged from family studies and, more recently, from identification of genes involved. In the majority of cases, the mode of inheritance seems to be autosomal dominant (AD), with incomplete penetrance and variable expressivity. A significantly higher frequency of hypodontia appeared in the parents (41%) and siblings (26%) of the hypodontic patients than in the general population (6%) (Grahnen, 1956), and the AD inheritance pattern was observed in families regarding the frequency of missing (or peg-shaped) upper lateral incisors (Alvesalo and Portin, 1969). Detection of the two mutated genes *MSX1* and *PAX9* has offered direct evidence of AD tooth agenesis (Vastardis et al., 1996; Stockton et al., 2000; Nieminen et al., 2001). Furthermore, autosomal recessive

(Ahmad et al., 1998), X-linked (Huskins, 1930), and polygenic inheritance models (Suarez and Spence, 1974; Chosack et al., 1975), as well as multifactorial models linking genetic and environmental factors (Brook, 1984; Brook et al., 2002), have been suggested.

Hypodontia prevalence ranges from the 0.3% reported for children in Jerusalem (Rosenzweig and Garbarski, 1965), to the 36.5% for a North American religious and genetic isolate, the Dariusleut Hutterites of Western Canada (Mahaney et al., 1990). In a recent meta-analysis of prevalence of tooth agenesis in Caucasian populations in Europe, North America, and Australia, rates differed by continent and gender. With third molars excluded, the highest prevalence of 6.3% (7.6% for females, 5.5% for males) was found in Australia, followed by 5.5% (6.3%, 4.6%) in Europe, and 3.9% (4.6%, 3.2%) in North America. The risk ratio for tooth agenesis in females, derived from all the studies included in the meta-analysis, was 1.37 of that of males (Polder et al., 2004). In Finland, hypodontia prevalence, excluding third molars, was 8% (9.5% in females, 6.5% in males). Third molar agenesis in the same Finnish sample was 21% (Haavikko, 1971), in accordance with the 20 to 30% reported elsewhere (Grahnen, 1956; Lavelle et al., 1970; Lynham, 1990).

2.2.3. Characteristics of isolated tooth agenesis

According to a meta-analysis of 24 studies involving about 112 000 individuals, the most commonly missing tooth (excluding the third molar) was the mandibular second premolar, representing 41% of the missing teeth (Polder et al., 2004), almost the same (42%) as in the Finnish study (Haavikko, 1971). In that meta-analysis, maxillary lateral incisors and maxillary second premolars occupied the second and third places, but in a Finnish sample the sequence was reversed. Overall agenesis did not differ significantly between maxilla and mandible. More details are presented in Table 1.

Among those with hypodontia, agenesis of one tooth was the most frequent phenomenon both in the meta-analysis (48%) (Polder et al., 2004) and in the Finnish sample (55%) (Haavikko, 1971), with two teeth missing in 35% and 33% of the affected patients, respectively. Oligodontia, in the meta-analysis defined as agenesis of six or more teeth, appeared in 2.6% of the affected patients, but overall prevalence was only 0.14%. Unilateral tooth agenesis slightly exceeded bilateral occurrence (Polder et al., 2004). The maximum number of missing teeth was 6 in the Finnish study, involving 2.4% of the affected patients (overall prevalence 0.19%). Contrary to the meta-analysis findings, bilateral tooth agenesis was more frequent (55%) than were unilaterally missing teeth (36%). The rest of the patients had “combined” bi- and unilateral tooth agenesis, for instance, bilateral in the maxilla and unilateral in the mandible (Haavikko, 1971).

Table 1. Distribution of tooth agenesis according to the meta-analysis of 24 studies (Polder et al., 2004) and a Finnish sample (Haavikko, 1971).

Tooth	Maxilla		Mandible	
	Percentage of missing teeth Polder et al.	Haavikko	Percentage of missing teeth Polder et al.	Haavikko
I1	0.2	0	3.5	2.7
I2	22.9	18.7	2.5	1.4
C	1.3	0	0.3	0.7
P1	2.8	3.5	1.4	0
P2	21.2	29.2	41.0	42.4
M1	0.7	0	0.3	0
M2	0.6	0.7	1.2	0.7
Total	49.7	52.1	50.3	47.9

I1: central incisor; I2: lateral incisor; C: canine; P1: first premolar; P2: second premolar; M1: first molar; M2: second molar

2.3. Reduced size and microdontia of permanent teeth

2.3.1. Terminology and diagnosis

The term *microdontia* describes reduced size of teeth, but no unequivocal definition for microdontia exists. At least one of the reasons is that in healthy populations tooth size varies between ethnic groups and sexes (Lavelle, 1972), and “normal” tooth size is difficult to determine. In practice, microdontia seems to refer mostly to a tooth of a size small enough to be obvious without measurements; diagnoses have been based on subjective clinical decisions (Dahllöf et al., 1988; Näsman et al., 1994, 1997a; Tsai and King, 1998). *Reduced tooth size* (mostly meaning reduced crown size) is a term frequently used when size reduction is not so obvious, and results are based on crown measurements from dental casts. Tooth size can be considered small if more than 2 SD below the average for a corresponding population (Koch and Thesleff, 2001). A 3.5 SD limit has also been used to separate small teeth from “normal” ones (Ooshima et al., 1996). One view is that a size discrepancy greater than 1 mm from the norm can easily be detected by the naked eye; consequently, this limit has also proven useful (Tsai and King, 1998).

2.3.2. Etiology of the reduced permanent tooth size and microdontia

Tooth size—like agenesis—is under genetic control that may be modified by environmental factors. Although microdontia associated with syndromes is not further discussed here, the fact that it reflects the genetic background involved in most of the cases is worth mentioning. Estimates of heritability for tooth crown size in non-syndromic patients have ranged around 60% (Alvesalo and Tigerstedt, 1974; Townsend and Brown, 1978), and even higher (up to 82%) in twins (Townsend et al., 2003). Heritability estimates of more than 80% for several crown measurements were reported in another twin study (Dempsey and Townsend, 2001). Reduced tooth size or microdontia is often considered a dental anomaly associated with hypodontia and, as such, suggested to be the differing expressions of one autosomal dominant gene causing agenesis (Grahnen, 1956; Alvesalo and Portin, 1969), with penetrance of 72% (Alvesalo and Portin, 1969). Interestingly, although agenesis and peg-shaped upper lateral

incisors seem to be different expressions of the same gene, other factors may cause strongly mesiodistally reduced teeth (Alvesalo and Portin, 1969). In support of the genetic etiology, reduced mesio-distal tooth crown dimensions have occurred in hypodontia and oligodontia patients and their relatives (Lysell, 1953; Lavelle et al., 1970; Brook, 1984; Schalk-van der Weide and Bosman, 1996; Brook et al., 2002). Moreover, the more severe the hypodontia, the smaller the teeth formed (Garn and Lewis, 1970; Schalk-van der Weide and Bosman, 1996; Brook et al., 2002). Brook proposed a model of a multifactorial “continuum” of tooth size reduction and tooth agenesis: The size of a tooth diminishes until below a certain threshold it no longer forms, resulting in agenesis (Brook, 1984). However, this idea is much older: Grüneberg noticed the association between missing third molars and reduced size of remaining molars in mice, speculating that the smaller the available piece of dental lamina, the less likely it is to form a tooth (Grüneberg, 1951).

Environmental factors are involved in determination of tooth size. In addition to genetics, a twin study emphasized also the substantial influence of environment on tooth crown size of up to 29% (Dempsey and Townsend, 2001). Crown diameters may be affected during prenatal life: Low infant birth weight and maternal smoking during pregnancy reduced some crown dimensions of deciduous and permanent teeth (Garn et al., 1980; Heikkinen et al., 1992; Heikkinen et al., 1994). Reduction in tooth size due to irradiation and chemotherapy is a clinically evident late adverse effect of pediatric anticancer therapy (Section 5.2.2.). The role of environmental pollutants (e.g., dioxin) in determination of human tooth size is unknown, although dioxins have reduced tooth size in animals both in vivo and in vitro (Section 2.1.2.).

2.3.3. Characteristics and prevalence of microdontia in permanent teeth

Microdontic teeth may present either with rather usual morphology (merely smaller than normal) or with peg-shaped crowns, tapering towards the incisal edge (Tsai and King, 1998). The prevalence of generalized microdontia, as occasionally described in connection with syndromes, is rare, with exact figures unknown. Some studies report microdontia (or peg-shaped teeth) occurring more frequently in females (Meskin and Gorlin, 1963; Chung et al., 1972), while others find the prevalence equal for both genders (Ooshima et al., 1996). In a healthy population, the most frequently affected tooth is a maxillary lateral incisor. Peg-shaped maxillary lateral incisors have occurred in 1.3 to 1.7% of subjects in Sweden but, reflecting a common genetic background of agenesis and microdontia, in 3.1% to 6.7% of parents and siblings of the hypodontia patients (Grahnen, 1956). In a Finnish study, 1.3% of the study population had at least one peg-shaped and 2.3% had at least one strongly mesiodistally reduced (no exact criterion delineated) upper lateral incisor (Alvesalo and Portin, 1969). The microdontia prevalence for maxillary lateral incisors has been 3.3% in southern China (peg-shaped or mesiodistal reduction more than 1 mm) (Tsai and King, 1998). Prevalence of peg-shaped laterals among ethnic groups in Hawaii (according to ancestor) was 1.6% in Japanese, 1.9% in Chinese, 3.1% in Filipino, and as high as 7.5% in Puerto Rican adolescents (Chung et al., 1972). A percentage of 1.9% was reported for native Japanese subjects in Japan (mesiodistally crowns deviated more than 3.5 SD from the sex-specific mean) (Ooshima et al., 1996).

2.4. Length of permanent teeth and their roots

2.4.1. Terminology and studies

Length of teeth, including crown and root lengths, has been studied for at least 100 years. Generally, data are sparse or lacking for methods (anatomic measurements and radiographic studies; various reference points), study materials, or both, making exact comparisons impossible. Common trends, however, are clear: For instance, in the maxilla, diminishing tooth length runs in the order: canines, central incisors, lateral incisors, second premolars, first molars, first premolars, second and third molars (Verhoeven et al., 1979). Root lengths are not usually reported separately. Instead, a so-called *relative root length*, also called a *root-crown (R/C) ratio* (Lind, 1972; Jakobsson and Lind, 1973), a *ratio of crown length to root length* (Brook and Holt, 1978), or a *crown-root index* (Carlsen, 1987) have served for study of relative root length in healthy populations. Because none of the studies reported the R/C ratios for all teeth, information on (relative) root length in different populations is very limited.

The mean length of each tooth type (measured from extracted teeth) was greater in the Netherlands for men than for women (Verhoeven et al., 1979). Sexual dimorphism of the order of 6% was reported in root length (longer roots in males) in one radiographic study (Garn et al., 1978), but no gender difference occurred in the two studies reporting R/C ratios of maxillary central incisors (Lind, 1972; Jakobsson and Lind, 1973)

2.4.2. Etiology, prevalence, and characteristics of shortened root length

Resorption may cause dental root shortening following originally normal development, or roots may be developmentally short, never reaching their “expected” length. Numerous environmental factors are able to cause the disturbed dental root development that may affect the size or shape of the roots or both, because roots, like other anatomic features, follow a normal variation. Short roots may also have a genetic background.

Deficient development

Systemic *anticancer chemotherapy or radiotherapy* that occurs when teeth are developing is able to disturb root development (Section 5.2.3.).

Occasionally, shortened roots or diminished relative root lengths have occurred in connection with *systemic diseases*. Some case reports include a patient history of Stevens-Johnson syndrome (a severe form of erythema multiforme) with generalized abnormal root development or with varying degrees of arrested root development in the permanent dentition (de Man, 1979; Ranalli et al., 1984; Brook, 1994). Histologic abnormalities in Stevens-Johnson syndrome involve the dermis and epidermis, and may be assumed to involve the epithelial cells of HERS, resulting in arrested root development (de Man, 1979). Patients with hypoparathyroidism show aberrant root development (and eruption of teeth) (Sunde and Hals, 1961; Jensen et al., 1981). In some cases, the etiology remains *idiopathic* (Lerman and Gold, 1977).

Short root anomaly (SR anomaly, SRA) designates a dental anomaly characterized by developmentally short and blunted roots, most often involving maxillary central incisors in healthy persons (Lind, 1972). In 15% of these patients, premolars and canines were also

affected. Etiology is still unknown, although the familial nature of SRA, indicating a genetic factor, was promptly noticed (Lind, 1972), and has been confirmed (Edwards and Roberts, 1990; Apajalahti et al., 1999). Using the criterion “root of the same size or smaller than the crown,” a high 10% prevalence of SRA was reported in Japan (Ando et al., 1967). In Caucasians, the prevalence of SRA has been lower, from 1.3 to 2.7%, with girls more frequently affected (Jakobsson and Lind, 1973; Brook and Holt, 1978; Apajalahti et al., 2002).

Resorption

The ability of *dental trauma* and *orthodontic treatment* to induce root resorption of different grades is well known (Brezniak and Wasserstein, 1993; Blake et al., 1995; Janson et al., 2000; Andreassen and Jacobsen, 2001). Risk for inflammatory or replacement resorption following extrusive or lateral luxation is about 5% or less, but as high as 50 to 70% after intrusive luxation or avulsion (Andreassen and Jacobsen, 2001). Short roots due to this kind of resorption are rare, however, as serious tooth injuries constitute only about 5% of dental traumas (Borssén and Holm, 2000). Furthermore, following trauma, root resorption seldom starts at the apical area, and consequently does not initially affect the root length.

Orthodontic tooth movement always induces root resorption, but this “normal” resorption does not change root length. More severe circumferential external apical root resorption leads to root shortening. Etiological factors behind orthodontic root resorption (ORR) may be patient- or treatment-related. Although none of the patient-related factors studied (e.g., general health, hormonal balance, gender, age, nutrition, habits, type of malocclusion) win unanimous support as regards their effect on ORR, adults are often considered more susceptible to root resorption than are children (Brezniak and Wasserstein, 1993, 2002). Teeth with deviant root morphology, such as pipette-shaped, pointed, or dilacerated roots, may be at increased risk for the root resorption that occurs primarily in the maxillary anterior teeth (Levander and Malmgren, 1988; Sameshima and Sinclair, 2001, 2004). A genetic contribution to individual root resorption susceptibility emerged in a family study showing siblings to experience similar levels of ORR. Moderately high heritability (about 60-80%) was attributed to this phenomenon in maxillary central incisors and mandibular first molars (Harris et al., 1997). Furthermore, it was recently demonstrated that those homozygous for the IL-1 β allele 1 have a 5.6- fold (95% CI 1.9-21.2) increased risk for ORR greater than 2 mm compared with those not homozygous for this allele (Al-Qawasmi et al., 2003). Of all treatment-related factors affecting ORR, a recent meta-analysis showed that only total distance of apical displacement and total active treatment time were highly correlated with mean amount of apical root resorption (reviewed by Segal et al., 2004).

In ORR, average root resorption has usually been less than 2 to 2.5 mm (Linge and Linge, 1983, 1991; Mirabella and Artun, 1995; Harris et al., 1997; Mavragani et al., 2000; Sameshima and Sinclair, 2001) or, in percentages, has varied between 4.6 and 14.0% (Blake et al., 1995; Mavragani et al., 2000). Resorption exceeding 2.5 mm has occurred in one or more maxillary incisors in 16.5% of the patients aged 11.5 to 25 years (Linge and Linge, 1991). Severe resorption, exceeding 4 mm or reaching one-third of the original root length, has been seen in 1.0 to 2.6% of teeth in general (Linge and Linge, 1983; Levander and Malmgren, 1988; McNab et al., 1999; Janson et al., 2000).

3. Cancer in children

3.1. Incidence of childhood cancer

Cancer in children is very rare worldwide. In industrialized countries, including Finland, only about 0.5% to 1% of all cancers occur in children under 15 years (Finnish Cancer Registry, 2004; reviewed by Stiller, 2004). Types of pediatric cancer differ markedly from those of adults. Most childhood malignancies are of mesodermal origin (about 90%), with the ectoderm-derived cancers most frequent (> 85%) in adults only occasionally occurring in children (Pihkala, 2004). Three predominant groups of childhood cancers make up the majority of the diagnosed cases: leukemia, tumors of the central nervous system (CNS), and lymphomas (Figure 4).

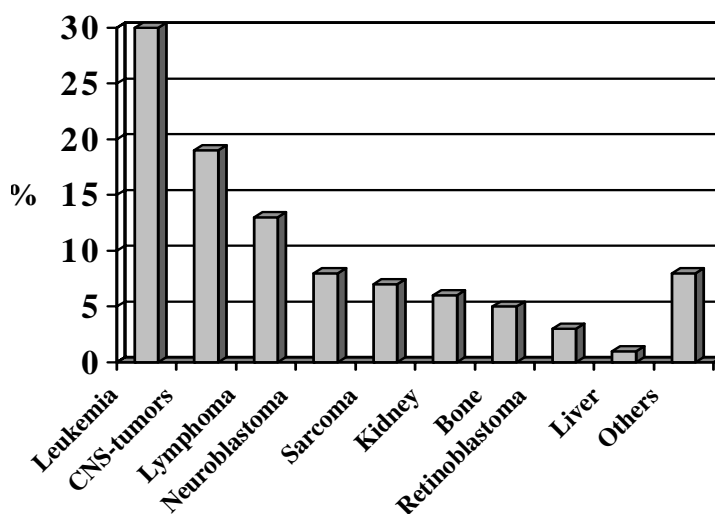


Figure 4. Distribution of childhood malignancies in children less than 15 years of age in Finland (Pihkala, 2004).

Internationally, variations in pediatric cancer incidence and distribution of cancer types occur. Total incidence ranges from 70 to 160 per million children (International Agency for Research on Cancer, IARC, 2003; reviewed by Stiller, 2004). In Finland, about 150 to 160 new cancers are diagnosed annually in children, with nearly 60% of these in males. The peak incidence rate occurs under 5 years of age, and the overall incidence rate in 0 to 14-year-olds is about 15/100 000 children (Finnish Cancer Registry, 2004).

3.2. Etiology of childhood cancer

For most cases of childhood cancer, the cause remains unknown, although some associated endogenous (mainly genetic) and exogenous (physical, chemical and biological agents) factors are known or suspected to be involved in their etiology. As regards this thesis, dealing with developmental dental defects after anticancer therapy and stem cell transplantation (SCT), cancer etiology is relevant only if the same factor might cause both cancer and

disturbances in tooth development. Since evidence for this is deficient, it can be passed over briefly with a few theoretically common etiological factors.

Ionizing radiation after the Chernobyl accident in 1986 raised the incidence of thyroid cancer, which was almost six-fold in children with the highest estimated doses of 1 Gy versus < 0.3 Gy doses (Astakhova et al., 1998). A small increase in incidence of childhood leukemia was also observed in Europe (reviewed by Moysich et al., 2002), but not in Finland (Auvinen et al., 1994). Environmental pollutants may play a role in the etiology of some childhood malignancies, although causal relationships are not very definitive. After the dioxin accident in Seveso (Italy) in 1976, relative risks for thyroid cancer and myeloid leukemia were 4.6 and 2.7 (Pesatori et al., 1993). Ionizing radiation and exposure to dioxins may both disturb tooth development (Sections 5.2. and 2.1.2.).

The role of genetics as a common etiologic factor for cancer and disturbed tooth development was recently shown for the first time in humans when a mutation in the Wnt-signaling regulator *AXIN2* appeared to predispose humans to oligodontia and to colorectal cancer (Lammi et al., 2004). As signaling pathways are shared with different organs, it is possible that comparable etiologic factors exist.

3.3. Principles of anticancer therapy

Improvements in anticancer therapy have led to an increased overall 5-year survival rate for childhood cancer from less than 30% in the 1960s up to 75 to 80% today (Smith and Gloeckler Ries, 2002). Surgery and radiotherapy (RT) were able to cure some children in the early days, but the introduction of combination CT in the late 1960s led to the dramatic increase in long-term remissions. Cancers of children consist of a diverse group of diagnoses—and within a single diagnosis, several subtypes. The intensity of anticancer therapy is modified according to characteristics of the disease. Today, treatment has become very intensive if the disease is aggressive, but less intensive protocols are used for cancer types considered less aggressive. The aim of therapy stratification is to minimize treatment-related late effects. All modes of anticancer therapy—surgery, RT, and CT—are still used in different combinations.

3.3.1. Surgery

The oldest form of curative therapy for local solid tumors is surgery, which nowadays is usually combined with other treatment modalities. Surgery plays a crucial role, for instance, in therapy for most solid tumors, such as Wilms' tumor, neuroblastoma, some brain tumors, soft tissue sarcomas, and bone tumors (Shamberger et al., 2002; reviewed by Weinstein et al., 2003; reviewed by Kalapurakal et al., 2004).

Biopsy is needed for diagnosis and staging, and in some cases, a solid tumor may be totally removed in this primary surgery. Alleviation of symptoms (palliative surgery) and resection of metastases may also be indications for surgery. Often, after biopsy, however, CT (and sometimes RT) primarily serves to reduce tumor size, which may allow total resection of an originally non-resectable tumor later, and diminish risk of injury to vital structures (Shamberger et al., 2002).

3.3.2. Radiation therapy

Radiation therapy aims at achieving local and regional control of a malignancy with acceptable damage to adjacent normal tissues. This is a demanding task, especially in pediatric patients, since radiation is targeted to growing tissues more susceptible to damage than are adult tissues. Sometimes RT may serve to provide symptomatic relief to patients with incurable disease. Tumor type, volume, location, and patient's age determine use and dose of RT, which continuously plays an important role, in combination with other treatment modalities, in several pediatric malignancies (Wilms' tumor, neuroblastoma, Hodgkin's disease, sarcomas, brain tumors). Three-dimensional localization of the tumor by computed tomography and magnetic resonance imaging has improved the possibility to protect normal tissues and target the radiation dose more precisely (conformal radiation). Total dose of radiation, size of the dose per treatment session (fraction), and dose rate can be varied to achieve the optimal balance between tumor control and complications threatening the surrounding normal tissues (Kouri et al., 1999; Tarbell and Kooy, 2002). Total body irradiation (TBI) is a special form of RT, frequently used prior to SCT (see also Section 3.4.).

Ionizing radiation is able to modify cellular DNA or break one or both of the cell's DNA strands. The inherent radiosensitivity of both tumor cells and normal cells varies. In general, quickly proliferating cells (basal cells of oral mucosa, hematopoietic stem cells, many tumor cells) are damaged easier than are differentiated, non-dividing cells (neurons, mature hematopoietic cells). Cells possess repair mechanisms that, in most cases, can restore the original structure of the DNA molecule in a few hours (sublethal cell damage). More extensive damage may be lethal: That cells are unable to recover is noticed at one of the checkpoints of the cell cycle, and these damaged cells are directed to programmed cell death (apoptosis), or they die during the cell division (Figure 5). Besides shielding, toxic effects on normal tissues can be reduced by use of fractionated doses (total radiation dose divided into smaller fragments given at intervals). This is beneficial, since normal tissues have a greater capacity to repair sublethal radiation-induced injuries than do most malignant cells. Damage is worse in tumor cells with a high proliferation rate, as these cells reach new sensitive phases in their cell cycle before the next radiation dose. Increasing oxygen concentration in the tumor between the doses also enhances the cell-killing effect of the radiation (Servomaa and Rytömaa, 1997; Kouri et al., 1999; Tarbell and Kooy, 2002).

3.3.3. Chemotherapy

Chemotherapy is the backbone therapy in many pediatric malignancies that earlier were inevitably fatal. After inducing short-term remissions with a single-agent CT, the cure of some patients with leukemias and Hodgkin's disease with combination CT in the 1960s proved that drugs could cure some percentage of human cancers. The cytotoxic effects of anticancer CT are directed both at the malignant and normal cells, which necessitates a trade-off between maximal anticancer efficacy and tolerable side effects. The maximally tolerated dose rate of anticancer drugs has increased over time when, for instance, with administration of hematopoietic growth factors and stem cell transplants, drug toxicity has been alleviated (Balis et al., 2002; Margolin et al., 2002).

The cell cycle of dividing cells has four phases (Figure 5). Most anticancer drugs exert their cytotoxic influence on a specific phase (phase-specific), while others are active during several phases (non-phase-specific). Some chemotherapeutic agents prevent cell proliferation by interfering with DNA or RNA synthesis or by breaking their strands; others exert their influence via enzymes or cell membrane structures, resulting in disrupted cell division and/or cell death (Table 2). In general, an apoptotic or cytotoxic effect on tumor cells increases with increasing *in vivo* concentration of the drug, resulting, however, in acute or long-term toxicities in normal host cells. The best-known acute toxicities caused by anticancer drugs are probably alopecia and nausea, but myelosuppression and orointestinal mucositis are even more distressing (for toxicities, see Table 2). Combination CT, containing several cytostatic drugs that alone are also effective against the tumor being treated, has several advantages. More malignant cells can be eliminated at the susceptible phases by combining drugs with additive or synergistic mechanisms of action. Moreover, when the anticancer drugs have different toxicity profiles, damage to healthy tissues is diminished, and risk for drug resistance decreased (Elonen and Järviluoma, 1998; Balis et al., 2002). Maximal dose intensity (maximum tolerated dose given at the shortest possible intervals) correlates in most cases with better disease outcome (Balis et al., 2002).

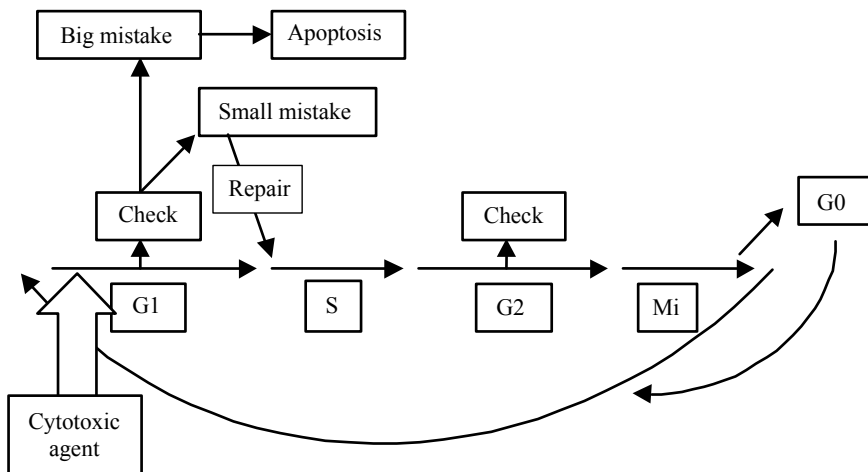


Figure 5. Phases of the cell cycle. G1: Cells produce RNA and proteins and prepare for DNA synthesis. S (Synthesis): DNA replicates. G2: Cells prepare for mitosis. Mi (Mitosis): Cells divide. G0 (resting phase): Cells temporarily cease to divide but are able to move again to phase G1. After cytotoxic exposure, the checkpoints are critical: Directions are given there for moving forward in the cell cycle after repair procedures, or committing to apoptosis (programmed cell death) (Elonen and Järviluoma, 1998; Balis et al., 2002).

Table 2. Classification of anticancer drugs frequently used in pediatric hematology and oncology, mechanisms of action, toxicity profile, and main indications (Elonen and Järviuoma, 1998; Balis et al., 2002; Hande, 2004).

Drug	Main mechanisms of action	Toxicity	Common anti-tumor spectrum
Alkylating agents			
Busulfan	DNA breaks and crosslinking	M; HD: pulm, hep, muc, N, V, NT	CML, HDC/SCT
Cyclophosphamide (Cy)	Cross-links DNA strands	M, A, N, V, cyst; HD: card	Lymph, ALL, NBL, Wilms, sar, HDC/SCT
Ifosfamide (IFOS)	Cross-links DNA strands	M, A, N, V, cyst, ren, NT; HD: card	Sar, germ cell
Melphalan (L-PAM)	DNA breaks and crosslinking	M, N, V; HD: muc, diarr	Sar, NBL, HDC/SCT
Thiotepa	DNA breaks and crosslinking	M, ST	HDC/SCT
Non-classical alkylating agents			
Dacarbazine (DTIC)	DNA methylation	M (mild), N, V, hep	NBL, sar, Hodgkin
Antimetabolites			
Cytarabine (ara-C) (pyrimidine analog)	Binds to DNA, RNA; inhibits DNA polymerase	M, N, V, muc, GI, ocular, hep; HD: NT	ALL, AML, lymph, HDC/SCT
Hydroxyurea	Enzyme inhibition (ribonucleotide reductase)	M, muc, skin	CML
Mercaptopurine (6-MP) (purine analog)	Binds to DNA, RNA; blocks purine synthesis	M, hep	ALL, CML
Methotrexate (MTX) (folic acid antagonist)	Blocks purine and thymidine synthesis by interfering with folate metabolism	M (mild), muc, hep; HD: NT, ren	ALL, lymph, OS
Plant alkaloids			
Etoposide (VP-16) (podophyllotoxin)	Binds to topoisomerase II; causes DNA breaks	M, A, N, V, muc, HSR, NT (mild), ANLL	ALL, AML, lymph, NBL, sar, brain, HDC/SCT
Teniposide (VM-26) (podophyllotoxin)	Binds to topoisomerase II; causes DNA breaks	M, A, N, V, muc, HT, HSR, NT (mild), ANLL	ALL, AML, (HDC/SCT)
Vinblastine (vinca alkaloid)	Binds to microtubulin, prevents mitosis	M, A, muc, NT (mild)	Histiocytosis, Hodgkin
Vincristine (VCR) (antimicrotubule agent)	Binds to microtubulin, prevents mitosis	NT, A, HT	ALL, lymph, most solid
Antitumor antibiotics			
Daunorubicin (anthracycline)	Topoisomerase inhibition, DNA damage (intercalation)	M, muc, N, V, diarr, A, card (acute, chronic)	ALL, AML, lymph
Doxorubicin (anthracycline)	Topoisomerase inhibition, DNA damage (intercalation)	M, muc, N, V, diarr, A, card (acute, chronic)	ALL, AML, lymph, most solid
Ildarubicin (anthracycline)	Topoisomerase inhibition, DNA damage (intercalation)	M, muc, N, V, diarr, A, card (acute, chronic)	ALL, AML, lymph
Bleomycin	Free radicals result in DNA breaks	Pulm, skin, muc, HSR	Lymph, germ cell
Dactinomycin (Actinomycin-D)	Topoisomerase inhibition, DNA damage (intercalation)	M, N, V, A, muc	Wilms, sar
Platinum analogs			
Carboplatin	DNA cross-links with platinum	M, N, V, hep (mild)	Brain, germ cell, NBL, sar
Cisplatin	DNA cross-links with platinum	M (mild), A, N, V, ren, oto, NT	Brain, germ cell, NBL, OS
Enzyme			
Asparaginase	Asparagine depletion; lymphoblasts disturbed	HSR, coagulopathy, N, V, NT, hep, pancreas	ALL, lymph

Abbreviations (toxicity): A, alopecia; ANLL, acute non-lymphoblastic leukemia; card, cardiac; cyst, cystitis; diarr, diarrhea; GI, gastrointestinal; HD, high dose; HDC high-dose chemotherapy; hep, hepatic; HSR, hypersensitivity reaction; HT, hypotension; M, myelosuppression; muc, mucosa; N, nausea; NT, neurotoxic; oto, ototoxicity; pulm, pulmonary; ren, renal; ST, stomatitis; V, vomiting

Abbreviations (anti-tumor spectrum): ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML chronic myeloid leukemia; HDC/SCT, high-dose chemotherapy prior to stem cell transplantation; lymph, lymphoma; NBL, neuroblastoma; OS, osteosarcoma; sar, sarcoma

3.4. Hematopoietic stem cell transplantation

Hematopoietic stem cells (HSCs), derived from totipotent *embryonal stem cells*, are immature cells that are already determined to a certain tissue lineage but are still *pluripotent* (= multipotent), i.e., able to give rise to all cells of the myeloid and lymphoid lineages (reviewed by Sell, 2004). They can reconstitute hematopoiesis in patients suffering from diseases primarily affecting hematopoietic or lymphatic systems (*stem cell transplantation*, SCT), or rescue patients whose treatment necessitates very intensive and consequently toxic therapy that secondarily destroys hematopoiesis (*stem cell rescue*, SCR) (Guinan et al., 2002). Bone marrow has been a traditional source of HSCs (therefore the term “bone marrow transplantation,” BMT), but nowadays HSCs are more frequently harvested from peripheral or umbilical cord blood. The terms are SCT, SCR, and also peripheral blood stem cell transplantation (PBSCT) and (umbilical) cord blood transplantation (CBSCT). Identification of HSCs is based on the cell-surface protein, CD34 antigen, expressed primarily on hematopoietic progenitor cells representing about 1 to 5% of marrow cells and 0.1 to 0.2% of peripheral blood cells (reviewed by Little and Storb, 2002; Nash, 2004). From the first unsuccessful efforts at human BMT in 1957 (reviewed by Little and Storb, 2002), hematopoietic stem cell transplantation (HSCT = SCT in this thesis) has become a successful treatment in several otherwise fatal hematologic, oncologic, and immunologic diseases in adults and in children.

The transplant is called *autologous* if the source of HSCs is the patient him-/herself, and *allogeneic* when HSCs have been harvested from another person. The availability of autologous HSCs may be compromised or ruled out in patients who have earlier been exposed to intensive anticancer therapy or when contamination of the transplant with malignant cells is probable. The preferred donors of allogeneic HSCs are human leucocyte antigen (HLA) - matched siblings, but for patients who lack such a sibling or other closely HLA-matched family donor, a matched unrelated donor is sought from donor registries. The possibility of finding an unrelated donor for Caucasian patients is about 80%, although it is dependent on the definition of “suitable HLA matching” (Guinan et al., 2002; reviewed by Little and Storb, 2002).

Prior to the SCT procedure, remission is induced with anticancer CT (excluding patients with immunological deficiencies). Preparative regimens for SCT include myeloablative high-dose chemotherapy (HDC; examples in Table 4, page 56), often combined with total body irradiation (TBI), both to eradicate possible residual disease, to create “marrow space,” and—when allogeneic stem cells are used—to suppress the patient’s immune system to avoid rejection (Guinan et al., 2002; reviewed by Little and Storb, 2002). This treatment causes irreversible bone marrow toxicity that without infusion of the new hematopoietic progenitor cells would be lethal. After SCT, successful engraftment usually occurs in 10 to 28 days. The post-transplant period may be complicated by bacterial, viral, and fungal infections; intensive supportive care is necessary (Guinan et al., 2002).

Autologous SCT (SCR) is used in treatment of several originally metastasized or recurrent solid tumors with poor prognosis such as neuroblastoma (Saarinen et al., 1996; Matthay et al., 1999), Wilms’ tumor (Campbell et al., 2004), and Hodgkin’s disease (Lieskovsky et al., 2004). Event-free survival rates from 40 to 60% have been reported after follow-ups of 3 to 5 years. Allogeneic transplants are commonly used in the treatment of

patients with high-risk ALL and AML in the first complete remission (CR), or in the second CR for the relapsed patients. The best treatment results have been achieved with allogeneic SCT also in chronic myeloid leukemia (CML). Moreover, congenital hematopoietic (e.g., thalassemia) or immunologic defects (e.g., severe combined immunodeficiency) and acquired disorders of marrow function (e.g., severe aplastic anemia), may require allogeneic SCT (Guinan et al., 2002; Nash, 2004).

3.4.1. Graft-versus-host disease

Patients receiving an allogeneic SCT are at risk for graft-versus-host disease (GVHD), which occurs when genetically disparate, immunocompetent donor lymphocytes are transferred into a recipient who is immunocompromised and incapable of rejecting the donor graft. Despite prophylactic immunosuppressive therapy for GVHD, the donor T-cells may recognize the recipient's diverse histocompatibility antigens and attack recipient cells, resulting in signs and symptoms of acute GVHD, involving primarily the skin, gastrointestinal tract, and liver (Guinan et al., 2002). It has, however, been noticed that leukemia patients who develop GVHD have a lower risk for recurrent disease after transplantation. This advantage, the "*graft-versus-leukemia-effect*," is often successfully utilized in treatment of the patients with a high risk for recurrent leukemia.

Symptoms of chronic GVHD may occur about 3 months after transplantation, but may develop much later, even a year after allogeneic SCT. Incidence of chronic GVHD runs from 25 to 80%. The rate is usually lower in children than in adults, lower after cord blood transplantation, and lower after having a transplant from a matched sibling donor (reviewed by Farag, 2004; reviewed by Higman and Vogelsang, 2004). Chronic GVHD most commonly involves the skin (65-80%), mouth (48-72%), liver (40-73%), and eyes (18-47%). Other possible involvement sites are the gastrointestinal tract (16-26%), lungs (10-15%), and joints (2-12%). Malnutrition is common, and even hair loss, destruction of sweat glands, and vertical ridges on the nails are possible (reviewed by Higman and Vogelsang, 2004). Salivary gland involvement is characterized by reduced saliva quantity and its altered quality (reviewed by Nagler and Nagler, 2004). Both the disease and its treatment with immunosuppressive medications (such as methotrexate, corticosteroids and cyclosporin A) increase risk for life-threatening infections. Immunosuppressive therapy can be discontinued in most cases after 9 to 18 months, but must be prolonged in patients with clinically extensive chronic GVHD (reviewed by Farag, 2004; reviewed by Higman and Vogelsang, 2004).

3.5. Key characteristics of selected childhood malignancies

3.5.1. Leukemia

Leukemia is the most common childhood cancer, and in Finland accounted for about 32% of all cancers occurring before age 15 during the period 1968 to 1998. *Acute lymphoblastic leukemia* (ALL) comprises about three-fourths of the leukemia diagnoses, and *acute myeloid leukemia* (AML) accounts for the majority of the remaining cases (International Agency for Research on Cancer, IARC, 2003). In general, the peak incidence in ALL (not in AML)

occurs at approximately age 2 to 3 (4) years and is higher in males (Smith et al., 1999; Margolin et al., 2002).

ALL is thought to arise in the bone marrow via a malignant transformation of a single lymphoid progenitor cell that proliferates and replaces the normal hematopoietic cells. Production of the normal marrow elements is disturbed, resulting in anemia, thrombocytopenia, and neutropenia of varying degrees. In addition to the bone marrow, leukemic blasts may already be present and proliferating in liver, spleen, thymus, lymph nodes, testes, and the central nervous system (CNS) at the time of diagnosis. ALL is a biologically heterogeneous disorder. Increased knowledge of the importance of clinical and biological prognostic factors (initial white blood cell count, age, sex, cell morphology, response to induction therapy, immunochemistry, cytogenetic markers) has fine-tuned the treatment strategies. Treatment of high-risk patients has become extremely intensive, but the less intensive treatment of low-risk patients aims at a cure with minimal treatment-related adverse effects (Margolin et al., 2002; Pui et al., 2004; Saarinen-Pihkala et al., 2004; Whitlock and Gaynon, 2004). The average duration of therapy for children with ALL is 2 to 2.5 years, and 5-year survival rates are 75 to 80% (Smith et al., 1999; Gustafsson et al., 2000; reviewed by Pui et al., 2001).

Treatment of ALL consists of remission induction, consolidation, (intensification), and maintenance therapy. Combination CT and (preventive) CNS therapy with craniospinal irradiation (CRI) have been the cornerstones of treatment. However, with the development of other treatment methods, CRI has been limited to avoid numerous late adverse effects (Saarinen-Pihkala et al., 2004). Allogeneic SCT, the most aggressive mode of therapy, is utilized for some very high-risk patients even in the first CR but, most frequently, in relapsed patients in the second CR (reviewed by Pui et al., 2001; Margolin et al., 2002). Finland, as all other Nordic countries, employs the protocols of the Nordic Society of Pediatric Hematology and Oncology (NOPHO). Patients are stratified to several risk groups: standard risk, intermediate risk, and high risk (the latter includes intensive, very intensive, and extra intensive therapy groups), and their treatment varies according to NOPHO-ALL 2000 protocols. Therapeutic or prophylactic CRI of 18 to 24 Gy may be given as a part of intensive or very intensive therapy, but the need is considered separately in very young children (< 4-5 years). The extra intensive therapy includes an allogeneic SCT in the first CR. In the Nordic countries (Denmark, Finland, Iceland, Norway, Sweden) during the 1990s, the survival rate for ALL patients at 5 years was 77.6% (Gustafsson et al., 2000). The 9-year event-free survival of high-risk ALL patients was lower, 61%, including subgroups of very high-risk patients with low survival rates (e.g., 25% for patients with precursor-B cell ALL with a high initial white blood cell count of $\geq 200 \times 10^9/\text{liter}$) (Saarinen-Pihkala et al., 2004).

AML includes a heterogeneous group of hematologic malignancies arising as a transformation of myeloid lineage progenitor cells. The incidence of AML is stable in childhood, with males and females equally affected (Golub and Arceci, 2002). About 10 children per year are diagnosed in Finland (Pihkala, 2004). Exposure to radiation and chemotherapeutic (alkylating) agents are risk factors for AML. For instance, after the atomic bombs in Japan, a 20-fold increased incidence of AML (and ALL) occurred (Smith et al., 1999; Golub and Arceci, 2002). AML presents with several subtypes, and currently the standard and high-risk criteria are based on cytogenetic and molecular genetic characteristics and response to therapy. In Finland, AML patients have been treated according to NOPHO

protocols since the 1980s, and in the latest version (NOPHO-AML03), the treatment begins with two chemotherapy courses (induction), followed by stratification of the patients to standard and high-risk. In standard risk patients, consolidation therapy continues with several courses of conventional chemotherapy (CCT) following each other at 3- to 4-week intervals. The treatment period is shorter than in ALL, usually 7 to 10 months. Allogeneic SCT is recommended for the high-risk patients, preferentially after the first course of consolidation CT. Overall 7-year survival rate in the NOPHO-AML93 study was 64% (Lie et al., 2003), which internationally represents one of the best results reported (Pui et al., 2004). In the absence of a suitable donor, treatment with autologous stem cell support has also been used in children with recurrent disease. This salvage therapy seemed to be useful, especially in patients with a long (> 12 months) first CR (Godder et al., 2004).

3.5.2. Lymphomas

Lymphomas, originating from various cells of the lymphocyte lineage, are the third most frequent types of cancers in children (Figure 4). The two predominant lymphomas are non-Hodgkin lymphoma (NHL), and Hodgkin's disease (HD), both further divided into subgroups. Together, lymphomas constitute about 10 to 15% of childhood malignancies. In young children, NHL is more frequent than HD, with the reverse in adolescents. HD is uncommon in children younger than 10, but its incidence increases during the early teen and adolescent years. In Finland, about 10 new NHL cases are diagnosed in children annually, and the number of HD diagnoses is even lower, about 4 per year (Percy et al., 1999; Pihkala, 1999).

Some risk factors for lymphomas exist. Patients with congenital or acquired immunodeficiency syndromes and patients with immunosuppressive medication after organ or stem cell transplantation are at increased risk for NHL (post transplant lymphoproliferative disease). The Epstein-Barr virus has been associated with HD, and clustering of HD cases in families and the higher risk in monozygotic than dizygotic twins for HD suggest a genetic predisposition or a common environmental factor (Percy et al., 1999; Hudson and Donaldson, 2002; Magrath, 2002).

Treatment varies according to type of lymphoma and extent of disease at diagnosis. Patients with a local NHL have an excellent prognosis after a short course of multiagent chemotherapy. NHL in children is often disseminated (lymphoblastic lymphoma) from the outset, and more intensive therapy is necessary, especially for patients with bone marrow or CNS involvement. The borderline between ALL and NHL involving the bone marrow is unstable. Thus, the treatment of disseminated NHL follows, or is identical to, the regimens designed for the treatment of ALL, extending up to allogeneic SCT after myeloablative therapy. RT is not considered to provide any extra benefit for cure and is used for children only in special cases. Prognosis for disseminated NHL resembles that for ALL: 60 to 80% of patients can be cured. With allogeneic or autologous SCT, survival rates of 30 to 60% have been reported after recurrent disease (Percy et al., 1999; Pihkala, 1999).

RT-related adverse effects have promoted interest in the combined-modality therapy for HD. Radiation dose and volume have been reduced in growing children, and the intensity and duration of multiagent CT has been tailored according to anatomic disease stage and presence or absence of symptoms at diagnosis. Many pediatric treatment protocols use six cycles of

combination CT and irradiation (15-25 Gy) of the involved field (Percy et al., 1999; Hudson and Donaldson, 2002). Approximately 90 to 95% of children with non-disseminated HD recover, as well as 70 to 90% of patients with disseminated disease. In recurrent HD, autologous (sometimes allogeneic) SCT can cure 20 to 30% of patients with a short and 50 to 80% of patients with a long (> 12 months) primary remission (Percy et al., 1999; Lieskovsky et al., 2004).

3.5.3. Neuroblastoma

Neuroblastoma (NBL), a tumor of the sympathetic nervous system, is derived from primordial neural crest cells that later populate sympathetic ganglia and adrenal medulla; the neoplasm thus originates from a sympathetic ganglion along the spine or in the adrenal gland. NBL etiology is unknown. It comprises 8 to 10% of childhood malignancies and is the most common cancer diagnosed in the first year of life. About 80% of the patients are less than 4 years at diagnosis (Goodman et al., 1999; Brodeur and Maris, 2002). NBL spreads early to the lymph nodes, bone marrow, bone, and liver, with approximately 70% of patients having metastases at diagnosis in this biologically heterogeneous neoplasm with its highly variable behavior. In very young children (< 1 year), spontaneous regression may occur, but in older children with metastatic NBL the outcome is poor, even after intensive therapy (Brodeur and Maris, 2002; reviewed by Weinstein et al., 2003). In Finland, about 10 new cases are diagnosed annually; incidence is rather uniform, at least in industrialized countries (International Agency for Research on Cancer, IARC, 2003).

Patients can be divided into risk groups, for instance, according to age, stage of the disease, histology, and genetic features of the tumor. Amplification of the proto-oncogene *MYCN* (also known as *N-myc*) is especially associated with aggressive and metastatic disease. Treatment protocols comprising surgery, RT, and anticancer CT have been introduced for low-risk, intermediate-risk, and high-risk patients (risk-related therapy). In addition to surgery, light CT is used for some low-risk patients. In infants with 4S disease (localized primary tumor with dissemination limited to liver, skin, or bone marrow), spontaneous regression may occur, but minimal CT or local RT is sometimes used; high survival rates, usually reaching 80 to 90%, have been reported (Brodeur and Maris, 2002; reviewed by Weinstein et al., 2003). Intermediate-risk patients are usually treated with moderately aggressive CT and surgery, with RT used in selected cases. Survival rates vary greatly, between 10% and 94%, which probably is an indication of heterogeneous treatment groups (reviewed by Weinstein et al., 2003). Survival of high-risk patients with multiagent CT regimens has been only about 15% (Brodeur and Maris, 2002). For this group, maximal therapy is needed, meaning HDC, often TBI, and autologous SCR, sometimes two or even three times, in addition to surgery, CCT, and RT. Furthermore, administration of 13-cis-retinoic acid (known to reduce proliferation and induce differentiation in neuroblastoma cell lines) after CT or SCR seems to improve prognosis (Matthay et al., 1999; reviewed by Weinstein et al., 2003). After autologous SCR, the maximum long-term survival rates of 53% for high-risk NBL have been achieved in Finland (Saarinen et al., 1996).

3.5.4. Wilms' tumor

Wilms' tumor (WT, nephroblastoma), the most common renal cancer in children, derives from undifferentiated embryonal mesodermal tissue and represents about 6% of the cancer diagnoses in children aged under 15 years. In the first 2 years of life, WT incidence is highest. After 7 years of age, embryonic cells, which differentiate and essentially disappear with age, are very seldom a source of cancer. (Bernstein et al., 1999; Grundy et al., 2002; reviewed by Sell, 2004). Increased risk for WT appears in some congenital anomalies such as Beckwith-Widemann and Sotos syndromes, and also in Denys-Drash and WAGR (Wilms' tumor, aniridia, genitourinary malformation, mental retardation) syndromes, associated with the mutated WT suppressor gene *WT1*. Ethnic incidence variations and familial occurrence in a small subset of patients (about 1.5%) are considered indicative of the genetic component in the etiology. No conclusive evidence exists for a role played by environmental exposure (Bernstein et al., 1999; Grundy et al., 2002; reviewed by Stiller, 2004).

In most patients, WT is a unilateral neoplasm. The most common, and in about 80% the only site of metastases are the lungs, followed by liver and lymph nodes. The extent of the tumor at diagnosis is the basis for the five-grade staging: moving from stage I to stage IV means moving from a local tumor, limited to a kidney, to a tumor with distant metastases. In stage V, the tumor is bilateral. Stage and histology (favorable/unfavorable) are used in the risk assessment that guides the therapy planning, but molecular genetic features may further fine-tune the treatment protocols in the future (Grundy et al., 2002; reviewed by Kalapurakal et al., 2004).

Surgical resection (mostly nephrectomy), either as an initial therapy or after an initial CT remains important (reviewed by Kalapurakal et al., 2004). Percutaneous needle biopsy may be taken first to clarify the histology, followed by preoperative CT, the intensity of which is modulated by tumor stage and histology (Saarinen et al., 1991). Prognosis is poor after local recurrence and, to avoid this, postoperative RT to the tumor bed is often used. Whole-lung irradiation has been recommended for children with pulmonary metastases, but new CT-based treatments are sought to eliminate the adverse effects of RT. The trend is toward shorter and more gentle therapy for children with low-risk tumors and toward more intensive therapy for those with high-risk disease (Grundy et al., 2002; de Kraker et al., 2004; reviewed by Kalapurakal et al., 2004). In patients with relapsed WT (long-term survival < 30% after conventional therapy), disease-free survival 3 or 4 years after HDC and autologous SCR has been 30 to 50%, (Pein et al., 1998; Campbell et al., 2004). Overall 5-year survival in children with WT is 85 to 90% (International Agency for Research on Cancer, IARC, 2003).

3.5.5. Rhabdomyosarcoma

Soft tissue sarcomas (STS) include a diverse group of neoplasms that originate from embryonal mesenchyme and make up 4 to 8% of pediatric cancers. The most common is rhabdomyosarcoma (RMS), which arises from cells of skeletal muscle lineage and represents about half of all childhood STS. RMS can occur anywhere in the body, with the head and neck area most frequently affected (40%), followed by the genitourinary tract (25%). Metastases are primarily found in lungs, bone marrow, lymph nodes, and bones. In children less than 15 years, the annual incidence of RMS ranges from 4 to 7 cases per million, with the

highest incidence in small children (< 4 years) (Gurney et al., 1999; Wexler et al., 2002; International Agency for Research on Cancer, IARC, 2003; reviewed by Stevens, 2005). Although most RMS cases are sporadic, adult relatives of children with RMS have shown a high frequency of adrenocortical and breast carcinomas. An association has also been noticed with neurofibromatosis and some other familial syndromes (Wexler et al., 2002; reviewed by McDowell, 2003).

RMS is classified into histologic subtypes (the two most common being embryonal and alveolar) carrying specific molecular genetic abnormalities, and to stages according to primary site, size, and invasiveness of the tumor, involvement of lymph nodes, and presence or absence of metastases. Stage, site, and pathological subtype are the most important factors affecting treatment outcome (Wexler et al., 2002; reviewed by Stevens, 2005).

Although the treatment philosophy for RMS varies among centers, multimodal therapy consisting of surgery, RT, and CT is usual. Primary surgical removal of the tumor is performed whenever possible, but all patients need CT to eradicate residual disease. Often, after reduction of the size of the tumor with multiagent CT, secondary excision is necessary, to assess treatment results and to remove any residual tumor. RT is frequently delivered to diminish the chance of local recurrence or to control metastases. Late effects of RT have made it necessary to reassess the use of RT, but no agreement has been achieved thus far. Overall survival of RMS patients ranges from 60 to 75% (Gurney et al., 1999; Stiller et al., 2001; reviewed by Stevens, 2005), with great differences between diagnostic groups. The metastatic disease is still a major treatment challenge, since 3-year event-free survival rates are only about 20 to 30% (Williams et al., 2004). According to one meta-analysis, patients treated with HDC and SCT did not do better: Event-free survival at 3 to 6 years was 24 to 29% (Weigel et al., 2001).

4. Late effects of pediatric anticancer therapy

For young adults in 2010, the estimated number of childhood cancer survivors is 1:250 persons (in 2002 this was about 1:900) (Dreyer et al., 2002). Late adverse effects of both RT and CT are becoming more familiar, and their clinical implications are increasing. Dental aberrations belong among these late effects (Section 5.2.), but are only one small part of the entity. Many late adverse effects must be considered as regards clinical dental treatment, but most effects have no known associations with tooth development. Late effects of anticancer therapy are thus not covered in detail.

4.1. The mouth and related tissues

High-dose RT (usually 50-70 Gy) for oral cancer or primary tumors of nearby areas is a risk for trauma-related or spontaneous *osteoradionecrosis* (ORN) of the jaws. Because in childhood these tumors are rare, ORN is seldom of concern in a pediatric population. It is of note that radiation effects on bone do not diminish but rather increase over the course of time, as tissues become more fibrotic and hypovascular (reviewed by Vissink et al., 2003a).

RT directed to the salivary glands reduces *salivary flow rate*, pH, and buffering capacity, and saliva turns viscous. Qualitative and quantitative changes compromise several protective functions of saliva, impair oral functions such as speech and swallowing, and predispose teeth to rapidly progressing radiation caries (reviewed by Vissink et al., 2003b). Following CT and HDC of pediatric SCT patients, the mean salivary secretion rate was lowest about 3 to 6 months after SCT, but recovered in one year to the level before SCT, which already was lower than normal. After 4 years, the mean salivary secretion rate exceeded the baseline value by 49% and did not differ from healthy controls. TBI of 10 Gy prior to SCT caused a long-term reduction in the mean salivary secretion rate, which remained 18% below the baseline level in the 4-year follow-up (Dahllöf et al., 1997). RT-related salivary gland damage was confirmed later in a scintigraphic study (Bågesund et al., 2000). Dahllöf et al. (1997) found no reduction in salivary flow rate in patients with or without chronic GVHD. In contrast to that, salivary gland involvement in GVHD reduced the salivary flow rate as much as 55 to 90%, and elevated concentrations of sodium, immunoglobulin G, and albumin, and reduced immunoglobulin A (IgA). These changes may be due to T-cell infiltration of glandular parenchyma, resulting in salivary parenchymal atrophy (reviewed by Nagler and Nagler, 2004).

Despite salivary dysfunction, the *caries experience* of the SCT patients, of CT (+RT) patients, and of controls did not differ (Näsman et al., 1994; Dahllöf et al., 1997; reviewed by Leiper, 2002b). Findings on the effects of conventional anticancer therapy on dental and oral health are contradictory (reviewed by Leiper, 2002b). For instance, the caries experience of pediatric cancer patients was in some studies in the same range as in healthy controls (Maguire et al., 1987; Nunn et al., 1991; Oguz et al., 2004), in other studies cancer patients had more decayed and filled teeth than did healthy children (Purdell-Lewis et al., 1988; Pajari et al., 1988c, 1995; Dens et al., 1995).

4.2. Other late effects

Growth disturbances after CT are often temporary, but RT of cranial, spinal, and abdominal areas or of epiphyseal growth plates, and TBI as one part of preparative regimens for SCT, are risks for permanent and progressive growth disturbances (Hovi et al., 1991; Moell et al., 1994; Hovi et al., 1999). RT may primarily affect bone growth or act secondarily, causing hypothalamic-pituitary axis damage. Malnutrition prior to SCT, growth hormone deficiency after TBI and HDC, and glucocorticoids as a part of anticancer therapy or in the treatment of GVHD all decrease growth (Taskinen and Saarinen-Pihkala, 1998; Hovi et al., 1999; reviewed by Brennan and Shalet, 2002; Dreyer et al., 2002). RT to the neck region or to the cranium and also TBI prior to SCT may result in *thyroid dysfunction*, again by direct damage to the thyroid gland or disruption of the hypothalamic-pituitary-thyroid axis (reviewed by Brennan and Shalet, 2002; reviewed by Brougham et al., 2002). *Gonadal function* may be damaged after anticancer CT (especially after HDC with alkylating agents), and especially after RT. The ovaries are less vulnerable to irradiation and CT than are testes, although pubertal development and fertility are both severely endangered in both genders (reviewed by Brennan and Shalet, 2002; reviewed by Brougham et al., 2002). Decreased *bone mineral density* after childhood ALL has been described, as well as disturbed *lipid and glucose metabolism* after allogeneic SCT (Arikoski et al., 1998; Taskinen et al., 2000).

Mediastinal RT and administration of anthracyclines (daunorubicin, doxorubicin) and alkylating agents (Cy) expose patients to (often subclinical) *cardiac late effects*, such as impaired exercise tolerance, arrhythmias, and cardiomyopathy (Pihkala et al., 1995; reviewed by Pai and Nahata, 2000; reviewed by Leiper, 2002a). *Pulmonary dysfunction* (restrictive or obstructive disease or diffusion problems) has been associated with RT, some chemotherapeutic agents (e.g. bleomycin, Cy, Mel), and chronic GVHD. Pulmonary late effects are more frequent after very intensive CT and SCT beyond the second CR, as compared with SCT in the first CR (Dreyer et al., 2002; reviewed by Leiper, 2002a). *Renal toxicity* with an abnormal glomerular filtration rate and persistent tubular dysfunction has occurred after RT, and also after administration of some anticancer agents, especially cisplatin (reviewed by Leiper, 2002a; Pietilä et al., 2005). Additional deterioration may be induced after allogeneic SCT, for instance, by immunosuppressant cyclosporin A. Concomitant use of other nephrotoxic agents, such as amphotericin B, may further exacerbate renal dysfunction (Dreyer et al., 2002; reviewed by Leiper, 2002a). *Neurophysiologic and neurologic* abnormalities have appeared due to anticancer CT alone (methotrexate, vincristine), but addition of RT (CRI in leukemia, RT for brain tumors, TBI prior to SCT) may cause further impairment of cognitive or nerve function (Ilveskoski et al., 1996; Lehtinen et al., 2002; reviewed by Leiper, 2002a). In children treated for cancer, the risk for a *second malignant neoplasm* (SMN) is approximately 3 to 12% within 20 years of their first diagnosis. In a Nordic cohort, relative risk for SMN was 4.3, being highest after a latency of ≥ 10 years in those patients < 5 years at the treatment of the first malignant neoplasm. Latency was shortest (mean 5.4 years) for leukemias and longest (17-18 years) for tumors of breast and digestive tract. CT potentiated the carcinogenic effect of RT (Garwicz et al., 2000). Alkylating agents and topoisomerase II inhibitors (Table 2, page 30) have been associated with therapy-related AML, while thyroid cancer, breast cancer, and soft tissue sarcomas are often secondary to RT (Dreyer et al., 2002; reviewed by Leiper, 2002b). After pediatric allogeneic SCT, 40 to 50% of the second malignancies have been post-transplant lymphoproliferative disorders, with the highest risk occurring during the first year after therapy. Solid tumors usually appear several years after SCT, being 34 times more common than expected in the general population. The youngest children (< 5 years at therapy) are at the greatest risk, especially after TBI or CRI or both (reviewed by Leiper, 2002b).

5. Effects of anticancer therapy on tooth development

Effects of radiotherapy or anticancer chemotherapy on tooth-forming cells and tooth morphogenesis have been mainly studied in rodent incisors, which differ from human teeth: They grow continuously, and cell differentiation, matrix secretion, and calcification are in process also in full-grown animals. Consequently, in spite of the animal's age, studies reveal the effects of therapy on tooth-forming cells in various phases of differentiation, and on their precursors, as well as on secretion and calcification processes. Even if the results may be comparable at cellular level with developing (roots of) human teeth, the morphological outcome is not, since human teeth have only a limited growth capacity. Rodent molars and teeth of cats, dogs, and monkeys reach their maturity with the closure of the apices like

human teeth, so their morphological outcome can be better compared with humans. In these cases, the animals' age—actually the stage of tooth development—is of vital importance. Often, effects of anticancer therapy offer a spectrum from mild alterations to tooth agenesis, varying with timing of therapy in relation to tooth developmental stage, which varies among teeth and is species-characteristic.

Animal studies can give clues to the aberrations of human tooth development, but direct comparison must be carefully considered. For instance, the equivalent doses of radiation or chemotherapeutic agents for animal species and humans are unknown. Furthermore, in animal studies, alterations in tooth development can be studied after radiotherapy alone or after exposure of animals to a single chemotherapeutic agent. This is not the situation with children, who are treated with combination chemotherapy, often accompanied by radiotherapy. Infections and their treatment, as well as nutritional deficiencies common during anticancer therapy, may also contribute to aberrant tooth development in children (reviewed by Pindborg, 1982).

5.1. Animal studies and in vitro experiments

5.1.1. Radiotherapy

Tribondeau and Récamier were the first to recognize radiation effects on tooth development, back in 1905, by studying kittens (Bruce and Stafne, 1950). Since that time, numerous experiments have been made on rats and mice but also on hamsters, rabbits, dogs, and monkeys. Although the radiation sensitivity of tooth-related cells may differ between animal species and between animals and man, radiation-induced microscopic or macroscopic dental findings are markedly similar in them all (reviewed by Kimeldorf et al., 1963).

The first sign of injury, within hours after exposure, is nuclear fragmentation in precursors of odontoblasts and ameloblasts (reviewed by Kimeldorf et al., 1963). Other writers indicate the first adverse effect of radiation as being edema of the pulpal proliferative zone, often followed by cystic cavities as seen in the incisors of rats (Medak et al., 1952; Adkins, 1967) or Syrian hamsters (Medak et al., 1954). After a heavy irradiation, severe hemorrhage and thrombosis of the pulp vessels has been reported in dogs (Kalnins, 1954). Depending on dose, degenerative changes in or necrosis of differentiating (non-secretory) odontoblasts occur and typical dentin niches, described also following anticancer CT (Section 5.1.2.), develop as the sign of a temporary disturbance. Thin dentin and predentin zones, degenerated and depolarized odontoblasts, and osteodentin in the pulp characterize the classical dentin niche. Formation of the osteodentin, regarded as a sign of odontoblast damage, begins in the pulp adjacent to the niche in rat incisors (Medak et al., 1952; Collet and Thonard, 1965; Koppang, 1967; Koppang and Stokke, 1969; Lindvall et al., 1972), in rat molars (Collet and Thonard, 1965), and in monkeys (Gowgiel, 1961). Following the degenerative cellular changes and transient disturbances in dentinogenesis, a regenerative process is often seen after doses of 1500 to 4000 r in continuously growing rat incisors. This is indicated by development of new incisors separated from the “pre-irradiation teeth,” small in size but showing rather normal structure, although often a mottled surface (Medak et al., 1952; English et al., 1954; Hansen and English, 1957). However, in incisors of Syrian hamsters, proliferating odontoblasts are destroyed, with no recovery, after doses of 4200 or

4800 r. Differentiated “old” odontoblasts could still produce dentin (Medak et al., 1954) and were suggested to be among the most radio-resistant cells of the body (Kalnins, 1954).

Differentiated ameloblasts in mouse molars (Burstone, 1950) and rat incisors (Medak et al., 1952; English et al., 1954) were also remarkably radio-resistant. In the majority of the studies, in which amelogenesis was reported, ameloblasts were considered more radioresistant than were odontoblasts of a similar stage. Contradictory results have been reported: a low dose of 375 r (Dale, 1953) and somewhat higher 500 to 900 r doses (Lindvall et al., 1972) regularly caused changes in the enamel but not always in the dentin of rat incisors. However, there is agreement that at least high radiation doses (> 1500 r) can disturb odontogenic epithelium and differentiation of ameloblasts, or the cells may become necrotic, resulting in hypoplastic changes in the enamel. Amelogenesis can regenerate, at least in rat incisors after radiation-induced inhibition or cessation (Medak et al., 1952; Dale, 1953; English et al., 1954; Hansen and English, 1957). In Syrian hamster incisors, the odontogenic epithelium behaved differently: local proliferation formed knoblike structures that enlarged to keratin-filled cysts. Thus, the odontogenic epithelium did not resume its original function in the recovery process, indicating possible differences between species (Medak et al., 1954).

The typical acute and long-term dental changes after irradiation are dose-dependent. Mild radiation damage results only in temporary changes that may not be noticeable macroscopically. More severe insults may cause permanent changes in formation of the dental hard tissues, manifesting themselves during a long follow-up as altered tooth size and shape (short, tapered, or blunted roots, microdontia), ankylosis or, in extreme cases, as tooth agenesis. These changes resemble each other in the developing teeth of several species, for instance in macacus rhesus monkeys (Gowgiel, 1961), cats (Donohue and Perreault, 1964), mice (molars) (Burstone, 1950), and Syrian hamsters (molars) (Bruce, 1950). After irradiation of 2000 r (~ 20 Gy, given in two equal doses 7 days apart) to cats, cessation of root formation occurred in the developing teeth. The blunted apical ends of the roots were partially or completely sealed with oseodentine, and dystrophic calcification of pulps was visible. Some teeth remained embedded in the jaws (Donohue and Perreault, 1964). Higher irradiation doses (4500 r, 5500 r, and 7500 r; fractions given during 5.5 to 12.5 weeks) to monkeys with their permanent teeth in various stages of development inhibited further crown and root development and destroyed the germs of the third molars that were not yet visible in the pre-irradiation radiographs. Calcification of the crowns was unaffected. Osteodentin closed the apices, and pulp tissue was fibrotic. Most teeth—even those without roots—erupted, but some became ankylosed (Gowgiel, 1961). In mouse molars, a single irradiation dose of 1500 r, 3000 r, or 5000 r resulted in aberrations of tooth development in a dose- and time-dependent manner. Irradiation of young animals (< 1 week) resulted in deficient root development in the first and second molars, and the third molars, less developed at the time of irradiation, emerged in some cases only as irregular masses of dentinoid material. Ankylosis of the molars was evident also in mice, especially after high irradiation doses (Burstone, 1950). Stage of tooth development was crucial also in the dental outcome of Syrian hamsters after various irradiation doses of approximately 1300 to 2500 r to the molar region; tooth agenesis and dwarfing of tooth crowns or roots or both occurred (Bruce, 1950b).

The minimum radiation dose that causes acute or long-term alterations in tooth-related cells and tissues varies by animal species and irradiation design. Moreover, methods of the examination (e.g., macroscopic, microscopic, decalcified, or ground sections) affect the

possibility of recording any changes. In rat incisors, some nuclear changes have been reported at 6 hours following a radiation of 25 r, but only doses of 400 r or greater resulted in degenerative changes (reviewed by Kimeldorf et al., 1963). Dentinogenesis has been disturbed in 60% of rat incisors after 600 r, but after 300 r the treated rats did not differ from controls (Collet and Thonard, 1965). In other studies, however, dentin niches occurred in rat incisors even after doses of only 200 or 300 r (Koppang, 1967; Koppang and Stokke, 1969).

The mechanism(s) of action, by which the harmful effect of radiation turns into disturbances in dentinogenesis, has inspired speculation. Direct effects of radiation on the tooth-forming cells, especially on preodontoblasts and differentiating odontoblasts, has probably been the most accepted mode for this action, but indirect mechanisms have also been suggested. For instance, radiation-induced destruction of blood vessels, resulting in hemorrhages and edema in the pulp and thrombosis of the other vessels, causing further deprivation of the blood supply may result in secondary changes in odontoblasts (Medak et al., 1952; Kalnins, 1954), or may prevent differentiation of odontoblasts (Burstone, 1950). Some authors also remind us of the possibility that the original insult may be against the odontogenic epithelium needed in initiation of the odontoblast differentiation (Burstone, 1950; Hansen and English, 1957).

5.1.2. Anticancer chemotherapy

Alkylating agents

Several studies on rat incisors and molars have demonstrated dose-dependent adverse dental effects of cyclophosphamide (Cy), an important member of the group of alkylating agents (Table 2, page 30). Intraperitoneal Cy doses from 25 to 150 mg/kg body weight in rats caused disturbances in their maxillary and mandibular incisors. The two highest doses (150 and 125 mg/kg) caused interruption of odontogenesis in all mandibular incisors and lingual aspects of maxillary incisors, and osteodentin closed up the pulp cavity. Labial aspects of maxillary incisors were more resistant, although all teeth were affected by interrupted odontogenesis or constriction (reduction in width). The lingual aspects of mandibular incisors were the sites most sensitive to Cy toxicity, and after the dose of 50 mg/kg, all teeth were severely injured (70% interrupted odontogenesis, 30% constrictions). The most resistant labial aspects of maxillary incisors showed constrictions in 10% and less severe dentin niches (reduced thickness of the dentin wall, osteodentin) in 90% of teeth. Even the smallest dose caused dentin niches in more than half the teeth. Furthermore, cystic cavities with congested vessels and hemorrhage occurred dose-dependently in apical areas (Koppang, 1973a). Offspring of rats who had received a single dose of Cy (40 or 50 mg/kg) during pregnancy presented with similar but more frequent changes in their incisors than did the adults (constrictions, niches, and edema). In addition, external tooth resorptions and scalloping of the dentinoenamel junction, seldom seen in adult rats, were present (Koppang, 1978). (For comparison, see Table 4, page 56, for HDC doses in children).

After Cy administration of 40, 80, and 120 mg/kg in single peritoneal doses, acellularity of basal pulp and cessation of root growth occurred in rat incisors. The time period to re-establish root growth increased with increasing dose (Adatia, 1975). Reduction in the growth rate of rat incisors occurred after a Cy dose of 40 mg/kg, but this was followed by an

increased growth rate, so that no remarkable difference was eventually noticeable. The depth of the dentine niches corresponded with the niches that were seen after 950 rad of whole-body irradiation to rats, although Cy-induced niches were longer (Lindvall et al., 1972; Koppang, 1981).

The most sensitive tooth-forming cells in rat incisors were preodontoblasts that had not yet started protein production, and the resulting dentine niches developed during 24 hours after a Cy injection of 25 mg/kg. After 40 mg/kg of Cy, precursors of preodontoblasts were also injured, resulting in larger niches (Koppang, 1973b). Mature odontoblasts, ameloblast, or enamel formation were unaffected (Koppang, 1973a, 1973b). Other studies confirmed the presence of unaffected differentiated odontoblasts and the high sensitivity of preodontoblast precursors to Cy but, in contrast to the finding of uninjured enamel formation by Koppang (1973a, 1973b), a dose-dependent mild toxicity was noticed also in the odontogenic epithelium (Adatia, 1975; Orams, 1983).

Delayed drug-related mortality (70-180 days after Cy administration) in rats occurred following a single high-dose Cy injection (75 mg/kg) (Vahlsing et al., 1975, 1977). The authors considered the deaths to be due to dental abnormalities preventing the rats from eating. Some rats had short or missing maxillary incisors, others presented with overgrown often abnormally oriented incisors. Following the Cy exposure, interruption of odontogenesis resulted in formation of pre- and post-experimental incisors separated from each other. The pre-experimental incisors erupted until lost, and when the post-experimental incisors erupted they were often morphologically aberrant and overgrown, preventing normal eating. These clinical findings were later confirmed (Reade and Roberts, 1978).

Two successive peritoneal Cy injections (both 30 mg/kg body weight) were given to rats at the ages of 10 and 13 days. At 10 days, the crowns of the first molars have developed and root formation is just beginning. In the second molars, approximately two-thirds of the crowns have formed, and the third molars have reached the bell stage, where dentin and enamel formation has begun in the occlusal part. Cy injections resulted in wide cell-free areas in the cervical region of the pulp of the third molars, close to the epithelium, followed by osteodentin formation. In the first and second molars the number of cells was reduced in the apical region, but no large cell-free areas appeared. Osteodentin formation closed the apical foramina and resulted in shortened root length. Even bacteria penetrated the osteodentin, causing pulpal and periodontal infection, considered a sign of poor-quality dentin (Näsman and Hammarström, 1996). In scanning electron microscopy these findings were confirmed. Crown morphology and size of the first and second molars were not affected since they were already fixed when Cy was injected, but the less developed bell-stage third molars were small with diverging cusp morphology and a porous structure. Root lengths of the first and third maxillary molars in the treated animals varied from 41 to 70% of control values. Again, the less-developed third molars suffered more than the first molars. Roots were also thin and tapered when compared to controls' roots. The authors remind us that these changes are very similar to those found in children after pediatric anticancer therapy (Näsman et al., 1997b).

Many other anticancer agents of this group are used in pediatric cancer treatment, but no studies are available as regards tooth development.

Antimetabolites

Several cytotoxic agents of this group are widely used in pediatric hematology and oncology (Table 2, page 30) but have been very little studied as regards tooth development. Hamster molar tooth germs were exposed in organ cultures to methotrexate (MTX) concentrations running from as high as 10^{-3} M to 10^{-7} M. Only the highest concentration tested (10^{-3} M) induced a small, insignificant decrease in cell mass, but with no histologic changes evident. The authors suggest that MTX causes no harm to the developing teeth of children (Wöltgens et al., 1998). The pyrimidine analogue antimetabolite 5-fluorouracil (5-FU), given in a single injection to 8-week-old mice (140 mg/kg estimated 10% lethal dose), caused moderate cell death of the epithelial sheath in maxillary incisors. Consequently, local dentin defects arose, but normal odontogenesis was established later, leaving a transient dentin injury (Satoh et al., 2001).

Plant alkaloids

Two antimicrotubular agents, vinblastine (VBL) and vincristine (VCR) (vinca alkaloids, Table 2, page 30), inhibit mitosis by binding to tubulin but also by interfering with other cellular functions, e.g., transport of proteins. Microtubules play an important role in enamel maturation when ameloblasts transport organic components from the enamel matrix. They also function in maintaining cell shapes. Thus, effects of antimicrotubular agents on ameloblast morphology and function occurred following VBL or VCR exposures. A single intravenous VBL injection (4 mg/kg) to rats caused a decrease in the number of microtubules of maturing ameloblasts in their incisors at one hour, and at 2 hours the microtubules almost disappeared. Reduction in ruffle-ended ameloblasts occurred, or the ruffles became shorter, with dislocation of nuclei, mitochondria, and endoplasmic reticulum (Akita et al., 1983). Changes in cell morphology and polarity, as well as disappearance of microtubules in both secretory and maturation stage ameloblasts after VBL administration, were later confirmed (Yamamoto et al., 1997). A decrease in immunopositive staining for α -tubulin, a structural component of microtubules, appeared from 1 to 6 hours after vinblastine injection. In 48 hours, most changes had recovered (Yamamoto et al., 1997). Furthermore, VBL decreased Ca^{2+} , Mg^{2+} -adenosine triphosphatase activity in both ruffle-ended ameloblasts and membrane-associated calcium (Eisenmann et al., 1992). This was thought to demonstrate VBL interference with the normal calcium influx into the enamel (McKee and Warshawsky, 1986). Irregular enamel formation occurred in mice incisors, continued at least 15 days after VBL injection, but recovered by day 60 (Satoh et al., 2001).

VBL had acute and protracted injurious effects in rat incisors also on preodontoblasts and odontoblasts. Both showed minor changes (displaced nuclei, grains in cytoplasm) 6 hours after the injection (2 mg/kg) but in 24 hours young odontoblasts, located incisal to the site of enamel secretion, were greatly changed in shape or destroyed, and old odontoblasts showed changes in morphology. In 3 days, the number of preodontoblasts was reduced and odontoblasts still presented with anomalous shapes. After 7 days, preodontoblasts and young odontoblasts had recovered and dentin niches and osteodentin appeared in the area of old odontoblasts (Mikkelsen, 1978). Moreover, VCR, injected at different doses, produced mitotic disturbances in preodontoblasts and their mesenchymal precursors, and these increased with increasing doses. High VCR doses also affected the differentiated

odontoblasts, causing morphologic derangement and having lethal effect even on some non-dividing cells. VCR interferes with the dentin matrix production by inhibiting the synthesis of collagen precursors or by reducing odontoblasts' ability to secrete them or both, leading to irregular predentin production (Stene, 1978; Stene and Koppang, 1980). With a longer follow-up, dentin niches due to reduced dentin production appeared, and their size increased with increasing doses (Stene, 1979).

Compared to vinca alkaloids, epipodophyllotoxins (etoposide, teniposide) have a different mechanism of action. To my knowledge, these have never been studied *in vivo* or *in vitro* as regards tooth development (Table 2).

Antitumor antibiotics

Doxorubicin (= adriamycin), commonly used in pediatric anticancer therapy, belongs to the anthracycline group (Table 2, page 30). Its effects on odontogenesis have been widely studied in rat experiments. One day following a single doxorubicin injection of 5 mg/kg body weight, destruction of preodontoblasts and some adjacent "progenitor" pulp mesenchymal cells occurred in incisors. The older the odontoblasts became, the more resistant they were to the toxic effects of the drug, and old, fully differentiated odontoblasts were unaffected (Dahl, 1984; Karim, 1985a). At the follow-up from 3 to 7 days, mesenchymal cell aggregations were first visible near the areas of destroyed cells, and at these sites osteodentin formation began in the pulp (Karim, 1985b). Most of the changes—reduced dentin production, altered odontoblast morphology, irregular dentin deposition, and formation of osteodentin in the pulp—were visible after doses of 5 mg/kg, with changes being more prominent with increasing (10 and 20 mg/kg) doxorubicin doses (Dahl, 1984; Karim and Eddy, 1984; Dahl and Stromme Koppang, 1985). No recovery of early preodontoblasts, the most sensitive to doxorubicin, occurred after any of the doses during the observation periods. When the total dose of doxorubicin was divided into several smaller doses, its odontogenic cytotoxicity increased, and regeneration was greatly delayed when compared with single-dose administration (Dahl, 1985).

The same kind of acute changes have appeared in incisors after an adriamycin dose of 5.3 mg/kg to 8-day-old mice (Sato et al., 2001). In follow-up, irregular dentinogenesis (osteodentin), cell debris, edema, disorganized odontoblasts, and inflammation of the alveolar bone surrounding the tooth apex were still apparent on days 10 to 18. These alterations did not recover during the observation period of 60 days. Ameloblasts and enamel formation remained unaffected (Sato et al., 2001). Exposure of golden hamster 3-day-old (bell-stage) maxillary second molars to adriamycin (1 mg/liter) during the first 2 hours of cell culture resulted in the development of teeth smaller than normal. Growth in the cervical loop area was inhibited, with mild necrosis within the apical pulp. With an adriamycin concentration of 5 mg/liter, necrosis was more extensive, and preodontoblasts and their precursors were destroyed and, furthermore, with 10 mg/liter, even some young odontoblasts and ameloblasts died. When molars were treated with adriamycin (5 mg/liter) and then cultured for 1 day, necrosis appeared in the pulp, and in the young cusps, necrosis destroyed all preodontoblasts and their precursors (Karim et al., 1989).

Dactinomycin (actinomycin-D) injection (0.375 µg/kg) has caused considerable necrosis of preodontoblasts and peripheral pulpal cells in developing rat incisors. Later,

osteodentin formed in the pulp, and decreased dentine production manifested itself as a sharply defined dentine niche. Dentine was normal both incisally and apically of the niche. No evidence appeared of the interference of dactinomycin with the differentiation of ameloblasts or the functions of the enamel organ (Adkins, 1972). In organ culture, several constant dactinomycin concentrations reduced dose-dependently the dry weight of the developing tooth germs of hamsters, but these disturbed the mineralization only at the highest concentration tested (5×10^{-5} M). This dose was cytotoxic to all odontogenic cells, including the secretory odontoblasts and ameloblasts. Cell proliferation rate was reduced even at low concentrations, with the most sensitive cells being preodontoblasts that (based on the nuclear morphology) appeared to undergo apoptotic cell death. Cell death was described as apoptotic at concentrations $< 10^{-7}$ M, but as necrotic due to acute cytotoxicity at higher doses ($> 10^{-6}$ M) (Lyaruu et al., 1997).

The effects of daunorubicin on hamster tooth germs resembled those of dactinomycin. Exceptionally, the most sensitive cells were preameloblasts, followed by preodontoblasts, with the mode of cell death being apoptotic at low daunorubicin concentrations, but toxic at high concentrations (Lyaruu et al., 1999).

Platinum analogs

The only agent reported in relation to tooth development is cisplatin, studied in one experiment on mice. Only minimal cell death occurred in precursor cells of the epithelial sheath on day 3 after the cisplatin injection (7.5 mg/kg), with no changes during follow-up until day 60 (Satoh et al., 2001).

5.2. Development of permanent teeth after anticancer therapy during childhood

The effect of radiotherapy (RT) on developing human teeth has become evident from case reports, one of which described five patients whose oral or facial hemangiomas or lymphangiomas were treated with irradiation at an early age (Bruce and Stafne, 1950). The authors listed the effects of irradiation on teeth: It “1) can injure a tooth germ to the extent that a tooth will not form; 2) causes dwarfing of permanent teeth; 3) effects dwarfing of the roots of permanent teeth that are undergoing development during irradiation; 4) effects premature completion of calcification of permanent teeth and 5) may cause early eruption of permanent teeth.” These findings, except the early eruption, and often with hypocalcification added, have been confirmed (Gorlin and Meskin, 1963; Donohue et al., 1965; Pietrokovski and Menczel, 1966; Lines et al., 1979; Folwaczny and Hickel, 2000).

RT is usually combined with anticancer CT. If the dental area is irradiated or receives scattered irradiation, it is difficult or impossible to distinguish between CT and RT effects. In most cases, however, irradiation fields do not include the teeth, which allows for studies in which CT effects can be examined separately. The information received from these studies varies by study population, treatment protocol, and study method. Dental consequences in the children most heavily treated with HDC, with or without TBI, and with SCT have not been widely studied, but when compared to children treated with CCT, the latter were less affected (Näsman et al., 1994, 1997a; Duggal, 2003).

Teeth extracted from patients having been cured of childhood cancer have also been histologically studied. The distribution of prominent incremental lines in their dentine corresponded with the periods of intravenous VCR therapy. VCR probably disturbed the microtubular function of odontoblasts and resulted in the decreased amount of dentine matrix that became hypercalcified when the “normal” amount of calcium was deposited in this decreased matrix (Macleod et al., 1987; Maguire et al., 1987). No changes appeared in the enamel (Macleod et al., 1987). In contrast, incremental lines occurred also in the enamel in two teeth of a patient exposed to multiagent CT, HDC, and TBI (10 Gy, single fraction) prior to SCT. Furthermore, gross hypoplasia appeared in the cervical area developing at the time of TBI (Dahllöf et al., 1994).

The key results of selected clinical studies of developmental dental defects (especially tooth agenesis, microdontia, and root development) are summarized in Table 3.

5.2.1. *Tooth agenesis*

After CCT, the percentage of patients with tooth agenesis varied from 5 to 28% (mean 16% in the Table 3 studies). The lowest figure can be explained by the method used: The most commonly missing teeth, second premolars and third molars, were excluded, because these teeth quite frequently are lacking also in a healthy population (Sonis et al., 1990). The highest percentage included the third molars (Maguire et al., 1987), at least one of which is missing in 20 to 30% of healthy people. The prevalence of tooth agenesis, excluding third molars, usually ranges from 4 to 8% (Section 2.2.2.). Thus, tooth agenesis seems to be slightly elevated after CT.

In the three studies on BMT recipients, which reported the prevalence of tooth agenesis, percentages were 56%, 58% (Näsman et al., 1994, 1997a), and 11% (Uderzo et al., 1997). It remains unclear whether the third molars were included in the Näsman studies, but obviously they were excluded from the latter. These studies failed to tell the age at which the diagnosis of tooth agenesis for various teeth was made. Thus, detailed information of tooth agenesis in SCT recipients is lacking (Table 3).

5.2.2. *Microdontia*

No subjective or objective criteria are given for the microdontia assessments in which, after CCT, microdontia prevalence varied from 10 to 38% (mean 20%) (Table 3). The importance of age at treatment as regards dental outcome was noticed in microdontia studies, with young patients being more vulnerable (Maguire et al., 1987; Sonis et al., 1990; Kaste et al., 1998).

Prevalence of microdontia in the three BMT groups was 25%, 68%, and 75% (Dahllöf et al., 1988; Näsman et al., 1994, 1997a) (Table 3).

5.2.3. *Root development of permanent teeth*

Since solid criteria or features expressing “disturbed” dental root development are lacking, several assessment methods, most of which have been based on subjective visual judgment, lead to disparate results. Treatment with CCT or CT and CRI disturbed root development in 15 to 28% of patients. In the two studies (Rosenberg et al., 1987; Sonis et al., 1990) in which

some kind of grading or measuring was done, figures ranged from 77 to 94%. Except for canines in the earlier 1988 study, root areas and ratios of root and crown areas were smaller in cancer patients than in controls (Pajari et al., 1988a; Duggal, 2003) (Table 3).

Root development is disturbed in almost all BMT patients (Dahllöf et al., 1988; Näsman et al., 1994, 1997a), and BMT patients are also more severely affected than are patients treated with CT (and sometimes with CRI). However, one study with 27 BMT patients gives a rather small prevalence, 33%, for “root hypoplasia” (Uderzo et al., 1997).

Table 3. Selected studies on tooth agenesis, microdontia, and dental root development in patients treated for cancer in childhood. Asterisks in the reference column indicate those studies with a considerable number of SCT patients.

Reference	N, pats	Age	Diagnoses (N, pats)	Therapy (N, pats)	Follow-up	Tooth agenesis	Microdontia	Deficient root development	Methods, miscellaneous
Jaffe et al., 1984	68	RT + CT pats: 0.5-17 (median 6.5) yrs at treatment; CT pats: not reported	RT + CT pats: HL (14), ALL (14), RMS (9), some others (8); CT pats (23): not reported	Head and neck RT + CT (43) or RT (2); RT 16-65 Gy; CT (23)	2-24 yrs (median 12 yrs)	Tooth agenesis not mentioned. RT + CT and RT group: HL: radiation effects in 13 of the 14 pats (93%); ALL: 6/14 (43%) had RT and/or CT sequelae; RMS: 5/9 (56%) had radiation effects on dental roots and crowns; Miscellaneous: 5/8 (63%) had radiation sequelae. CT group: 5/23 (22%) had tooth abnormalities (microdontia, thinning of roots)	19/82 (23%), 38% of pats ≤ 3 yrs at therapy; maxillary teeth more affected; ALL group tended to be more affected; ST group affected	23/82 (28%); 50% of pats treated at ≤ 9 yrs affected; mandibular teeth slightly more affected; ST group more affected	PRG, clinical examination; no criteria for the subjective or objective dg of DDD; no detailed information
Maguire et al., 1987	52 pats studied for this report; 30 pats earlier	Not reported at dg; 3-22 yrs for the 52 pats; no age for the group of 30 pats	ALL (44); ST (38); not involving the head and neck	ALL: CT + 24 Gy CRI; ST: not reported	Not reported	23/82 (28%); third molar agenesis in 17/82 (20%); maxillary teeth slightly more affected; ST group more affected	Tooth agenesis and microdontia not reported. Only premolars studied for root shortening, thinning also in first molars and canines. Subjectively, 76.5% of pats had DDD; 5/17 pats (29%) showed marked root shortening; 13/17 (76%) thinning of the apical portion; Quantitatively, in mandible: 23/32 (72%) of P2:M1 ratios < CI of controls; 19/30 (63%) of P1:M1 ratios < CI. In maxilla: 27/32 (84%) of P2:M1 ratios < CI; 21/29 (72%) of P1:M1 ratios < CI	PRG, clinical examination of 52 pats; sibling controls; agenesis dg not before 5 yrs. 11 months for second premolars, 10 yrs 11 months for third molars; no other objective criteria; 66% had some dental defects (hypoplasiae included)	
Rosenberg et al., 1987	17	3.2-8.9 yrs (mean 7 yrs 2 months) at dg	ALL	CT (16); CT + CRI (1)	Exact follow-up time not reported; Pats 14 yrs or older at examination	Tooth agenesis and microdontia not reported. Only premolars studied for root shortening, thinning also in first molars and canines. Subjectively, 76.5% of pats had DDD; 5/17 pats (29%) showed marked root shortening; 13/17 (76%) thinning of the apical portion; Quantitatively, in mandible: 23/32 (72%) of P2:M1 ratios < CI of controls; 19/30 (63%) of P1:M1 ratios < CI. In maxilla: 27/32 (84%) of P2:M1 ratios < CI; 21/29 (72%) of P1:M1 ratios < CI	PRG, periapical radiographs; Subjective assessment: guidelines for root tapering, "shortening", "blunting" and P2 length to M1 length compared to ratios of extracted teeth in historical controls; Note: If both teeth shortened, ratio may be normal		
Pajari et al., 1988a	38	1-15 (mean 5.7) yrs at dg	ALL (24); Others (14) (Wilms, lymphoma, NBL, sarcomas, CNS tumors)	ALL: CT (7); CT + 24 Gy CRI (16); CT + 10 Gy TBI + BMT (1); Others (14): CT or CT + RT	1-8 yrs after the cancer therapy	Not reported	Crown areas of permanent teeth not affected when compared to controls	PRG; 2 healthy controls/patient; areas of roots and crowns and root/crown ratios of left, fully developed permanent mandibular teeth; incisors pooled; premolars pooled; third molars excluded	

Reference	N, pats	Age	Diagnoses (N, pats)	Therapy (N, pats)	Follow-up	Tooth agenesis	Microdontia	Deficient root development	Methods, miscellaneous
* Dahljöf et al., 1988	16 BMT pats	1.0-11.6 (mean 7.1) yrs at BMT	ALL, ANLL (12); SAA (3); Gaucher (1)	A(NLL): CT + HDC + 10 Gy TBI (9) + 24-25 Gy CRI (3); SAA: HDC; Gaucher: HDC + 8 Gy TBI	2.7-6.0 (mean 3.9) yrs	Not reported (not present?)	4/16 (25%); all microdontia pats received TBI and were 1-5.4 yrs at BMT	Short, V-shaped roots (I): all pats with and 1/3 of pats without TBI; arrested root development (II): 5/16 (31%), all 5 with TBI	PRG: subjective classification: I, short, V-shaped roots; II, arrested root development and premature apical closure; III, enamel hypoplasia; IV, microdontia; Pats with TBI and < 6 yrs at BMT had the most severe disturbances
Sonis et al., 1990	97	Diagnosed < 10 yrs; treatment groups (1, 2, 3) divided by age at dg into < 5 yrs (1A, 2A, 3A) or ≥ 5 yrs (1B, 2B, 3B)	ALL	1A, 1B) CT + IT MTX (19); 2A, 2B) CT + IT MTX + 18 Gy CRI (27); 3A, 3B) CT + IT MTX + 24 Gy CRI (51)	At least 5 yrs after dg	5/97 (5%); Group 3A, 5/25 (25%) affected; No tooth agenesis in other groups	21/97 (22%); In groups 2A, 3/13 (23%), 3A, 15/20 (75%) and 3B, 3/31 (9.7%) pats others not affected	1A, 2A, 3A: All affected, pats in group 3A most severely; 1B: 5/11 (45%) affected; 2B, 3B: all affected	PRG: defects in maxillary lateral incisors, third molar development and agenesis of second premolars excluded; classification and severity rating scale; diagnostic criteria subjective (e.g., < 50% of normal root length; "normal" was a subjective visual guess)
Nunn et al., 1991	52	57-291 months at examination; age at dg 39-96 months for the pats with DDD	Childhood cancer (ALL, others)	CT or CT + CRI	Not reported	8/52 (15%)	5/52 (9.6%)	8/52 (15.4%)	PRG, BW, clinical examination; sibling controls; subjective assessment; no criteria for DDD; 14/52 (27%) of pats had DDD (hypoplasiae included)
* Näsman et al., 1994	57 CT pats; 19 BMT pats	CT: mean age 5.1 ± 3.3 yrs at dg; BMT: mean age 6.5 ± 3.5 yrs at BMT	CT: Hem. malign. (AL, NHL, 30); various ST (27); BMT: AL, CML (15), Gaucher (2), NHL (1), SCID (1)	CT or CT + CRI; HDC + TBI (7-10 Gy, single dose)	CT: 3-17.5 (mean 6.6) yrs from dg; BMT: 3-8.6 (mean 5.4) yrs after BMT	Hem.: 3/30 (10%); ST: 8/27 (30%); BMT: 11/19 (58%)	Hem.: 3/30 (10%); ST: 4/27 (15%); BMT: 13/19 (68%)	Short, V-shaped: Hem.: 8/30 (27%); ST: 2/27 (7%); BMT: 18/19 (95%) Arrested: Hem.: 2/30 (7%); ST: 2/27 (7%); BMT: 11/19 (58%)	PRG, clinical examination; healthy controls for BMT pats; in subjective assessment Dahljöf et al. method (1988) with aplasia (V); mean number of teeth with disturbed root development: controls 0.4 ± 0.7, CT pats 1.2 ± 1.6, BMT pats 15.9 ± 8.2; most extensive DDD in pats ≤ 5.4 yrs at BMT
Holtgrave et al., 1995	60	3-52 months at dg	ALL (34); ST (25), no craniofacial	CT + 18 Gy (23) or 24 Gy CRI (11) for ALL; CT for ST	64-189 months from treatment	ALL: 3 teeth; ST: 3 teeth; number of pats with tooth agenesis not given	ALL: 32 teeth; ST: 5 teeth; number of pats not given	ALL: 28 teeth (26 pats studied); ST: 24 teeth (13 pats studied); number of pats with DDD lacking	PRG, clinical examination; healthy controls; no criteria for tooth agenesis and microdontia diagnoses; root length/tooth length index used

Reference	N, pats	Age	Diagnoses (N, pats)	Therapy (N, pats)	Follow-up	Tooth agenesis	Microdontia	Deficient root development	Methods, miscellaneous
* Näsman et al., 1997	52 CT pats; 16 BMT pats	CT: mean age 5.1 ± 3.3 yrs at dg; BMT: mean age 6.3 ± 3.5 yrs probably at BMT	CT: AL (19), lymph. (9), ST (24); BMT: hem. malign.	CT or CT + 18-24 Gy CRI (13); HDC + 10 Gy TBI + BMT	CT: 3.0-17.5 (mean 6.6) yrs from dg; BMT: 3.0-8.6 (mean 5.7) yrs from BMT	CT: 11/52 (21%); BMT: 9/16 (56%)	CT: 7/52 (13%); BMT: 12/16 (75%)	Short V-shaped: CT: 10/52 (19%); BMT: 15/16 (94%) Arrested: CT: 4/52 (8%); BMT: 11/16 (69%)	PRG, clinical examination; 3 healthy controls/CT pat; DDD assessed as in 1994; areas of teeth and roots, ratios of C/R areas of mandibular teeth; mean tooth and root areas in BMT pats < CT pats < controls; ratios of C/R areas did not always differ, roots deviated more than crowns
* Uderzo et al., 1997	27 BMT pats	0.7-14.7 (mean 6.6) yrs at dg; 1.1-17.9 (mean 9.0) yrs at BMT	ALL (14), NHL (1), AML (10), CML (2)	CT or CT + 18 Gy CRI (11); HDC (27) + 12 Gy TBI (25) + BMT	0.8-7.7 (mean 2.7) yrs; (12/27 < 1.5 yrs)	3/27 (11%); third molar agenesis "obviously not counted as agenesis"	Microdontia not reported separately. "Root hypoplasia," present in 9/27 (33%) of pats, determined as "arrested root development with shortened or tapered V-shaped roots, smaller than normal or crowded teeth"	PRG, clinical examination; subjective assessment, no criteria for DDD; many short follow-up periods; "Tooth abnormalities (e.g., enamel opacities) or agenesis in 62.9% of patients."	
Kaste et al., 1997	423	0.6-13 (median 4.8) yrs at dg; pats divided by age: ≤ 8 or > 8 yrs at dg	ALL	CT or CT + 18-24 Gy CRI	Age at follow-up 2.4-20.3 (median 9.5) yrs	36/423 (8.5%)	80/423 (19%); (5.7% lateral maxillary incisors; 6.2% third molars)	Root stunting: 103/423 (24%); 82% of these pats received CRI and 86% were ≤ 8 yrs at dg	PRG: subjective assessment, no criteria; DDD detected (taurodontia included); pats ≤ 8 yrs, 42%, and > 8 yrs at dg, 32%; Pats with CRI, 50%, and without CRI, 25%
Kaste et al., 1998	52	3 days-7.2 yrs (median 1.5) yrs	NBL	CT; CT + head/neck RT (6); CT + RT + BMT (2)	1.9-19.3 (median 5.0) yrs	In text: 9/52 (17%); (in Table: 6/52, 11.5%)	20/52 (38%)	Root stunting: 9/52 (17%)	PRG, dental records; subjective assessment, no criteria; third molars included; dental defects in 71% of pats (hypoplasiae, excessive caries also in primary teeth included)
* Hölttä et al., 2002	18 SCT pats	Non-TBI: 1.0-5.8 (mean 2.3) yrs at SCT; TBI: 1.4-4.1 (mean 2.7) yrs at SCT	NBL	Non-TBI (8): CT or CT + RT (not dental area) + HDC + SCT; TBI (10): CT or CT + RT (not dental area) + HDC + TBI + SCT	1.9-14.6 (mean 7.9) yrs after SCT	15 pats; Non-TBI: 2/5 (40%) of pats; 6/122 (5%) of teeth; TBI: 10/10 (100%) of pats; 66/238 (28%) of teeth	17 pats; Non-TBI: 6/7 (86%) of pats; 12/126 (9.5%) of teeth; TBI: 8/10 (80%) of pats; 22/238 (9%) of teeth	15 pats; Non-TBI: 4/5 (80%) of pats; 27/126 (21%) of teeth; TBI: 10/10 (100%) of pats; 137/238 (58%) of teeth	PRG, clinical examination; healthy group for testing Defect Index (Del); age criteria for tooth agenesis; subjective limit for microdontia; R/C ratios measured and limits for mild, severe, very severe alterations; overall damage expressed with Del

Reference	N, pats	Age	Diagnoses (N, pats)	Therapy (N, pats)	Follow-up	Tooth agenesis	Microdontia	Deficient root development	Methods, miscellaneous
Duggal, 2003	69 (7 BMT pats)	Not reported	ALL (43%), Wilms (14%), HL (9%), others	CT (24) or jaw RT (13), or CT + 16-22 Gy CRI (25), or CT + TBI + BMT (7)	In remission at least 5 yrs	Tooth agenesis and microdontia not studied. Root areas smallest in TBI + BMT pats, followed by those with jaw radiation. Root areas did not differ between CT and CT + CRI groups, were smaller than in controls. Age at dg not significant in terms of root surface area.			PRG; 1 healthy control/patient; root surface areas of left mandibular teeth studied with a video camera – image analysis – computer system
*	55 SCT pats	1.0-9.4 (mean 4.3) yrs at SCT; Non-TBI: mean 4.1 yrs at SCT; TBI: mean 4.4 yrs at SCT	ALL (12), AML (9), NBL (19), Wilms (5), others (10); Non-TBI (16); TBI (39)	CT + (CRI) + HDC + SCT (16); CT + (CRI) + HDC + TBI + SCT (39)	1.0-20.6 (mean 7.4) yrs after SCT	16/52 (31%) without, 19/29 (62%) with third molars; most frequent (77-83%) when ≤ 3 yrs at SCT; TBI not significant	44% without third molars; most frequent when ≤ 3 yrs (75%) or 3.1-5.0 yrs (60%) at SCT; TBI not significant	Not reported	PRG, clinical examination; tooth agenesis without or with third molars, age criteria for tooth agenesis; subjective limit for microdontia; effects of TBI and age at SCT studied; young age (≤ 5 yrs) a stronger risk factor for defects than TBI
*	52 SCT pats	1.0-9.4 (mean 4.4) yrs at SCT	ALL (12), AML (8), NBL (18), Wilms (5), others (9); Non-TBI (14); TBI (38)	CT + (CRI) + HDC + SCT (14); CT + (CRI) + HDC + TBI + SCT (38)	1.0-20.6 (mean 7.2) yrs after SCT	Only SDSs of R/C ratios reported (others earlier). At patient level: 52/52 pats (100%) had R/C ratios deviating more than ±2 SDSs; TBI impaired, age less important. At tooth level, the worst mean R/C ratios (-4.4 SDS) at the SCT age of 3.1-5.0 yrs; TBI impaired at all ages. Non-TBI: 55% of R/C ratios outside ±2 SDSs; TBI: 85% of R/C ratios outside ±2 SDSs			PRG, clinical examination; R/C ratios and their SDSs (based on values in healthy people) for permanent teeth assessed (no third molars), effects of TBI and age at SCT studied; pats at the greatest risk for root aberrations 3.1-5.0 yrs at SCT

Abbreviations: AL, acute leukemia (ALL or ANLL = AML); ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplantation; BW, bite-wing radiograph; CI, confidence interval; CML, chronic myeloid leukemia; CRI, cranial/craniospinal irradiation = central nervous system (CNS) radiation; C/R, crown/root; CT, chemotherapy; DDD, developmental dental defect; dg, diagnosis; Gy, Gray; HDC, high-dose chemotherapy; Hem. malign., hematological malignancies; HL, Hodgkin's disease; IT MTX, intrathecal methotrexate; M1, permanent first molar; NBL, neuroblastoma; NHL, non-Hodgkin lymphoma; P1, first premolar; P2, second premolar; pat(s), patient(s); PRG, panoramic radiograph; R/C, root/crown; RMS, rhabdomyosarcoma; RT, radiotherapy; SCID, severe combined immunodeficiency; SCT, stem cell transplantation; SDSs, standard deviation scores; ST, solid tumors; TBI, total body irradiation; yrs, years

AIMS OF THE STUDY

The purpose of the present study was to investigate the development of permanent teeth in a group of pediatric stem cell transplant (SCT) recipients. Another goal was to develop a simple and objective method to express numerically the quantity of dental disturbances.

Specifically, the aims were to examine the effects of TBI and age at SCT on

- tooth agenesis
- microdontia
- dental root development

and

- to develop an objective method to study root development in SCT patients, and to compare results to corresponding values in healthy people
- to develop and test a method by which the sum of developmental disturbances of individual patients' permanent teeth can be expressed with one index figure

SUBJECTS AND METHODS

6. Study population

6.1. Stem cell transplant recipients (I, III, IV)

Eligible patients were all those of Finnish decent who had undergone SCT under the age of 10 years at the Hospital for Children and Adolescents, University of Helsinki, between 1980 and 1999. The minimum follow-up period after SCT was one year. Of the 85 eligible patients, 13 did not wish to take part in the study (mean age at SCT 6.0, range 1.5 to 9.7 years), and 8 patients were not reachable (mean age at SCT 3.7, range 1.1-7.9 years). Furthermore, 8 patients originally willing to take part in the study were excluded due to their death (N = 2) or terminal care (N = 1), other severe health problems (N = 1), cooperation problems and/or to very young age (N = 4). Thus, 56 patients (mean age at SCT 4.3, range 1.0-9.4 years), 66% of the original eligible survivors, were included in the final study group. The Medical Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki, approved the study protocol. Table 4 lists study patients with key characteristics. Further details on the mean ages (ranges), numbers of patients and teeth examined, and patient groupings appear in Tables 5 and 6.

6.1.1. Treatment of patients before stem cell transplantation

Conventional multiagent chemotherapy was started for those children diagnosed with cancer. Patients with ALL, AML, and NHL were treated according to Nordic protocols (Gustafsson et al., 2000; Lie et al., 2003), including prednisolone, vincristine, doxorubicin, methotrexate, L-asparaginase, cyclophosphamide, cytosine arabinoside, and 6-mercaptopurine. Anticancer chemotherapy for the NBL patients consisted of vincristine, cyclophosphamide, dacarbazine, cisplatin, and doxorubicin, in individual cases modified with ifosfamide and etoposide (Saarinen et al., 1996). Wilms' tumor patients received vincristine, actinomycin D, cyclophosphamide, and doxorubicin, which were also used for the one RMS patient, with ifosfamide and etoposide added. The two patients with CML were treated with hydroxyurea, while the patients with myelodysplastic syndrome (MDS) and severe aplastic anemia (SAA) received no conventional chemotherapy before the SCT procedure. The patient with yolk sac tumor was treated with chemotherapy courses consisting of bleomycin, etoposide, and cisplatin.

Radiotherapy, targeted to the tumor bed or local metastases, was administered to 17 patients. Six patients received RT to the skull area: CRI (12 to 24 Gy) to three leukemia patients and local irradiation to three NBL patients for metastases situated in the left frontal bone (20 Gy), right orbital area (20 Gy), and left temporal bone (6 Gy). The effect of minor scattered irradiation on developing teeth was considered insignificant, especially when 5 of the 6 patients later also received TBI. In the remaining 11 patients, the RT targets were far from the developing teeth.

6.1.2. Conditioning for stem cell transplantation

In all patients, the preparative regimen for SCT included HDC. Chemotherapeutic agents with dosages are listed in Table 4. In addition, 39 of 56 patients received TBI of 10 to 14 Gy, overlapping with developing teeth. TBI of 10 Gy was delivered to one patient in a single fraction; all the others had TBI in 2 Gy fractions during 3 days. Two patients with TBI excluding the head, and one patient with total nodal irradiation (see Table 8, pages 69-70) were considered “non-TBI” patients as regards tooth development.

6.2. Healthy adolescents and young adults (I, II)

The healthy subjects in Studies I and II participated in a longitudinal investigation initiated at the Department of Pedodontics and Orthodontics in 1967 with the permission of the National Board of Health. The main aim of that study was to gather information on tooth development and craniofacial growth in Finnish children (Nyström, 1982). During the years 1967 to 1969 maternity nurses at five Mother and Child Welfare Centers in Helsinki informed women carrying their first children about the study. These mothers volunteered to bring 382 children to their first dental examination at 6 months, and the number of children increased to 435 when mothers later brought 53 siblings of the first-born babies. These children, born between 1967 and 1973, were all of Finnish descent. They were examined clinically also at 9, 12, and 18 months, then semiannually until 1980, and annually thereafter. At the age of 16 years, 187 adolescents were still participating in the study (Nyström et al., 2001). Three to four PRGs per patient were taken during the course of the study. The study subjects were healthy, with no history of severe childhood diseases or treatments known to affect tooth development. Among these subjects, 18 PRGs were randomly picked to test a scale called the “Defect Index” (DeI), created for the group of SCT recipients (I). For the study of root-crown (R/C) ratios of permanent teeth in a normal population (II), study records and PRGs of all 187 subjects were examined. After excluding those with the history of orthodontic treatment (N = 36), the PRGs with reference points for tooth measurements not clearly visible in several teeth (N = 27), and PRGs technically unacceptable (N = 16), the study material comprised PRGs of 108 healthy Finnish adolescents. Age and grouping information is in Tables 5 and 6.

Table 4. Key characteristics of the stem cell transplantation (SCT) patients, who participated in the studies. Follow-up from SCT to most recent panoramic radiograph.

Patient	Gender	Diagnosis	Age at SCT, yrs	Follow-up, yrs	TBI	HDC	Study
1.	F(emale)	NBL	1.0	13.6	No	Mel	I; III; IV
2.	M(ale)	ALL	1.1	13.2	Yes	Ara-C	III; IV
3.	F	NBL	1.2	2.0	No	Mel	(I)
4.	M	NBL	1.4	9.0	Yes	VMP	I; III; IV
5.	F	NBL	1.4	6.0	No	Mel	I; III; IV
6.	F	NBL	1.5	14.3	No	Mel	I; III; IV
7.	M	NBL	1.9	3.8	No	ECT	I; III
8.	F	NBL	2.0	9.1	Yes	VMP	I; III; IV
9.	F	NBL	2.0	9.3	Yes	VMP	I; III; IV
10.	F	NBL	2.2	10.1	Yes	VMP	I; III; IV
11.	M	AML	2.2	7.0	Yes	Cy	III; IV
12.	F	MDS	2.2	5.9	Yes	Cy	III; IV
13.	M	NBL	2.3	9.6	Yes	VMP	I; III; IV
14.	F	NBL	2.4	2.2	Yes	VMP	III; IV
15.	M	AML	2.5	8.8	Yes	Ara-C	III; IV
16.	F	Yolk sac tumor	2.5	3.1	No	ECT	III;
17.	M	NBL	2.7	14.7	No	Mel	I; III; IV
18.	M	AML	3.1	4.4	Yes	Ara-C	III;
19.	M	NBL	3.1	13.7	Yes	VMP	I; III; IV
20.	F	NBL	3.1	5.7	Yes	VMP	I; III; IV
21.	M	NBL	3.2	10.7	Yes	VMP	I; III; IV
22.	F	NBL	3.2	7.5	No	VMP	I; III; IV
23.	F	ALL	3.5	6.0	Yes	Mel	III; IV
24.	M	ALL	3.6	4.3	Yes	Ara-C	III; IV
25.	M	SAA	3.9	12.3	Yes	Cy	III; IV
26.	F	NBL	3.9	10.4	Yes	VMP	I; III; IV
27.	M	NBL	4.1	13.1	Yes	VMP	I; III; IV
28.	F	AML	4.2	14.1	Yes	Cy	III; IV
29.	M	Wilms' tumor	4.2	4.7	No	Mel	III; IV
30.	F	RMS	4.7	7.2	No	ECT	III; IV
31.	F	Wilms' tumor	5.0	6.2	No	Mel	III; IV
32.	F	ALL	5.0	9.0	Yes	Ara-C	III; IV
33.	F	Wilms' tumor	5.1	4.1	No	Mel	III; IV
34.	F	ALL	5.2	3.8	Yes	Ara-C	III; IV
35.	F	AML	5.2	20.6	Yes	Cy	III; IV
36.	F	AML	5.2	7.9	Yes	Cy	III; IV
37.	M	CML	5.2	7.3	Yes	Cy	III; IV
38.	M	ALL	5.4	3.4	Yes	Ara-C	III; IV
39.	M	ALL	5.4	4.5	Yes	Ara-C	III; IV
40.	M	ALL	5.4	5.5	Yes	Ara-C	III; IV
41.	F	CML	5.5	1.8	Yes	Ara-C	III; IV
42.	F	ALL	5.6	8.5	Yes	Cy	III; IV
43.	M	NHL	5.7	2.1	Yes	Cy	III; IV
44.	M	NBL	5.8	7.7	No	Mel	I; III; IV
45.	M	ALL	5.8	4.0	Yes	Mel	III; IV
46.	M	ALL	5.9	2.7	Yes	Mel	III; IV
47.	M	Wilms' tumor	6.1	8.0	No	Mel	III; IV
48.	M	SAA	6.2	7.2	No	Cy	III; IV
49.	M	ALL	6.3	1.5	Yes	Cy	III; IV
50.	M	Wilms' tumor	6.4	8.3	No	Mel	III; IV
51.	F	NHL	6.4	5.0	Yes	Ara-C	III; IV
52.	F	AML	6.8	3.0	Yes	Mel	III; IV
53.	M	NBL	7.9	1.0	No	VMP	III; IV
54.	F	AML	8.7	4.3	Yes	Ara-C	III; IV
55.	F	AML	9.0	10.7	Yes	Cy	III; IV
56.	M	MDS	9.4	3.1	Yes	Cy	III; IV

Abbreviations: TBI: total body irradiation; HDC: high-dose chemotherapy; NBL: neuroblastoma; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; NHL: non-Hodgkin lymphoma; SAA: severe aplastic anemia; RMS: rhabdomyosarcoma; Ara-C: cytosine arabinoside 3 g/m² x 12; Cy: cyclophosphamide 120 to 200 mg/kg; Mel: melphalan 140-180 mg/m²; VMP: etoposide 300 mg/m², melphalan 140 + 70 mg/m², cisplatin 90 mg/m²; ECT: etoposide 250 mg/m² x 3, carboplatin 500 mg/m² x 3, thiotepa 300 mg/m² x 3

Table 5. Mean ages of patients at stem cell transplantation (SCT) with follow-up periods after SCT, and mean ages of study subjects at panoramic radiography (PRG).

	Study I	Study II	Study III	Study IV
Mean age (years) of SCT patients at SCT (range)	2.6 (1.0-5.8)		4.3 (1.0-9.4)	4.4 (1.0-9.4)
PRG (range)	10.5 (3.2-17.3)		11.7 (4.7-25.7)	11.7 (4.7-25.7)
Mean follow-up (years) after SCT (range)	7.9 (1.9-14.6)		7.4 (1.0-20.6)	7.2 (1.0-20.6)
Mean age (years) of healthy subjects at PRG (range)	18.7 (14.5-24.3)	18.3 (12.6-25.4)		

7. Methods

7.1. Radiographic and clinical examinations

Radiographic and clinical examinations of the 53 SCT recipients were performed at the Institute of Dentistry, University of Helsinki. The PRGs of these patients were taken by two experienced radiographers using either the Orthopantomograph® OP 100 (GE Healthcare, Tuusula, Finland) or the PM 2002 CC (Planmeca Co., Helsinki, Finland), with respective magnification factors of 1.3 and 1.2. Lateral cephalograms were also taken. The PRGs (and other dental information available) for three patients came from their local health centers. The author of this thesis performed all other clinical examinations.

Clinical examination of the SCT patients included recording of caries (also initial), hypomineralization lesions, fillings, extractions, eruption of teeth, occlusion, periodontal tissues (not probing depth), and oral mucosa. Alginate impressions for plaster casts were taken whenever possible, i.e., when children agreed to undergo the procedure. Patients or their guardians were asked about the child's dental care (professional and home care), symptoms occurring in teeth and other oral tissues (pain, dryness), as well as their opinion of the child's ability to chew all kinds of food. They were asked if they knew any relatives with missing teeth or other dental disturbances. Since the results of the clinical examination are not analyzed in this thesis, they are not described in detail.

The study protocol was repeated in 2 to 3 years for the SCT patients with developing dentitions. If dentition was fully developed at the time of the first examination (except for third molars), no further visits were planned. Many patients (or guardians) or the children's local dentists contacted the author and hoped for "extra" visits when some specific dental problems occurred. Several patients were thus clinically examined 3 to 5 times during the course of the study, but other parts of the study protocol were carried out only when necessary for treatment or treatment planning. If "extra" PRGs were taken, they were utilized in the study.

PRGs of the healthy subjects were also taken at the Institute of Dentistry, University of Helsinki (Nyström et al., 2001), with either an Orthopantomograph 5 (Palomex Co, Tuusula, Finland) or Cranex DC (Sorodex Co, Helsinki, Finland), each with a magnification factor of 1.3. The same two radiographers took the PRGs of healthy subjects and SCT patients. The author, seeking, in particular, information on childhood diseases, dental trauma, tooth extractions, and orthodontic treatment, reviewed all files concerning clinical examinations of the healthy subjects.

7.2. Assessment of permanent tooth agenesis from panoramic radiographs (I, III)

Since the developmental schedules of different teeth vary, specific age limits were set for the recording of tooth agenesis (Section 1.4., Figure 3). Permanent incisors, canines, and first molars were always considered eligible for the tooth agenesis assessment. First premolars were not judged as lacking before the age of 5 years. The age limit for the second premolars and permanent second molars was 6 years. In Study I, third molars (wisdom teeth) were considered missing only at the age of 13 or later. Some characteristics of the poor-risk NBL patients, examined for tooth agenesis in Study I, are presented in Tables 5 and 6.

Age limits for assessment of tooth agenesis in Study III, and in the thesis Results were the same as in Study I (see above) except for the third molars, for which the limit was reset to 12 years. The effects of HDC, TBI, and age at SCT on tooth agenesis were studied in two separate assessments, excluding or including the third molars, with study groups formed accordingly (Table 6; more details in Tables 4 and 5). Many of the NBL patients included in both Studies I and III had longer follow-up times in Study III, which was carried out only when most patients had already attended their second examination.

7.3. Assessment of microdontia from panoramic radiographs (I, III)

Microdontia was assessed by subjective visual judgment. Only the remarkably small teeth, in which crown size (mesio-distal dimension seen on PRGs) was estimated to be half or less than “normal,” were considered microdontic. For characteristics of study groups see Tables 5 and 6.

Table 6. Study topics and grouping of study subjects in Studies I-IV and in Results of the thesis, with numbers of patients examined in each (sub)group. Numbers of root-crown (R/C) ratios investigated also given.

Study no.; study topic	N, patients, perspective(s) of the study	Patient grouping (N)	No. of R/C ratios studied
Study I			
Tooth agenesis	18 NBL patients (3 excluded), 18 healthy test subjects, third molars included	TBI group (10) Non-TBI group (5) Healthy test group (18)	
Microdontia	18 NBL patients (1 excluded), 18 healthy test subjects, third molars included	TBI group (10) Non-TBI group (7) Healthy test group (18)	
R/C ratio	18 NBL patients (3 excluded), 18 healthy test subjects, third molars included	TBI group (10) Non-TBI group (5) Healthy test group (18)	238 126 464
Defect Index (Del)	18 NBL patients (1 excluded), 18 healthy test subjects, third molars included	TBI group (10) Non-TBI group (7) Healthy test group (18)	
Study II			
R/C ratio	108 healthy subjects, third molars excluded	Males (53) Females (55)	1382 1397
Study III			
Tooth agenesis	#1) 52 SCT recipients, third molars excluded #2) 29 (updated N = 32 in the thesis) SCT recipients \geq 12 yrs, third molars included	TBI group (#1 = 38; #2 = 18) Non-TBI group (#1 = 14; #2 = 11) Group Y: \leq 3.0 yrs at SCT (#1 = 13; #2 = 6) Group M: 3.1-5.0 yrs at SCT (#1 = 15; #2 = 9) Group O: \geq 5.1 yrs at SCT (#1 = 24; #2 = 14)	
Microdontia	55 SCT recipients, third molars excluded	TBI group (39)/non-TBI group (16) Group Y: \leq 3.0 yrs at SCT (16) Group M: 3.1-5.0 yrs at SCT (15) Group O: \geq 5.1 yrs at SCT (24)	
Study IV			
SDS of the R/C ratio	52 SCT recipients, third molars and microdontic teeth excluded	Total (52) TBI group (38) Non-TBI group (14) Group Y: \leq 3.0 yrs at SCT (14) TBI/non-TBI (10/4) Group M: 3.1-5.0 yrs at SCT (14) TBI/non-TBI (10/4) Group O: \geq 5.1 yrs at SCT (24) TBI/non-TBI (18/6)	945 674 271 226 154/72 262 185/77 457 355/122
	39 SCT recipients with advanced tooth development (= more than 50% of the teeth fully developed), third molars and microdontic teeth excluded	TBI group (29) Non-TBI group (10) Group Y: \leq 3.0 yrs at SCT (11) TBI/non-TBI (8/3) Group M: 3.1-5.0 yrs at SCT (12) TBI/non-TBI (9/3) Group O: \geq 5.1 yrs at SCT (16) TBI/non-TBI (12/4)	605 237 215 145/70 242 175/67 385 285/100
Thesis			
Individual Defect Index (IDel)	55 SCT recipients; third molars excluded	Total (55) TBI group (39)/non-TBI group (16)	
	36 SCT recipients with advanced tooth development (= at minimum 20 teeth of 28 included in the index), third molars excluded	TBI group (27)/non-TBI group (9) Group Y: \leq 3.0 yrs at SCT (11) TBI/non-TBI (8/3) Group M: 3.1-5.0 yrs at SCT (10) TBI/non-TBI (8/2) Group O: \geq 5.1 yrs at SCT (15) TBI/non-TBI (11/4)	

7.4. Assessment of root-crown ratios from panoramic radiographs (I, II, IV)

A previously described method, originally used to study the root-crown (R/C) ratios (also called “relative root length”) of upper maxillary incisors (Lind, 1972), was adapted for the assessment of R/C ratios in all permanent teeth. The heights of the crowns and the lengths of the roots were measured according to the same measurement principles in Studies I, II, and IV (Figure 6). The R/C ratios of individual teeth were calculated by dividing root length by crown height. A tooth was excluded from R/C ratio assessment if the apex was not closed, reference points used in the measurements were not reliably visible, roots were unusually curved, there existed a history of dental trauma, or attrition or abrasion of the crown was detectable.

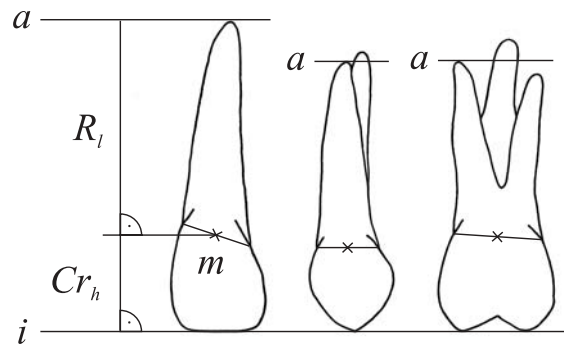


Figure 6. Method for measuring crown height and root length. In teeth with two buccal roots, the longer one was measured. Palatal roots were omitted.

m: visually determined midpoint of a straight line connecting the points of intersection between the contours of the root and crown;

Crown height (Cr_h): perpendicular line from point m to the incisal/occlusal reference line (i). In canines and most premolars, line i was set by placing one of the parallel lines of the measuring grid visually perpendicular to the long axis of the tooth and tangential to an incisal tip or to a buccal cusp. In incisors and molars, line i was set by placing the line of the grid so as to follow the incisal edge or to connect the buccal cusps, respectively.

Root length (R_l): distance from point m perpendicular to the apical tangent a , which was parallel to the incisal/occlusal reference line i .

A transparent plastic measuring grid (PM 2002 CC, RadioDent Co, Helsinki, Finland), with parallel lines at intervals of one and two millimeters (Figure 7), was used in the measurements, which were rounded to the nearest half or whole millimeter. Instead of the absolute linear measurements (millimeters) of root lengths and crown heights, only ratios were used in the studies to eliminate the effect of the varying magnification factors between different machines and regions of the same radiograph (Welander et al., 1989; Thanyakarn et al., 1992a).

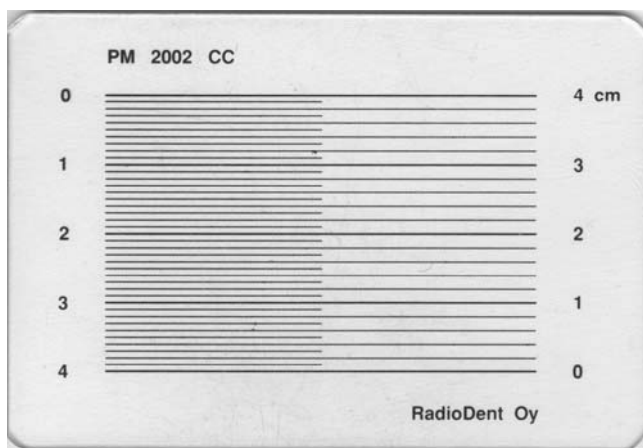


Figure 7. Plastic measuring grid.

7.4.1. Categories of root-crown ratios in Study I

The categories of R/C ratios in Study I were based on the “normal” R/C value of 1.6 for maxillary central incisors (Lind, 1972) and on the author’s clinical judgment as to the extent of injury. The first category, D0, included undisturbed teeth with R/C ratios > 1.6 ; in D1-category (mild disturbance) the R/C ratios were 1.2 to 1.6, in D2 (severe disturbance) 0.9 to 1.1, and in D3 (very severe disturbance) < 0.9 . The same limits were used for all permanent teeth. Category number expressed the number of defect points (1, 2, or 3), reflecting disturbances especially in root development.

7.4.2. Determination and reproducibility of root-crown ratios in Study II

After completion of Study I, it seemed probable that “normal” R/C ratios vary among teeth. To clarify the “normal” R/C ratios for individual teeth, these were measured in a group of healthy adolescents, separately in males and females (II) (Table 6). Furthermore, the measurement method was described in detail (Figure 6), and its reproducibility was tested both for intra-examiner (A1 and A2) and for inter-examiner (A1 and B) measurements. The error of the method (e_m) was calculated by the formula

$$e_m = \sqrt{\sum \frac{d^2}{2n}},$$

where d is the difference between double assessments (A1-A2 or A1-B), and n is the number of double determinations. Error variances (e_m^2) were calculated for all teeth and compared to the biological variance of the corresponding tooth which came from the standard deviations (SD) of the repeated measurements by the formula

$$\text{biological variance} = \frac{SD_{A1}^2 + SD_{A2}^2 + SD_B^2}{3}.$$

7.4.3. Treatment of root-crown ratios in Study IV

R/C ratios of fully developed permanent teeth were calculated in the group of SCT recipients, and—based on gender-specific R/C ratios in a healthy population (II)—standard deviation scores (SDSs) were calculated for all R/C ratios of the SCT patients. SDS, also called a z-score, indicates how much and in what direction the patient's value deviates from the mean value of the normal population, expressed in units of the population's standard deviation. SDSs were calculated according to the formula

$$\text{SDS} = \frac{\text{RC}_{\text{pat}} - \text{RC}_{\text{mean}}}{\text{SD}},$$

where RC_{pat} is the R/C ratio of a patient's tooth, and RC_{mean} and SD are the tooth- and gender-specific mean R/C ratio and standard deviation of the corresponding tooth in a healthy population. The effects of TBI and age at SCT on SDSs were studied in several subgroups (Table 6).

7.5. Defect indices (I, Results of the thesis)

Defect Index (DeI) combines tooth agenesis (D5; gives 5 defect points), microdontia (D4; 4 defect points), and abnormalities in the R/C ratio (D1, D2, D3; 1, 2, and 3 defect points, respectively; see Section 7.4.1.) in a single figure expressing severity of damage in a dentition. The following formula was used in the calculation:

$$\text{DeI} = (N_{\text{D1}} \times 1) + (N_{\text{D2}} \times 2) + (N_{\text{D3}} \times 3) + (N_{\text{D4}} \times 4) + (N_{\text{D5}} \times 5),$$

where N is the number of teeth in the respective disturbance category D1, D2, D3, D4, or D5 (consequently, the higher the final figure, the more severe the dental damage). The theoretical maximum of the DeI was 160 in Study I with third molars included.

Individual Defect Index (IDeI), a more precise second version of the DeI, based on the gender-specific R/C ratios in a healthy population (Section 7.4.3.), was developed later. After the calculation of SDSs of the R/C ratios, new limits were set for the determination of defect points according to SD score. Defect points for the IDeI were as follows:

- Tooth agenesis; defect points: 5
- Microdontia; defect points: 4
- SDS of R/C ratio inside ± 2 ; defect points: 0
- SDS of R/C ratio between -2.1 and -3.0 or 2.1 and 3.0 ; defect points: 1
- SDS of R/C ratio between -3.1 and -4.0 or 3.1 and 4.0 ; defect points: 2
- SDS of R/C ratio between -4.1 and -6.0 or 4.1 and 6.0 ; defect points: 3
- SDS of R/C ratio between -6.1 or less or 6.1 or more; defect points: 4

The sum of the defect points described the entire damage to the dentition. The theoretical maximum (all teeth missing, including third molars) of the IDeI is 160. The main findings concerning the use of IDeI are presented in Results of the thesis. Patient-groupings are in Table 6.

7.6. Statistical analyses (I-IV)

The Statistical Package for the Social Sciences (SPSS for Windows), versions 9.0, 10.0, and 11.0 (SPSS, Inc., Chicago, Illinois, USA) were used in statistical analyses. A statistical method was selected as appropriate depending on study design, type of test variable (continuous, categorical), and distribution of this variable (normal, non-normal). A non-parametric Mann-Whitney test was used on several occasions, since the study groups were small and did not follow a normal distribution. For example, the number of developmental defects and DeI (continuous variables) between TBI and non-TBI groups (I) and the number of missing and/or microdontic teeth between the TBI/non-TBI and age groups (III) were compared with the Mann-Whitney U-test. It was utilized also in comparing differences in SDSs and other continuous variables between study groups (IV). The statistical significance of the categorical variables between groups (e.g., presence or absence of tooth agenesis and microdontia; number of teeth with R/C ratios outside ± 2 or ± 3 SDS limits) was studied with the Pearson's chi-square (χ^2) test or the Fisher exact test (III, IV). The Pearson correlation coefficient and linear regression analysis were used to study associations of TBI and age at SCT with number of missing and microdontic teeth (III). Pearson correlation coefficients were also calculated for repeated intra- and inter-examiner R/C ratio assessments, and their paired differences were tested with the paired t-test (II). The independent sample t-test was applied when the R/C ratios were compared between males and females (II). P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

8. Agenesis of permanent teeth

Tooth agenesis was assessed in 52 SCT patients (Study III), including also the NBL patients of Study I. During follow-up, new PRGs were obtained and the most recent ones were used when study results were analyzed. In this Results section, the information is again in part updated from the newest PRGs, and with small differences appearing in absolute numbers of teeth when compared to results in the original publications. New information is also presented.

8.1. Tooth agenesis in stem cell transplant recipients (I, III, and updated)

8.1.1. Prevalence of tooth agenesis in permanent teeth

Third molars excluded

In the SCT patient group (N = 52; III), the prevalence of tooth agenesis was 31% (16 of 52 patients). TBI had no effect on the agenesis prevalence that was 32% and 29% in the TBI and non-TBI groups, respectively (Table 7). In the group of 17 NBL patients, prevalence of tooth agenesis was 76%, with a significant difference between TBI and non-TBI patients ($p = 0.015$) (Table 7).

The effect of age on prevalence of tooth agenesis was investigated by dividing the patients into three age groups: Y (Youngest; ≤ 3.0 years), M (Middle; 3.1-5.0 years), and O (Oldest; ≥ 5.1 years), according to age at SCT. Teeth were lacking in 10 of 13 (77%) patients in Group Y, with no difference between TBI- and non-TBI groups. It is of note that 3 of 4 patients lacked teeth following anticancer CT alone (Table 7). The possibility that anticancer chemotherapy (CCT and HDC) caused tooth agenesis was especially high, if children were less than 2 years old at the beginning of treatment (Table 8). Group M was less affected, with a 40% prevalence of tooth agenesis. In Group O, no teeth were missing (Table 7).

Third molars included

The final results for tooth agenesis can be seen only after the third molars are included in the assessment. In a subgroup of the SCT recipients who were ≥ 12 years at PRG (agenesis of the third molars not confirmed before age 12), or who were younger but with the third molars present, an agenesis assessment including all teeth was performed (in Study III, N = 29; updated N = 32). The prevalence of tooth agenesis was high, 66%, with a tendency toward higher prevalence among TBI patients (75%) than among non-TBI patients (50%). The difference was not significant, however (Table 7). At least one of the third molars was missing in 17 of 32 (53%) patients.

Most of the patients (77%) treated for NBL presented with tooth agenesis (Table 7). Teeth were missing from all (100%) TBI patients but from only 3 of 7 (43%) non-TBI patients ($p = 0.07$) (Table 7). The mean age of the NBL patients was low, 2.4 years at diagnosis and 3.0 years at SCT (medians 2.2 and 2.7 years), which mostly explains this high prevalence of tooth agenesis.

Prevalence of tooth agenesis examined for age groups Y, M, and O was 88%, 78%, and 47%. All patients in the youngest TBI group and more than half the children in the oldest TBI group were affected (Table 7). The earlier observations that both young age at SCT (or diagnosis) and preparative regimen including TBI elevated the risk for tooth agenesis were confirmed.

8.1.2. Number of missing teeth

Third molars excluded

In the SCT patients (N = 52), the mean number of missing teeth was slightly higher in the ones with TBI than in the non-TBI patients (1.7 vs. 0.8), but this difference was non-significant (Table 7). The patients treated for NBL were more severely affected than were SCT recipients on average. The mean number of missing teeth in the NBL patients was 3.7 (range 0-11), and rose to 5.4 in the TBI group, which significantly differed from the non-TBI patients (Table 7).

Patient age at SCT had a negative correlation with number of missing teeth ($r = -0.580$; $p < 0.001$). Mean number of missing teeth was highest (3.9) in Group Y, whereas no teeth were missing in Group O. Both younger groups (Y and M) differed from Group O. The mean number of missing teeth did not differ significantly between the TBI and non-TBI groups, although a clear tendency toward higher numbers was evident among TBI patients (e.g., 4.7 vs. 2.0 in Group Y) (Table 7; Figure 8).

In the Finnish population, the mean number of missing teeth in patients with hypodontia has been 1.7 (Haavikko, 1971). Anticancer therapy made the number of missing teeth higher than that number found in genetic hypodontia; in the 16 SCT patients with tooth agenesis, mean numbers of missing teeth were 4.8 (SD 2.4) in the whole group, and 5.5 (SD 2.4) and 2.8 (SD 1.0) in the TBI (N = 12) and non-TBI (N = 4) groups, respectively ($p = 0.020$). The difference between the NBL patients in the TBI and non-TBI groups further affirmed the fact that risk for tooth agenesis was associated not only with age, but was also increased due to TBI. However, all the patients with tooth agenesis had been less than 5 years old at SCT (Table 8; Figure 8).

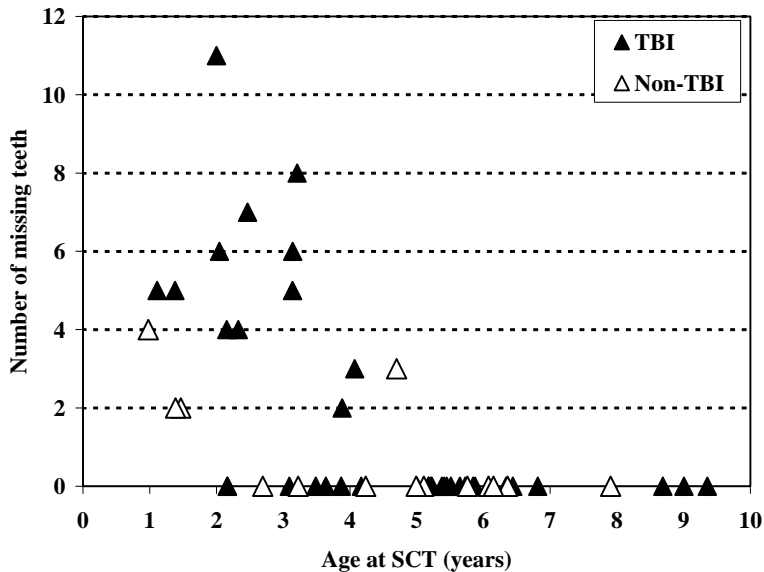


Figure 8. Numbers of missing teeth (third molars excluded) in individual patients (N = 52), according to age at stem cell transplantation (SCT). Patients were with or without total body irradiation (TBI and non-TBI patients). Note that all patients less than 2 years old at SCT were missing teeth.

Third molars included

In a subgroup of 32 SCT recipients, the detrimental effect of TBI was indicated by the higher mean number of missing teeth in the TBI than in the non-TBI patients (4.2 vs. 1.1; $p = 0.026$). The mean age of the non-TBI patients was slightly lower than that of the TBI patients, but median age tended to be higher in the non-TBI group. This demonstrates the higher number of older individuals, not so susceptible to tooth agenesis, among the non-TBI patients (Table 7). Thus, in addition to irradiation, this age distribution might have had some effect on results.

In the group of NBL patients (N = 13), the mean number of missing teeth was 5.3, with a significant difference between TBI (8.3) and non-TBI (1.3) patients (Table 7). The NBL patients, typically young at diagnosis, are at high risk for extensive agenesis of permanent teeth.

The mean number of missing teeth in Groups Y and M was equal (4.9) and differed significantly from that of Group O (Table 7). The highest mean number of missing teeth (7.8) was in the TBI children who were ≤ 3.0 years at SCT (Group Y) (Table 7). All missing teeth in Group O were third molars. Patient age at SCT showed a negative correlation with number of missing teeth ($r = -0.585$; $p < 0.001$). Age explained 58% and 32% of the variation in number of missing teeth in the TBI and non-TBI groups, respectively (Figure 9). Thus, when all teeth were included, age at SCT had a strong effect on number of missing teeth, especially in the TBI group.

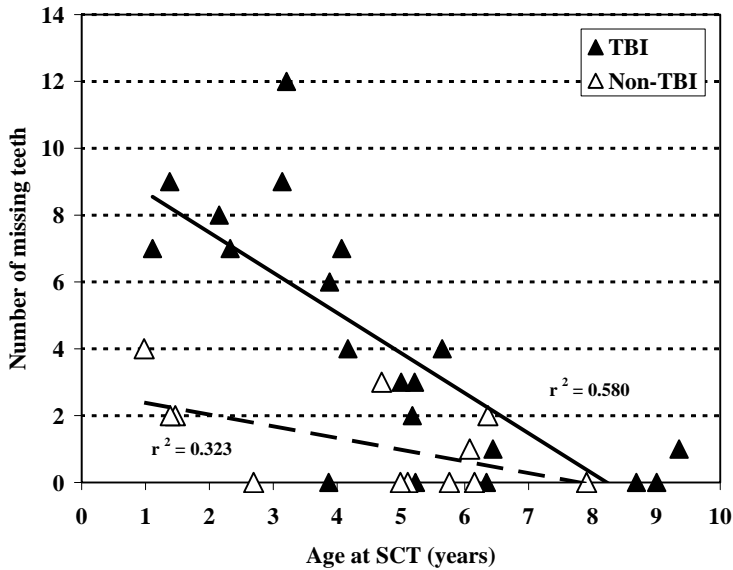


Figure 9. Number of missing teeth in relation to total body irradiation (TBI) and age at stem cell transplantation (SCT). Only those 32 patients ≥ 12 years old at PRG were included. Third molars were included.

At the most recent examination, 25 of all 52 patients presented with tooth agenesis (all teeth included according to age rules; see Section 7.2.). Mean number of missing teeth in these 25 patients was 5.1 (SD 3.2), with means of 6.0 (SD 3.2) in TBI (N = 19) and 2.3 (SD 1.0) in non-TBI patients (N = 6) ($p = 0.011$).

In Figure 10, PRGs show examples of tooth agenesis in SCT patients (cases 36, 13, 8, and 19).

Table 7. Agenesis of permanent teeth expressed as number of patients with agenesis and mean number of missing teeth in the study group and in selected subgroups. Mean ages of the patients at SCT also given.

Group (N; TBI/nonTBI)	Mean age at SCT (SD)			Patients with tooth agenesis (%)			Mean number of missing teeth (SD)					
	All	TBI	Non-TBI	Sig. TBI/ non-TBI	All	TBI	Non-TBI	Sig. TBI/ non-TBI	All	TBI	Non-TBI	Sig. TBI/ non-TBI
#1) All (52; 38/14)	4.5 (2.0)	4.5 (2.0)	4.4 (2.1)	NS	16 (31)	12 (32)	4 (29)	NS	1.5 (2.6)	1.7 (2.9)	0.8 (1.4)	NS
Median	4.8	4.6	4.8						0	0	0	
#2) All (32; 20/12)	4.7 (2.3)	4.8 (2.4)	4.5 (2.3)	NS	21 (66)	15 (75)	6 (50)	NS	3.0 (3.3)	4.2 (3.7)	1.2 (1.4)	0.026
Median	5.0	4.6	5.0						2.0	3.5	0.5	
#1) Group Y (13; 9/4)	1.9 (0.5)	2.0 (0.4)	1.6 (0.7)	NS	10 (77)	7 (78)	3 (75)	NS	3.9 (3.2)	4.7 (3.4)	2.0 (1.6)	NS
#1) Group M (15; 11/4)	3.9 (0.7)	3.7 (0.6)	4.3 (0.8)	NS	6 (40)	5 (45)	1 (25)	NS	1.8 (2.7)	2.2 (2.9)	0.8 (1.5)	NS
#1) Group O (24; 18/6)	6.2 (2.0)	6.2 (1.4)	6.2 (0.9)	NS	0 (0)	0	0	-	0	0	0	-
Sig. Y/M					0.055	NS	NS		0.08	NS	NS	
Sig. M/O					0.002	0.004	NS		0.038	0.002	NS	
Sig. Y/O					0.001	< 0.001	0.033		0.001	< 0.001	0.018	
#2) Group Y (8; 4/4)	1.7 (0.6)	1.7 (0.6)	1.6 (0.7)	NS	7 (88)	4 (100)	3 (75)	NS	4.9 (3.3)	7.8 (1.0)	2.0 (1.6)	0.029
#2) Group M (9; 7/2)	4.1 (0.7)	3.9 (0.6)	4.8 (0.2)	NS	7 (78)	6 (86)	1 (50)	NS	4.9 (4.0)	5.9 (4.0)	1.5 (2.1)	NS
#2) Group O (15; 9/6)	6.6 (1.5)	6.8 (1.7)	6.2 (0.9)	NS	7 (47)	5 (56)	2 (33)	NS	0.9 (1.3)	1.2 (1.5)	0.5 (0.8)	NS
Sig. Y/M					NS	NS	NS		NS	NS	NS	
Sig. M/O					NS	NS	NS		0.01	0.016	NS	
Sig. Y/O					0.069	NS	NS		0.004	0.003	NS	
#1) NBL (17; 10/7)	3.0 (1.7)	2.7 (0.9)	3.3 (2.6)	NS	13 (76)	10 (100)	3 (43)	0.015	3.7 (3.1)	5.4 (2.6)	1.1 (1.6)	0.001
Median	2.7	2.7	2.7						4.0	5.0	0	
#2) NBL (13; 7/6)	3.1 (2.0)	2.9 (1.0)	3.4 (2.8)	NS	10 (77)	7 (100)	3 (50)	0.07	5.3 (4.1)	8.3 (2.0)	1.3 (1.6)	0.001
Median	2.7	3.1	2.1						6.0	8.0	1.0	

Abbreviations: SCT: stem cell transplantation; SD: standard deviation; N: number; TBI: total body irradiation; Sig.: significance; #1) Third molars excluded; #2) Third molars included, agenesis not confirmed before the age of 12; NBL: neuroblastoma; NS: non-significant; Group Y: patients ≤ 3.0 years at SCT; Group M: patients 3.1 to 5.0 years at SCT; Group O: patients ≥ 5.1 years at SCT

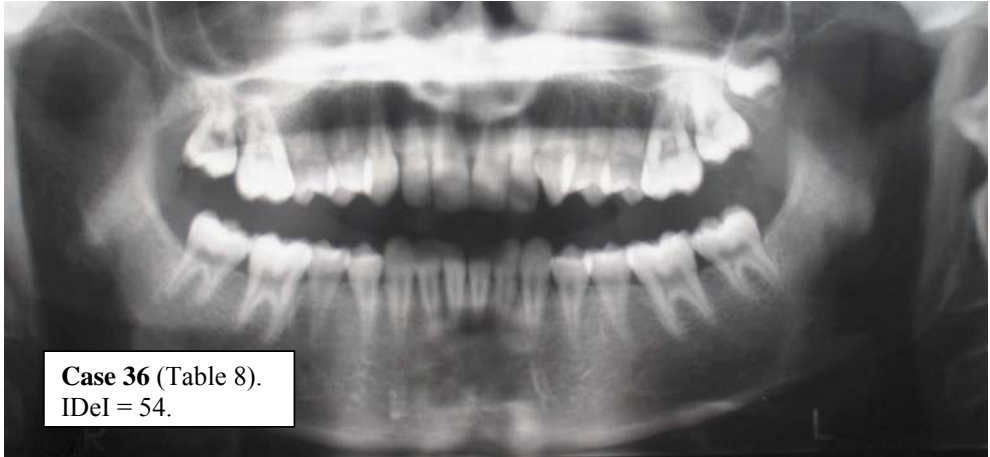
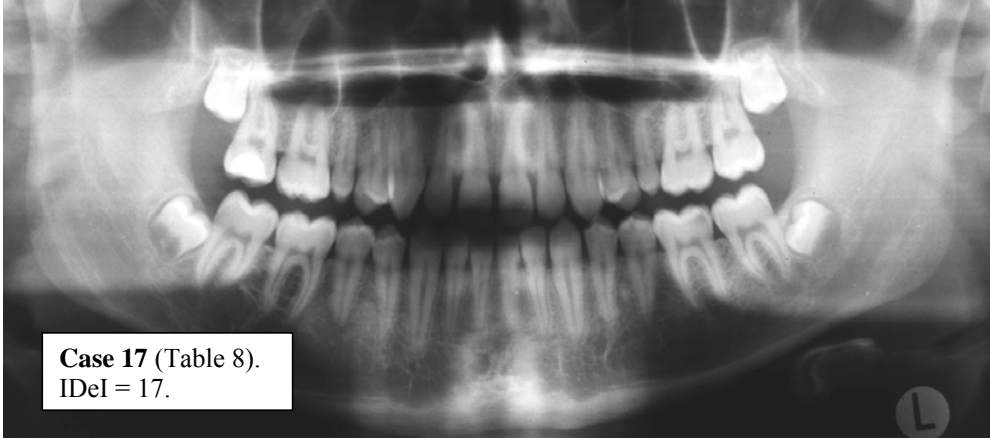
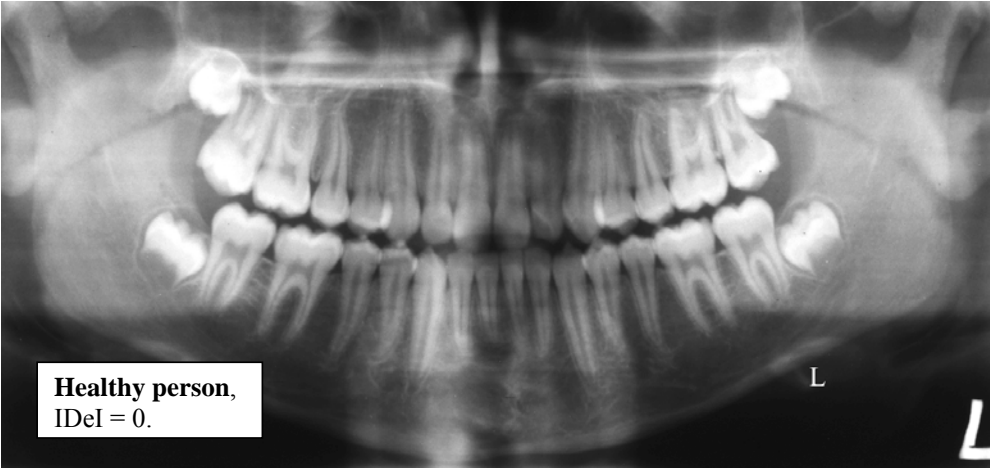
Table 8. Tooth agenesis and microdontia in individual SCT patients recorded according to age at SCT. Age at diagnosis is given, since it may explain dental findings. Some subjective observations also listed. Empty spaces in tooth agenesis and microdontia columns mean that patients were excluded from analysis due to young age. Third molars classified as missing only in patients ≥ 12 years. Age groups Y, M, and O were used in Study III on tooth agenesis and microdontia as shown here (data updated in thesis Results). IDel score is the sum of dental injuries (tooth agenesis, microdontia, and aberrant root development; Section 12).

Patient; Gender	Diagnosis	Age at dg.	Age at SCT	TBI/ Gy	N, missing teeth; third molars excluded (teeth affected) ☼	N, missing teeth; third molars included (third molars affected) ☼	N, microdontic teeth; third molars excluded (teeth affected) ☼	Findings based on subjective clinical and/or radiographic observations ☼	IDel score
Group Y: age ≤ 3.0 years at SCT									
1. F	NBL	0.2	1.0	0	4 (15, 25, 35, 45)	4	2 (12, 22)	Taurodontism	32
2. M	ALL #	0.5	1.1	12	5 (15, 22, 25, 35, 45)	7 (38, 48)	0	12: small; 37, 47: single-rooted teeth	62
3. F	Patient excluded from studies due to young age. She is still listed to maintain the original numbering system.								
4. M	NBL	0.9	1.4	10	5 (17, 15, 25, 35, 45)	9 (18, 28, 38, 48)	4 (14, 24, 34, 44)	12, 22: small	71
5. F	NBL	0.4	1.4	0	2 (14, 24)	2	2 (34, 44)	16, 26: ectopic eruption	24*
6. F	NBL	0.9	1.5	0	2 (14, 24)	2	2 (34, 44)	24, 35, 45: small	34
7. M	NBL	1.4	1.9	0			3 (14, 34, 44)		12*
8. F	NBL	1.4	2.0	10	11 (17, 15, 14, 24, 25, 37, 35, 34, 44, 45, 47)		0	12, 22: small; at 11.1 yrs third molars not seen	91
9. F	NBL	1.6	2.0	10	6 (15, 25, 37, 35, 45, 47)	8 (18, 28, 38, 48)	4 (14, 24, 34, 44)	At 11.4 yrs third molars not seen	87
10. F	NBL	1.6	2.2	10	4 (15, 25, 35, 45)		0	12, 22: small; eruption problems	72
11. M	AML	1.8	2.2	12	0		0	Dilatations ("niches") in some pulp cavities	40
12. F	MDS	1.6	2.2	10	0		3 (15, 25, 45)	35: small	38*
13. M	NBL	2.0	2.3	10	4 (15, 27, 35, 45)	7 (28, 38, 48)	4 (14, 24, 34, 44)	Taurodontism, thin roots	76
14. F	NBL ^a	2.0	2.4	10			4 (14, 24, 34, 44)		20*
15. M	AML # s	1.4	2.5	12	7 (17, 25, 27, 37, 35, 45, 47)		1 (15)	At 11.2 yrs third molars not seen; 46: infra-position	103
16. F	Yolk sac	2.1	2.5	0			2 (17, 15)	At 5.6 yrs 25, 35, 45 not seen	8*
17. M	NBL	2.2	2.7	0	0	0	2 (15, 25)		17
Group M: age 3.1-5.0 years at SCT									
18. M	AML #	1.6	3.1	12	0		12 (17, 15, 14, 24, 25, 27, 37, 35, 34, 44, 45, 47)	Severe problems in tooth eruption	52*
19. M	NBL	2.5	3.1	12	5 (17, 15, 25, 35, 45)	9 (18, 28, 38, 48)	3 (27, 37, 47)	Very severely affected dentition in general	117
20. F	NBL	2.8	3.1	10	6 (15, 25, 37, 35, 45, 47)		1 (34)	14, 24, 44: small	56*
21. M	NBL	2.7	3.2	12	8 (17, 15, 25, 27, 37, 35, 45, 47)	12 (18, 28, 38, 48)	4 (14, 24, 34, 44)	Very severely affected dentition in general	112
22. F	NBL	2.8	3.2	0 •	0		0	17, 27, 37: small and single-rooted	43
23. F	ALL #	1.4	3.5	14	0		12 (17, 15, 14, 24, 25, 27, 37, 35, 34, 44, 45, 47)	Crowding	92
24. M	ALL	2.0	3.6	10	0		2 (27, 44)	17: small; 17, 27, 37: single-rooted; 13, 23: ectopic; at 11.4 yrs 28, 48 developing	27*

Patient; Gender	Diagnosis	Age at dg.	Age at SCT	TBI/ Gy	N, missing teeth; third molars excluded (teeth affected) ☼	N, missing teeth; third molars included (third molars affected) ☼	N, microdontic teeth; third molars excluded (teeth affected) ☼	Findings based on subjective clinical and/or radiographic observations ☼	IDel score
25. M	SAA	3.7	3.9	10.5	0	0	0	23: ectopic eruption, resorbed lateral incisor	59
26. F	NBL	3.4	3.9	10	2 (35, 45)	6 (18, 28, 38, 48)	2 (15, 25)	17: halted eruption	48
27. M	NBL	3.6	4.1	12	3 (12, 22, 35)	7 (18, 28, 38, 48)	3 (15, 25, 45)	Severely affected dentition; niches	101
28. F	AML	3.8	4.2	10	0	4 (18, 28, 38, 48)	0	47: infra-position; niches	67
29. M	Wilms	3.7	4.2	0	0	0	0	7*	7*
30. F	RMS #	1.6	4.7	0	3 (15, 25, 27)	3	3 (17, 37, 47)	17, 37, 47: one rooted teeth; niches	69
31. F	Wilms	3.8	5.0	0	0	0	0	Eruption problems; niches	19*
32. F	ALL	4.5	5.0	10	0	3 (18, 28, 38)	0	17, 27: single-rooted; niches	50
Group O: age ≥ 5.1 years at SCT									
33. F	Wilms	4.2	5.1	0	0	0	0	Taurodontism; niches	11*
34. F	ALL	4.5	5.2	10	0	0	0	Niches	54
35. F	AML	5.0	5.2	10	0	2 (38, 48)	0	17, 27: single-rooted	47
36. F	AML	4.7	5.2	12	0	3 (18, 38, 48)	0	Niches; mother: agenesis of 35, 45	54
37. M	CML □	4.7	5.2	10	0	0	0	17, 27: single-rooted; niches	27
38. M	ALL	4.6	5.4	10	0	0	0	Niches	13*
39. M	ALL#	2.3	5.4	10	0	0	0	16: infra-position; niches	41
40. M	ALL# s	3.4	5.4	10	0	0	0	17 (27, 37, 47): small; 26 single-rooted	28*
41. F	CML	5.0	5.5	12	0	0	0	47: less developed than other second molars	7*
42. F	ALL#	4.2	5.6	12	0	4 (18, 28, 38, 48)	1 (17)	27, 47: small; niches	84
43. M	NHL # □	4.1	5.7	12	0	0	0	Taurodontism	35*
44. M	NBL	4.8	5.8	0	0	0	2 (12, 22, 22*)	12, 22: Peg-shaped; 13, 23: ectopic	13
45. M	ALL#	1.9	5.8	14	0	0	2 (14, 24)	12: small	54
46. M	ALL#	3.5	5.9	12	0	0	0	Niches	33*
47. M	Wilms#	4.7	6.1	0	0	1 (38)	0	Taurodontism	29
48. M	SAA	6.0	6.2	0 □	0	0	0	Niches	21
49. M	ALL	5.5	6.3	12	0	0	0	28, 38: developing but small	1*
50. M	Wilms	5.0	6.4	0	0	2 (28, 48)	0	Taurodontism; niches	29
51. F	NHL	5.8	6.4	10	0	1 (38)	0	Niches	19
52. F	AML#	5.4	6.8	10	0	0	0	Niches	15*
53. M	NBL □	7.4	7.9	0 ●	0	0	0	27: single-rooted	37
54. F	AML	8.4	8.7	10	0	0	0	18, 28, 38: microdontia; niches	29
55. F	AML	8.5	9.0	10	0	0	0		3
56. M	MDS	9.0	9.4	10	0	1 (38)	0 →		3

☼, in tooth numbering FDI two-digit system (Keiser-Nielsen, 1971) used; #, Transplanted for a recurrent disease; □, released after SCT; s, second cancer after SCT; ◆, considering age at treatment, defect unrelated to treatment; *, < 20 teeth included in IDel score, which will increase in future; ●, patient received TBI of 10 Gy excluding the head and is included in non-TBI group; □, patient received total nodal irradiation of 6 Gy and is included in non-TBI group

Figure 10. Examples of panoramic radiographs. For Individual Defect Index (IDeI) see Section 12.





8.1.3. Characteristics of tooth agenesis in stem cell transplant recipients

Tooth agenesis (third molars omitted) was similarly distributed between maxilla and mandible both in the SCT patients and the Finnish population sample (Table 9). In both samples, the most frequently missing tooth was a lower second premolar, followed by an upper second premolar. Eye-catching differences were evident in maxillary lateral incisors, whose frequency of missing teeth in population samples usually occupies second or third place (Table 1, page 22), but were seldom lacking in SCT patients when counted as percentages of missing teeth. On the contrary, the percentage of missing second molars was considerably high in SCT patients but very low in the population (Table 9). These differences can be taken as indications of the differing etiology of tooth agenesis: environmental factors have modified the pattern of genetic hypodontia that was not excluded in the SCT group.

In the group of 25 SCT patients with tooth agenesis (all teeth included according to age rules; see Section 7.2.), 8 patients, all with TBI, had agenesis of some third molars (all four were missing in 6 patients) combined with agenesis of some other teeth, mainly second premolars (Table 8). Four patients had agenesis of some teeth, but their third molars were present. These patients had been very young, 1.6 years or less, at SCT or at diagnosis, and they all were non-TBI patients. Nine patients had agenesis of one or more third molars, but all the other teeth were present. Of these patients, 8 had been 5 years or more at SCT. In the remaining 4 patients, who were too young for assessment of third molars, 6 to 11 teeth were missing. They all had been young (2.0-3.1 years) at SCT and had received TBI (Table 8). Three of these individuals were more than 11 years at the latest PRG, and there is the strong possibility that the third molars will be also lacking.

Table 9. Distribution of tooth agenesis in the 52 SCT recipients and in a Finnish population sample of 1041 subjects (Haavikko, 1971).

Tooth	Maxilla		Mandible	
	Percentage of missing teeth Hölttä; SCT patients	Percentage of missing teeth Haavikko; Finnish population	Percentage of missing teeth Hölttä; SCT patients	Percentage of missing teeth Haavikko; Finnish population
I1	0	0	0	2.7
I2	3.9	18.7	0	1.4
C	0	0	0	0.7
P1	7.8	3.5	2.6	0
P2	28.6	29.2	32.4	42.4
M1	0	0	0	0
M2	11.7	0.7	13.0	0.7
Total	52.0	52.1	48.0	47.9

I1: central incisor; I2: lateral incisor; C: canine; P1: first premolar; P2: second premolar; M1: first molar; M2: second molar

One or more of the third molars was missing in 21% of the subjects (10% of third molars) in the Finnish population sample of Haavikko (1971). In the SCT patients, the corresponding figure was much higher, 53% (39% of third molars). Due to the large variation in development schedule, the age limit for the third molar agenesis was set at 12 years. However, the median age for follicle formation was about 9 years in the healthy population

(Haavikko, 1970), and in our SCT recipients, the first signs of third molar development were usually evident at 8 to 10 years. These facts suggest that the prevalence of third molar agenesis will be even higher when all patients become old enough for the analysis. At the moment, the prevalence would be 69% if all patients ≥ 11 years at PRG were included.

8.2. Discussion of tooth agenesis

8.2.1. *Effect of age at anticancer therapy*

Those insults that occur before tooth mineralization has advanced beyond its initial phases may destroy the tooth germ, but it is unlikely that attacks taking place later will result in agenesis. The earliest mineralization occurs around birth in the first molars, followed by the central incisors, lower lateral incisors, and canines at 3 to 4 (or 6) months (Logan and Kronfeldt, 1933; Schour and Massler, 1940). Anticancer therapy is very seldom delivered before that age, and no agenesis of these teeth was seen in the study patients, even though the earliest diagnoses were made at 0.2 and 0.4 years. In general, agenesis of these “early teeth” is very rare (Table 9) (Haavikko, 1971; Polder et al., 2004), and it is usually of genetic origin or is associated with syndromes (Arte, 2001). Calcification of the upper lateral incisors begins later, at 10 to 12 (or 15) months (Logan and Kronfeldt, 1933; Schour and Massler, 1940), and very early anticancer therapy might be responsible for the agenesis of these teeth. It may be impossible in certain circumstances to determine whether agenesis of the upper lateral incisor is of genetic or environmental origin (Table 8, Case 2). In one of the two patients with missing upper lateral incisors, etiology cannot be related to the anticancer therapy that started at the time that about three-quarters of the crown development should have been completed (Table 8, Case 27; Figure 3, page 17).

According to histologic findings, calcification of the first premolars begins at 1.5 to 2 years, and second premolars and second molars follow at 2 to 2.5 and 2.5 (or 2) to 3 years, respectively (Logan and Kronfeldt, 1933; Schour and Massler, 1940). Theoretically, based on this schedule, environmental insults that occur during the first 2 to 3 years of life may cause agenesis of these teeth. This seems to be fairly valid also in practice. Of the patients old enough at PRG for assessment of all their teeth, tooth agenesis was present in all patients who were ≤ 2 years at SCT, and in 15 of 16 (94%) whose anticancer CT started before age 3. However, in patients who were older than 3.5 at SCT, absence of teeth other than third molars was very rare (Table 8).

It is more difficult to explain why some patients less than 3 years at SCT and/or at the start of treatment, had no agenesis of premolars or second molars. For instance, cases 10, 11, and 12 (Table 8), each with a different disease, were diagnosed and transplanted at similar ages and received TBI, but only one had tooth agenesis. Moreover, number of missing teeth showed wide variation, especially in the TBI group. Several speculations can be presented. The MDS patient did not receive CCT before HDC, which probably diminished the total toxicity of her treatment. The tooth-forming cells were saved from the intermittent CT courses that might have killed the odontogenic cells, if previous regimens had already caused them some functional or structural injuries. This explanation does not apply to the AML patient who was treated also with CCT. Cyclophosphamide (Cy) was the chemotherapeutic agent used for HDC in both of the patients without tooth agenesis (Cases 11 and 12 in Table 4).

Perhaps the Cy concentrations around tooth buds did not cross the limit at which irreversible injuries could occur, and the tooth-forming cells escaped apoptotic or toxic death. It is possible that some other HDC regimens, for instance VMP, which consists of three drugs with different mechanisms of action, are more toxic to tooth germs than is Cy alone. No experimental studies yet confirm this, and in a small study series like ours it was impossible to separate the roles of different chemotherapeutic agents or HDC protocols in tooth agenesis. Cy alone dose-dependently disturbs the development of rat incisors and molars, but no tooth agenesis has been reported. This is to be expected, however, because the experimental animals have been “too old”, i.e., their tooth development had already passed the initial phases at which Cy could have theoretically destroyed tooth germs (Section 5.1.2., alkylating agents).

One more probable explanation can be offered for inter-individual differences in tooth agenesis: variations in the timing of tooth formation, as seen in PRGs, between individuals are common. Tooth development is a very complicated sequence, in which several signal molecules—via many steps—activate their target genes, that, in turn, control the next steps of tooth development (Section 1.3.; Figure 2). After reviewing those detailed mechanisms of tooth development, a schedule, measuring time in months, is of limited value. As regards the exact consequences of toxic environmental insults to teeth, timing may be a question of hours rather than days, weeks, or months. Thus, differences in tooth development between individual patients occur, even if they all have been treated with the same anticancer protocol at about the same age.

8.2.2. Effect of total body irradiation

The typical injurious effects of RT on human teeth (Section 5.2.) are confirmed in several animal experiments reporting dose-dependent and, to some extent, species-specific dental changes (Section 5.1.1.). RT first destroys the differentiating odontoblasts and their precursors, although the minimum harmful doses and exact molecular mechanisms of the resulting cell death remain unknown. Developing human teeth have shown some abnormalities even after a radiation dose of 4 Gy (Fromm et al., 1986). Timing of RT in relation to tooth development is important: tooth agenesis may be induced only if the irradiation is delivered before or at the initial phases of calcification, i.e., during the first 3 years of life (Section 1.4.; Figure 3). Calcification of the third molars begins late, at the age of 7 to 10 years (Schour and Massler, 1940), and RT may destroy the cells of the dental lamina prior to initiation of the third molars, or may destroy the developing tooth germs any time before calcification.

In the SCT patients (N = 52; third molars excluded) TBI did not cause an increase in the prevalence of tooth agenesis. Differences were small also when patients were grouped by age at SCT. Since the age groups differed from each other more than did the irradiation groups within each age group, it seems that age at SCT is a more serious risk for tooth agenesis than is TBI. Only among NBL patients did TBI significantly increase the prevalence of tooth agenesis (Table 7, page 68). This result should be treated with caution, however, since in a small group, individual patients have much weight. For instance, in the non-TBI group, two patients (Cases 44 and 53 in Table 8, pages 69-70) were so “old” at diagnosis (4.8 and 7.4 years) that treatment could not have caused them tooth agenesis (except of the third molars),

no matter whether they received TBI or not. Prevalence of tooth agenesis increased when third molars were included in the assessment (N = 32). The TBI groups tended to be more severely affected than were the non-TBI groups (Table 7).

The number of missing teeth in the TBI groups was significantly higher than in the non-TBI groups, when all patients or Group Y were observed (third molars included). This difference was especially clear in patients treated for NBL. The same tendency was clear in all other assessments, although the number of patients in the subgroups was small, and significance was not reached in most comparisons (Table 7). TBI thus appeared to cause the number of missing teeth to rise, which may have important clinical implications as indicated by the maximum number of missing teeth, which was 4 in the non-TBI and 12 in the TBI group (Tables 7 and 8; Figures 8 and 9, pages 66 and 67).

8.2.3. Agreement with previous clinical studies

Most earlier studies include only children treated with CCT, in several cases connected with CRI in ALL (Table 3, pages 49-52). Prevalence of tooth agenesis ranged between 5% (Sonis et al., 1990) and 30% (Näsman et al., 1994) (see Table 3 for more information). However, these figures cannot be directly compared to the prevalence of 31% in the SCT group (Study III), due to varying patient groups and methods used. In the 1990 study, abnormalities in maxillary lateral incisors, agenesis of second premolars and development of the third molars were excluded since, according to the authors, disturbances occur in these teeth at a relatively high frequency. Teeth were missing only in patients who had been treated at the age of < 5 years and had received CRI of 24 Gy (Sonis et al., 1990) (Table 3). If we had omitted these same teeth in the present SCT group, the agenesis prevalence would have been 21%, much higher than the 5% reported earlier. The age limit for tooth agenesis is well in accordance between these two studies. There is a slight contradiction as to the effect of irradiation on tooth agenesis, since in the previous study teeth were missing only in the irradiated patients (Sonis et al., 1990), but in the the current study (III) equally in irradiated and non-irradiated patients. The intensive HDC of the SCT patients may explain this difference.

Third molars were included in at least two studies, one by Maguire et al. (1987) and the other by Kaste et al. (1998), in which agenesis prevalences were 28% and 17%. These percentages were considerably smaller than the current figure, 66%. The 20% prevalence of third molar agenesis alone (Maguire et al., 1987) was about the same as the prevalence (21%) in a healthy Finnish population (Haavikko, 1971), but much lower than the 53% in the present SCT group. There are multiple possible reasons for these differences, although comparisons are difficult when many authors give neither the treatment protocols used, patients' age at the beginning of anticancer therapy, nor criteria for assessment of tooth agenesis (Table 3). Mean age or age distribution of the patients or both may affect results. The current SCT patients were more intensively treated with CCT and HDC, and they may have been younger at treatment than the patients in the older studies treated with CCT and sometimes CRI; this would make the present SCT group more susceptible to tooth agenesis. In addition, TBI, also focused on tooth germs, was delivered to most of the SCT patients in the present study, but only minor scattered irradiation affected some tooth germs when CRI was given to the leukemia patients treated with CCT.

In studies on SCT recipients, variations also occur in methodology, age of patients, and treatment protocols. In the groups of 19 and 16 BMT patients with TBI (7-10 Gy; single fraction at least to the patients in the 1994 study), agenesis prevalences were 58% and 56% (Näsman et al., 1994, 1997a). These figures probably include third molar agenesis, since many patients more than 5 years at SCT were lacking teeth (Näsman et al., 1994). The percentages were high when compared to the 31% (third molars excluded) in the present SCT patients. However, the earlier percentages probably should be compared to ours with the third molars included. In that case, the new (62% in Study III; updated figure 66%) and the older results would be in closer accordance. A higher prevalence in our study was expected, since the mean age of our patients at SCT was lower (4.3 years) than the mean ages at diagnosis in the patients (6.5 and 6.3 years) studied by Näsman et al. To my knowledge, the highest prevalence of tooth agenesis reported, 80% (third molars included), was in the 15 SCT patients treated for NBL (Study I). It can be speculated that treatment of NBL is more toxic to tooth germs than is treatment of other malignant diseases. This cannot, however, be confirmed in the current study, since the low mean age of these NBL patients at SCT (2.8 years; I) is a strong prognostic factor for tooth agenesis regardless of the treatment protocol.

One study of 27 hematological SCT patients, probably excluding the third molars, gave a low tooth agenesis prevalence of 11% (Uderzo et al., 1997). The median ages of those patients at diagnosis and at BMT were 6.6 and 9 years. Only 5 of the patients were less than 5 years at SCT, which makes the low prevalence of tooth agenesis obvious, even if 25 of the 27 patients received TBI of 12 Gy.

8.3. Conclusions on tooth agenesis

Based on the observations of the SCT recipients studied:

1. Prevalence of tooth agenesis was high, 31% (third molars excluded), in SCT recipients when compared to that of the Finnish population (8%). With all teeth included, agenesis prevalence was 66%.
2. Agenesis prevalence for third molars alone was 53% (21% in the population) and will be even higher when all patients are old enough for assessment.
3. Maxillary lateral incisors represented 3.9%, and permanent second molars represented 24.7% of all missing teeth. These teeth differed most from the “genetic hypodontia,” in which the reported proportions were 20.1% and 1.4%, respectively.
4. Mean number of missing teeth in those individuals with tooth agenesis was higher than reported for “genetic hypodontia.”
5. No missing teeth, except third molars, were observed in patients who were 4 years or older at the start of CT and 5 years or older at SCT.
6. Tooth agenesis occurred after CT alone, especially in patients ≤ 2 years at SCT.
7. Young age at SCT (or at the start of CT) was a stronger risk factor for the tooth agenesis than was TBI, although TBI caused additive impairment.
8. TBI led to increased numbers of missing teeth (third molars included), which was high in the SCT recipients ≤ 5 years at SCT.
9. The youngest TBI patients (≤ 3.2 years at SCT) displayed the most serious consequences of anticancer therapy as regards the prevalence of tooth agenesis and number of missing teeth.

9. Microdontia

9.1. Microdontia of permanent teeth in stem cell transplant recipients (I, III, and updated)

In the studies on prevalence of microdontia and number of microdontic teeth, only the assessments excluding third molars are presented. Third molars would not much change the results: Only three of them in one patient were microdontic (Case 56 in Table 8, pages 69-70). Results presented here have been slightly updated since the earlier reports (I, III).

9.1.1. Prevalence of microdontia

Third molars excluded

Microdontia was present in 24 of 55 SCT recipients (44%). Non-TBI patients tended to be slightly more affected (50%) than were TBI patients (41%). Three-quarters of all the patients in Group Y, including all children in the non-TBI group, presented with microdontia. Irradiation did not bring out marked differences within the age groups. Patients in Group M were also severely affected, and both younger groups differed from Group O when all the patients or only the TBI patients were included (Table 10).

Microdontia was very common in patients treated for NBL, with approximately 80% of the patients involved (I, Table 10). Young age at treatment probably was the main reason for the high microdontia frequency that occurred almost equally in the TBI and non-TBI patients.

Figure 10 (pages 71-72) presents examples of microdontia in SCT patients (cases 17, 13, and 19).

Table 10. Microdontia of permanent teeth (third molars excluded), expressed as number of patients with microdontia and mean number of microdontic teeth in the study group and in selected subgroups. Mean ages of the patients at SCT are also given.

Group (N; TBI/nonTBI)	Mean age at SCT (SD)				Patients with microdontia (%)				Mean number of microdontic teeth (SD)			
	All	TBI	Non-TBI	Sig. TBI/ non-TBI	All	TBI	Non-TBI	Sig. TBI/ non-TBI	All	TBI	Non-TBI	Sig. TBI/ non-TBI
Total (55; 39/16)	4.3 (2.1)	4.4 (2.0)	4.1 (2.1)	NS	24 (44)	16 (41)	8 (50)	NS	1.5 (2.5)	1.6 (2.9)	1.1 (1.2)	NS
Group Y (16; 10/6)	1.9 (0.5)	2.0 (0.4)	1.8 (0.7)	NS	12 (75)	6 (60)	6 (100)	NS	2.1 (1.5)	2.0 (1.9)	2.2 (0.4)	NS
Group M (15; 11/4)	3.9 (0.7)	3.7 (0.6)	4.3 (0.8)	NS	9 (60)	8 (73)	1 (25)	NS	2.8 (4.0)	3.6 (4.4)	0.8 (1.5)	NS
Group O (24; 18/6)	6.2 (1.2)	6.2 (1.4)	6.2 (0.9)	NS	3 (13)	2 (11)	1 (17)	NS	0.2 (0.6)	0.2 (0.5)	0.3 (0.8)	NS
Sig. Y/M					NS	NS	0.033		NS	NS	NS	
Sig. M/O					0.003	0.001	NS		0.005	0.002	NS	
Sig. Y/O					< 0.001	0.011	0.008		< 0.001	0.018	0.009	
NBL (19; 11/8)	2.9 (1.7)	2.7 (0.8)	3.3 (2.4)	NS	15 (79)	9 (82)	6 (75)	NS	2.2 (1.5)	2.6 (1.6)	1.6 (1.1)	NS

Abbreviations: SCT: stem cell transplantation; SD: standard deviation; N: number; TBI: total body irradiation; Sig.: significance (p); NS: non-significant; NBL: neuroblastoma; Group Y: patients \leq 3.0 years at SCT; Group M: patients 3.1 to 5.0 years at SCT; Group O: patients \geq 5.1 years at SCT

9.1.2. Number of microdontic teeth

In the SCT patients (N = 55), the mean number of microdontic teeth was 1.5, with no difference between TBI and non-TBI patients. Patient age was negatively correlated with mean number of microdontic teeth ($r = -0.388$; $p = 0.003$). Age explained only 14% and 40% of the variation in TBI and non-TBI groups, respectively (15% in the whole study group). Patients who had been 5 years or older at SCT (Group O) had significantly fewer microdontic teeth than did those in the two younger groups, Y and M (Table 10; Figure 11). Counting only the 24 patients with microdontia, the mean number of microdontic teeth was 3.3. TBI patients were more affected, with a mean number of 3.9 (SD 3.4; range 1-12) teeth, a difference not significant when compared to the non-TBI patients with 2.3 (SD 0.5; range 2-3) microdontic teeth. Patients in Group M were most severely affected (4.7; SD 4.2; range 1-12), although Group Y was not significantly different (2.8; SD 1.1; range 1-4). It should be noted, however, that in Group M were two patients treated for a recurrent disease, whose microdontia was not in line with the rest of the group's. Without these patients, whose treatment began at an early age, the number of microdontic teeth in Group M would be 2.6 (range 1-3) (Table 8, pages 69-70; Figure 11).

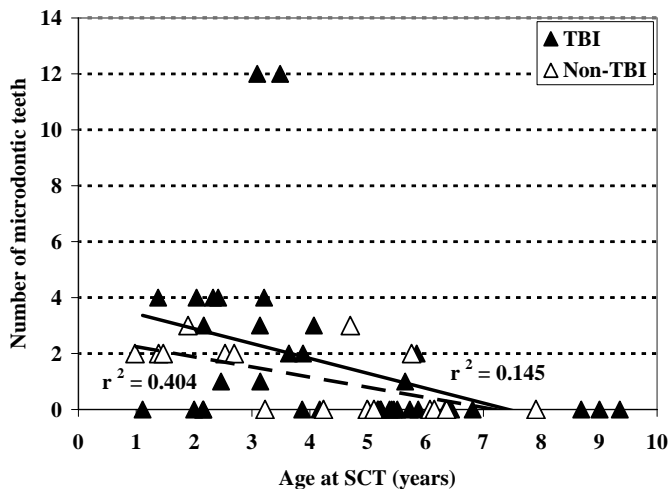


Figure 11. Number of microdontic teeth in relation to total body irradiation (TBI) and age at stem cell transplantation (SCT) (N = 55), with third molars excluded. The two patients with 12 microdontic teeth had been treated with conventional chemotherapy starting at ages 1.6 and 1.4 years, and their recurrent disease was treated with high-dose chemotherapy, TBI, and SCT.

9.1.3. Characteristics of microdontia in stem cell transplant recipients

Mandibular first premolars were most frequently microdontic (27.5%), and together with maxillary first premolars they represented almost half of all microdontic teeth (Table 11). Maxillary lateral incisors are the most frequently microdontic (often peg-shaped) teeth in population studies (Grahnen, 1956; Alvesalo and Portin, 1969) (see also Section 2.3.3), but these came after premolars and second molars in SCT recipients, with only two (5%) patients

affected. One of these patients was already 4.8 years at the start of CT, and the treatment could not be the reason for his peg-shaped maxillary lateral incisors that must have had a genetic background. Microdontia of the second molars is a rare phenomenon in healthy populations but turned out to be quite frequent in SCT recipients. Thus, the microdontia pattern after anticancer therapy differed much from the pattern in genetic microdontia, confirming the effect of anticancer therapy in microdontia. The relative proportion of microdontic teeth and their distribution in maxilla and mandible are presented in Table 11. As regards the combined proportion of tooth agenesis and microdontia, maxillary teeth were slightly more frequently affected than were mandibular teeth. Maxillary second premolars had been most vulnerable to treatment, followed by mandibular second premolars (Table 11).

Table 11. Relative proportions of teeth (in percentages) of the total number of microdontic, of missing, and of the combined microdontic and missing teeth in the group of 55 stem cell transplant recipients.

Tooth	Maxilla			Mandible		
	Microdontic teeth (%)	Missing teeth (%)	Microdontic + missing teeth (%)	Microdontic teeth (%)	Missing teeth (%)	Microdontic + missing teeth (%)
I1	0	0	0	0	0	0
I2	5.0	3.9	4.5	0	0	0
C	0	0	0	0	0	0
P1	21.3	7.8	14.6	27.5	2.6	15.3
P2	17.5	28.6	22.9	7.5	32.4	19.7
M1	0	0	0	0	0	0
M2	11.3	11.7	11.5	10.0	13.0	11.5
Total	55.0	52.0	53.5	45.0	48.0	46.5

I1: central incisor; I2: lateral incisor; C: canine; P1: first premolar; P2: second premolar; M1: first molar; M2: second molar

9.2. Discussion of microdontia

9.2.1. Tooth size and shape—theory and practice

Determination of tooth size is under genetic control, and genetic influence is always present in the background, even if some environmental factors may modify this predestined process (Section 2.3.2.). The size and shape of a tooth crown depends on the morphogenesis of the epithelium during cap and bell stages. Epithelial signaling centers, primary and secondary enamel knots, are considered to be of fundamental importance in regulating tooth shape by integrating the several linked signaling pathways. The primary enamel knot appears at the tip of the tooth bud, at the site where epithelial folding initiates tooth crown development (transition from bud to the cap stage). Enamel knot cells themselves do not proliferate but are able to stimulate cell division in the enamel epithelium and the dental papilla (Jernvall et al., 1994). They express more than ten signals whose functions are not well known (Vaahtokari et al., 1996a; reviewed by Thesleff and Mikkola, 2002; reviewed by Pispá and Thesleff, 2003). Sonic hedgehog is known to play a role in dental patterning: In its absence, the size and the shape of teeth in mice were severely affected (Dassule et al., 2000). Enamel knot signals affect both the epithelial and mesenchymal cells, maintaining the expression of earlier induced signals and transcription factors and inducing new ones (reviewed by Thesleff and Mikkola, 2002; Thesleff, 2003). The primary enamel knot is removed by apoptosis

(Vaahtokari et al., 1996b) and, soon after, follows the formation of the secondary enamel knots in teeth having several cusps, at the sites of future cusp tips. Numerous activating and inhibiting signals are required to determine the number and size of cusps and the distances between them. The first odontoblasts differentiate from the cells of dental papilla underlying the secondary enamel knots, and deposit the dentin matrix. The cusp pattern becomes fixed as soon as the dentin matrix mineralizes (reviewed by Thesleff et al., 2001). The epithelial folding and the growth of the cervical loops, along with the differentiation of odontoblasts and ameloblasts, “shape” the tooth crown by the end of the late bell stage.

Toxic compounds may at high doses destroy part of the tooth-forming cells by direct toxicity or, at lower doses, may trigger the apoptotic pathway (Lyaruu et al., 1997, 1999). Consequently, the decreased number of viable cells, a “labor shortage,” results in the building of a tooth of reduced size. Based on the signaling theory, environmental insults (e.g., anticancer CT and RT) that can disturb the formation of the primary or secondary enamel knots, or their signaling, may result in microdontia. The exact mechanisms are unknown. As seen in clinical practice and in animal studies, both anticancer CT and RT are capable of altering cell behavior or destroying the cells, depending on circumstances. Even a single chemotherapeutic agent, cyclophosphamide (Section 5.1.2., alkylating agents), is able, in rats, to reduce crown size and disturb cusp morphology of the developing third molars (Näsman and Hammarström, 1996). Studies on the toxicity of dioxins to developing embryonic mouse teeth give interesting results (Section 2.1.2). In teeth developed beyond the bud stage at the time of exposure, mesiodistal size is reduced dose-dependently (Kattainen et al., 2001; Miettinen et al., 2002). Smaller tooth size and disturbed cusp morphology has also been noticed in embryonic mouse teeth, developed beyond the initiation stage after *in vitro* dioxin exposure (Partanen et al., 2004). These changes highly resemble those following anticancer therapy, and might direct further research toward the biochemical mechanisms whose disturbances are responsible for reduced tooth size.

9.2.2. Microdontia in stem cell transplant recipients

Microdontia prevalence (44%) was high among the SCT recipients and was distributed among several teeth (Table 11). Studies on microdontia prevalence in general deal with maxillary lateral incisors, since other teeth are seldom involved. In Finland, at least one maxillary lateral incisor with a strongly mesiodistally reduced crown size has been found in 2.3% of one study population (Alvesalo and Portin, 1969). Consequently, it seems obvious that anticancer therapy is a credible reason for the reduced tooth size seen in SCT patients, although genetic microdontia was not excluded in the present study population.

The prevalence of microdontia would probably have been even higher, had mesiodistal crown sizes in the SCT group been measured and compared with the values in a healthy population. In several patients, maxillary lateral incisor(s) were evidently “small,” but to avoid overestimation in a subjective assessment, they were not judged microdontic. “Small” (not peg-shaped) maxillary lateral incisors occurred in patients less than 2 years old at diagnosis (Table 8, pages 69-70). Schedules of tooth mineralization (Figure 3, page 17) (Logan and Kronfeldt, 1933; Schour and Massler, 1940) turned out to be good guides for predicting teeth that were at risk for microdontia: First premolars were at risk before the second premolars and second molars that develop later. In general, risk for microdontia was

high in patients less than 4 years at SCT (Table 8; Figure 8, page 66). The age-group division of the patients showed that the highest prevalence of microdontia was in Group Y, but the highest mean number of microdontic teeth was in Group M (Table 10). However, in small patient groups like the present one, even a single diverging individual causes marked changes in study variables, so results should be treated with caution. For instance, in Group M, the mean value of microdontic teeth would have been lower than in Group Y, had the two children with 12 teeth involved been excluded. These two patients had already been treated with CCT, starting from 1.4 and 1.6 years, at the time when their first premolars were vulnerable to CT. Later, when their second premolars and second molars were susceptible to treatment, they received HDC and TBI prior to SCT, due to recurrent disease. No teeth were missing from these patients and, consequently, they all were subject to microdontia.

The clinical significance of microdontia varies by number of affected teeth and tooth size. Within the so-called “microdontic teeth” tooth size ranges from very small, scarcely visible “grains” to teeth that are normally shaped but small. Even the smallest teeth with no value from the occlusal point of view mostly erupt and resorb the primary teeth, which would have been more useful. Some type of microdontia classification, taking size alterations into account, would be practical in determining the clinical significance of the damage in individual patients.

9.2.3. Agreement with previous clinical studies

One common problem with microdontia studies is the subjective nature of their assessments, which makes comparisons questionable. The majority of the studies reporting microdontia do not even provide written criteria of microdontia. In a group of 82 children treated for ALL and solid tumors, the prevalence of microdontia was 23% (Maguire et al., 1987) (Table 3, pages 49-52). Age of patients during treatment was not given (at examination 3-22 years), but it seems probable that many were so old that there was only a small or no theoretical possibility for microdontia. The authors noticed that therapy during the first 3.5 years of life resulted in small teeth more likely than did later treatment, and maxillary teeth were affected more often than mandibular teeth. In the patients 3 years or younger at treatment, microdontia prevalence was 38%. This age finding and the more frequent damage in maxillary teeth were in good agreement with the current study (III), but the microdontia prevalence in our young patients was notably higher (75%). The intensive treatment of our SCT patients offers an explanation for this difference. The microdontia prevalence of 22% occurred in ALL patients, but only those patients with CRI were affected. In one subgroup of patients < 5 years at diagnosis and with CRI of 24 Gy, the prevalence was 75% (Sonis et al., 1990) (Table 3). In that study, surprisingly, CRI, in which teeth receive only a small, scattered irradiation dose, seemed to play an important role in the etiology of microdontia, whereas in the present study, TBI, directed also to tooth germs, had little effect.

Prevalence of microdontia in other studies ranged from about 10 to 38% (Nunn et al., 1991; Näsman et al., 1994, 1997a; Kaste et al., 1997), with the highest prevalence in patients treated for NBL (Kaste et al., 1998) (Table 3). A higher prevalence, 79%, occurred in the current study for the NBL patients (Table 10). This was an expected result, since all the patients had been intensively treated for poor-risk NBL (Stage III-IV), but the previous study

by Kaste et al. (1998) also included stage I-II NBL patients who were not treated with equal intensity. Only two of their patients received BMT (Table 3).

The microdontia prevalence among SCT recipients has been reported in only a few studies (Table 3). The pioneer work with 16 patients found 4 patients with microdontia (25%) (Dahllöf et al., 1988). They all were young (5.4 years or less) at SCT and had received TBI in a single fraction over a period of 4 hours. Only one patient in this age group, one treated without TBI, did not have microdontia. Other patients were older at treatment, so microdontia caused by therapy was unlikely, except in the third molars. These results seem to be in line with those of the present study when age at SCT is taken into account. Two other studies gave a microdontia prevalence of 68% and 75% for BMT patients (Näsman et al., 1994, 1997a), both higher than the 44% found here. Mean age of patients does not explain this difference: In previous studies patients were older, and consequently, their teeth should have been less vulnerable. At least many (perhaps all) of those patients received TBI in a single fraction, which might have been more injurious to teeth than was our fractionated TBI. The most probable explanation is, however, that criteria in the assessment of microdontia differed.

9.3. Conclusions on microdontia

Patient groups in our study were quite small, and it was not always possible to draw absolute conclusions as to the effects of age, CT, or irradiation on microdontia, although some tendencies were apparent.

1. Prevalence of microdontia was high, 44% (third molars excluded), in the SCT recipients when compared with that of a healthy population.
2. Microdontic teeth were seldom present in patients who were 5 years or older at SCT. Thus, young age at SCT (< 5 years) was a risk for microdontia.
3. Some 60 to 75% of the patients who were < 5 years at SCT later presented with microdontia.
4. CT alone caused microdontia, and TBI did not result in a higher prevalence.
5. TBI had no significant effect on number of microdontic teeth.
6. In the SCT patients, microdontia was differently distributed within tooth types than that reported for “genetic microdontia.” The most frequently microdontic teeth were first premolars, which represented almost half of all microdontic teeth. Second premolars and second molars were also affected more often than were the maxillary lateral incisors that are mostly affected in “genetic microdontia.”
7. Schedules of tooth mineralization were good guides in predicting the teeth at risk for microdontia during anticancer therapy.

10. Combined tooth agenesis and microdontia

10.1. Patients affected with tooth agenesis or microdontia or both

Agenesis or microdontia or both affected 49% of the 55 SCT recipients when third molars were excluded from assessment. With third molars included (N = 32), 72% were affected. More than 90% of the patients in Group Y had tooth agenesis or microdontia or both, and

these two contributed almost equally to the prevalence. All patients ≤ 3 years at SCT were affected when third molars were included in assessment. In Group M, microdontia was more prevalent than tooth agenesis, and third molar agenesis increased “injury prevalence” to 78%. Without third molars, the Group O patients were less frequently affected than were the other groups. In this group, however, third molars raised “injury prevalence” from 13 to 53%, and the difference between groups M and O disappeared (Figure 12).

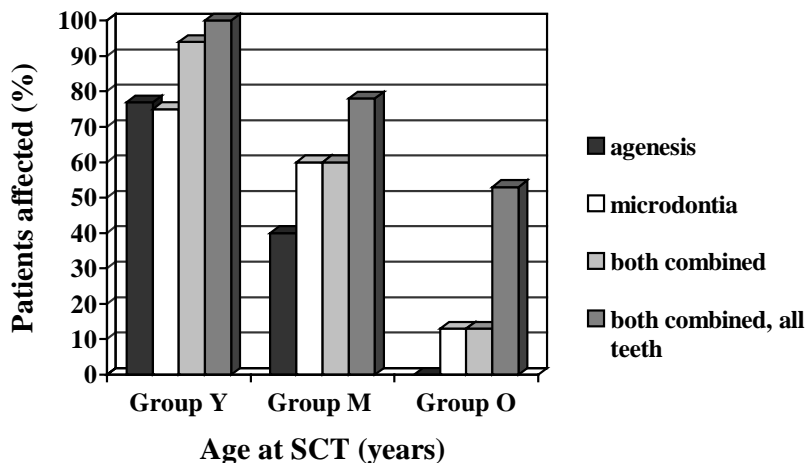


Figure 12. Percentages of patients with tooth agenesis, microdontia, combined agenesis and microdontia (third molars excluded), and combined agenesis and microdontia of all teeth (third molars included) in age groups Y (≤ 3 years at SCT), M (3.1-5.0 years at SCT), and O (≥ 5.1 years at SCT). Omitting third molars, prevalence of tooth agenesis and microdontia in group O differed from groups Y and M (agenesis: Y vs. O: $p < 0.001$; M vs. O: $p = 0.002$ and microdontia: Y vs. O: $p < 0.001$; M vs. O: $p = 0.003$). Each group differed from the others when tooth agenesis and microdontia were combined (Y vs. M: $p = 0.033$; Y vs. O: $p < 0.001$; M vs. O: $p = 0.003$). With all teeth included, prevalence of combined agenesis and microdontia differed significantly only between groups Y and O ($p = 0.026$).

10.2. Combined number of missing and microdontic teeth

The mean number of missing or microdontic teeth or both, when third molars were excluded, decreased from the youngest to the oldest group. Group O differed from the others in all comparisons (Figure 13). With all teeth included, Group M had the highest mean number of missing or microdontic teeth or both (6.6, median 6.0). However, the clinical significance of the tooth injuries in Group Y may be more marked than in Group M, since the number of damaged teeth other than third molars was higher. Missing third molars diminish the length of the dental arch, which seldom causes severe harm, but injuries to the teeth, situated more anteriorly on the alveolar ridge, result in gaps that may compromise occlusion. In short, all children who are 5 years or less at the time of SCT are at risk for severe dental damage due to anticancer therapy.

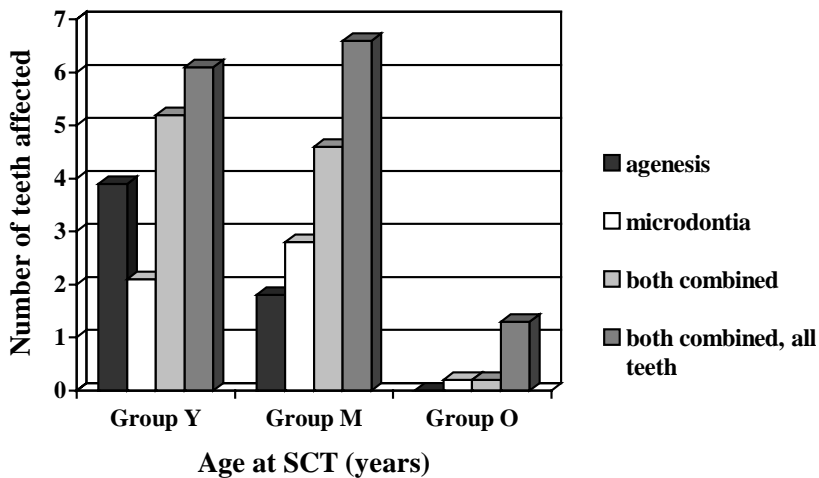


Figure 13. Mean number of missing, microdontic, combined missing and microdontic teeth (third molars excluded), and combined mean number of all missing and microdontic teeth (third molars included) per patient in age groups Y (≤ 3 years at SCT), M (3.1-5.0 years at SCT), and O (≥ 5.1 years at SCT). Without third molars, the mean number of affected teeth in group O differed from the other groups in terms of agenesia (Y vs. O: $p = 0.001$; M vs. O: $p = 0.038$), microdontia (Y vs. O: $p < 0.001$; M vs. O: $p = 0.005$), and combined tooth agenesia and microdontia (Y vs. O: $p < 0.001$; M vs. O: $p = 0.004$). With all teeth included, the combined mean number of damaged teeth was highest in Group M, although Group Y was almost equally affected. Group O differed from the others (Y vs. O: $p = 0.001$; M vs. O: $p = 0.015$).

11. Root-crown ratios of permanent teeth (I, II, IV)

11.1. Assessment method

Developing a simple, objective, yet valid and reproducible method for assessing defects of dental root development was the reason for the studies of root-crown (R/C) ratio. The measurement method, adapted from an earlier one (Lind, 1972), was used in the three studies with R/C ratios assessed from PRGs. In panoramic radiography, different magnification factors apply to the horizontal and vertical dimensions outside the central plane of the layer, and between different regions of the same radiograph (McDavid et al., 1985; Welander et al., 1989). The horizontal distortion is marked, but the vertical magnification factor between the central plane of the layer and the planes displaced ± 10 mm is less than $\pm 5\%$ (McDavid et al., 1985). Thus, the PRG method is considered acceptable for vertical measurements, which have been reproducible (Larheim et al., 1984; Carels et al., 1991; Thanyakarn et al., 1992a; Stramotas et al., 2000). We chose a method assessing R/C ratios instead of absolute linear measurements, because alterations in tooth angulation affect radiographic tooth length, but the change in the R/C ratio is negligible, because the root and the crown usually lie in almost the same vertical plane (Brook and Holt, 1978). In the present study, palatal roots were omitted due to their diverging inclination compared to that of the crown, which may result in

proportionately greater enlargement than that seen in buccal roots (Thanyakarn et al., 1992a). No further attention was paid to magnification factors, since use of the ratio eliminated their effect.

Assessment of R/C ratios is accurate and reproducible when the patients are correctly positioned (Stramotas et al., 2000). In the present study (II), reproducibility of the method was good in intra- (correlation 0.87, $p < 0.001$) and inter-examiner (correlation 0.83, $p < 0.001$) assessments. Furthermore, mean R/C ratios between the repeated measurements did not differ.

The error of the method is always present and consists of errors in the radiographic procedure and in the measurements by the observer(s). In Study II, the magnitude of the error of the method (e_m) in the intra- and inter-examiner R/C assessments varied among teeth from 1.8% (intra and inter) for maxillary lateral incisors to 4.3% for mandibular first premolars (intra) and to 4.7% for mandibular second premolars (inter). Biological variances in R/C ratios always exceeded the error variances (e_m^2) of the corresponding teeth. The mean e_m^2 percentage of biological variance was 13.2% in intra- and 18.9% in inter-examiner evaluations. The main source of the error has earlier been the difficulty in recognition of reference points (Larheim et al., 1984; Thanyakarn et al., 1992b). For the current studies, two very experienced radiographers had taken the PRGs, and patients were probably correctly positioned. Thus, the largest share of the e_m likely stems from observer performance, i.e., from difficulty in determining the intersection between the root and crown or, in some cases, in determining incisal or apical reference points. Changing of reference points would have had no advantage, since a similar difficulty in locating reference points was noted by investigators who used the cemento-enamel junction as the dividing line between crown and root (Mavragani et al., 2000; Sameshima and Asgarifar, 2001).

The PRG method has some limitations. Especially in the premolar region, a typical PRG-related problem is overlapping of teeth (Welander et al., 1989), which worsens the visibility of the reference points in the intersection of the crowns and the roots. The maxillary sinuses may also impair visibility. As a result, about 30% of the maxillary first premolars had to be excluded from the study. Another disadvantage, related to the method itself, is that only lengths (heights) of the roots and crowns are observed. This method leaves an anomaly unnoticed in teeth with thin but “normal” length roots. This disadvantage could be avoided with area measurements, which, however, make high demands on the PRGs: Horizontal measurements included in the area measurements are less reliable than vertical ones, except on the central plane of the layer (Rejebian, 1979; McDavid et al., 1985; Larheim and Svanaes, 1986; Welander et al., 1989). This problem can in part be overcome with a ratio of root and crown areas, which reduces the effect of image distortion on the results. Ratios, no matter whether based on length or area measurements, do not reveal dental damage if crowns and roots are equally affected.

As long as no perfect method for R/C assessment is available, some limitations and methodologic errors must be accepted. On the grounds of our results, R/C ratios of permanent teeth can be assessed from panoramic radiographs with acceptable accuracy and reproducibility. The method can be utilized, in addition to study of the effects of childhood disease or its treatment on R/C ratio, also in describing the amount of root shortening in some syndromes, or in following the amount of root resorption in orthodontic patients.

11.2. Root-crown ratios of permanent teeth in healthy subjects (II)

R/C data for the healthy Finnish population was planned to serve for comparison with various patient groups or individuals in study of root-crown aberrations. The R/C ratios of contralateral teeth were pooled in the results, since they did not differ significantly from each other. R/C ratios are given separately for maxillary and mandibular teeth both for males and females (Table 12). The highest mean R/C ratios were registered for the mandibular second premolars in both genders, closely followed by the mandibular first premolars. The lowest mean values were for central incisors. Ranking was the same for the corresponding maxillary teeth. R/C ratios were higher for the mandibular teeth than for the corresponding maxillary teeth. Some significant differences in R/C ratios appeared between males and females, but in most teeth these values were almost equal (Table 12). Our results suggest that, when studying developmental root deficiency, separate reference values for “normal” R/C ratios should be used for maxillary and mandibular teeth and even for males and females.

Table 12. Mean root-crown (R/C) ratios with standard deviations (SD) and 95% confidence intervals (CI) for mature permanent teeth in males (M) (N = 53) and females (F) (N = 55).

□ Teeth	N, M	N, F	Mean R/C, M	Mean R/C, F	SD, M	SD, F	95% CI, M	95% CI, F	Maxilla vs. mandible, M	Maxilla vs. mandible, F	M vs. F
11, 21	105	106	1.86	1.78	0.17	0.16	1.82-1.89	1.75-1.81	***		**
12, 22	102	105	2.04	1.97	0.21	0.18	2.01-2.09	1.93-2.00	NS		**
13, 23	103	102	2.10	2.10	0.22	0.21	2.06-2.15	2.06-2.14	***		NS
14, 24	78	68	2.16	2.15	0.22	0.22	2.11-2.21	2.10-2.20	***		NS
15, 25	88	92	2.19	2.21	0.22	0.25	2.14-2.24	2.15-2.26	***		NS
16, 26	101	92	1.87	1.80	0.15	0.15	1.84-1.90	1.76-1.83	***		**
17, 27	105	100	1.99	1.94	0.17	0.18	1.96-2.02	1.90-1.97	NS		*
31, 41	95	105	1.97	1.92	0.16	0.14	1.94-2.01	1.89-1.95		***	**
32, 42	102	107	2.05	2.02	0.17	0.16	2.01-2.08	1.99-2.05		*	NS
33, 43	92	101	2.22	2.23	0.23	0.20	2.17-2.27	2.19-2.27		***	NS
34, 44	101	105	2.43	2.42	0.27	0.25	2.38-2.49	2.37-2.47		***	NS
35, 45	99	105	2.44	2.46	0.26	0.24	2.39-2.49	2.41-2.51		***	NS
36, 46	105	101	2.11	2.07	0.17	0.18	2.09-2.15	2.03-2.10		***	NS
37, 47	106	108	2.01	1.98	0.18	0.19	1.97-2.04	1.94-2.01		NS	NS

□ In tooth numbering, FDI (Fédération Dentaire Internationale) two-digit system (Keiser-Nielsen, 1971) is used

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS, $p > 0.05$

Earlier information on “normal” R/C ratios (also called “relative root length” or “crown-root index”) was available but, due to the inconsistency in methods, comparison of the values was impossible. Materials and methods were seldom described in detail or the method was useless in a radiographic study, or both. For instance, extracted teeth were measured in some studies (Bjorndal et al., 1974; Verhoeven et al., 1979; Carlsen, 1987), and some reference points used, like vestibular cemento-enamel junction, may not be adequately recognizable on radiographs. Tooth measurements on different kinds of radiographs were performed in other studies, but all teeth were assessed in none of them (Lind, 1972; Jakobsson and Lind, 1973; Brook and Holt, 1978; Larheim et al., 1984; Carels et al., 1991; Thanyakarn et al., 1992a; Schalk-van der Weide et al., 1993; Midtbo and Halse, 1994). Thus, the results of the present study are not directly comparable to previous results, although some common tendencies are

noticeable. For instance, after excluding R/C ratios of molars that were seldom reported, all researchers found smallest R/C ratios (or crown-root index) to be for maxillary central incisors. The highest values were recorded for maxillary or mandibular second premolars (ratios calculated from the data of Bjorndal et al., 1974; Carlsen, 1987; Midtbo and Halse, 1994).

11.3. Root-crown ratios of permanent teeth in stem cell transplant recipients (I, IV)

In the pioneer study (I) on NBL patients (Table 6, page 59), determination of the R/C ratios was based on the R/C value obtained from the maxillary central incisors (Lind, 1972). The same limit served in classification of all teeth (Section 7.4.1.). All 10 patients who received TBI as part of their preparative regimen for SCT had deviations in their R/C ratios. According to the three-grade classification of 10 patients, 7, 8, and 9 had mild, severe, or very severe aberrations in R/C ratios, respectively. Teeth were differently affected: the same patient had, for instance, mild disturbances in some teeth and very severe ones in others. In the TBI patients with NBL, 58% of the permanent teeth whose R/C ratios were assessed had deviations in the R/C ratios. The non-TBI patients were less affected on the severity scale: Mild R/C deviations appeared in 4 of 5 patients, and one of these patients also had severe alterations. No aberrations were very severe. The non-TBI patients were less affected also as regards percentage of deviant R/C ratios, which was 21%.

In the course of the pioneer study it appeared probable that tooth- and perhaps gender-specific “normal” R/C ratios should be used in the comparison with the R/C values of SCT recipients. This idea served as a foundation for the second study on the R/C ratios of SCT recipients (IV). Instead of the severity classification used in Study I, SD-scores, expressing deviation from the reference values, were calculated for all teeth.

11.3.1. Root-crown ratios at patient level (IV)

Altered root development (examples in Figure 10, pages 71-72) occurred in all 52 pediatric SCT recipients studied, when R/C ratios outside ± 2 SDSs were considered “deviating.” The mean number of affected teeth per patient was 13.9 (range 1-27) with a mean number of 18.2 (range 1-28) teeth measured. Of 52 patients, 47 (90%) had R/C ratios deviating more than ± 3 SDSs. Some patients had been very young at PRG, and it might have been misleading if conclusions concerning these patients (at the patient level) had been based only on a few fully developed teeth that could be measured. Therefore, a subgroup of 39 SCT recipients with advanced tooth development (more than 50% of their teeth were fully developed) was studied separately. Setting the limit at ± 2 SDS, 34 of 39 patients (72%) had at least half, and 8 of 39 patients (21%) had all of their fully developed teeth affected. It is of note that these milder alterations of the R/C ratio were very common also in the non-TBI patients. For the percentage of affected R/C ratios (outside ± 2 SDS) per individual, in reference to TBI and age at SCT see Figure 2 in the original publication IV (black and white triangles represent TBI and non-TBI patients, respectively).

Of these 39 patients, 25 (64%) had more than half of their R/C ratios deviating more than ± 3 SDSs. TBI patients were more widely affected than non-TBI patients ($p < 0.001$) (Table 13). Using the same variable, the age groups Y, M, and O did not differ significantly,

although the R/C ratios in the patients ≤ 5 years at SCT tended to show greater damage. In each age group, more TBI than non-TBI patients had R/C ratios outside the ± 3 SDS limits in more than half their teeth. TBI patients were also more affected when the R/C ratios outside ± 3 SDSs were studied by age group (Table 13). For the percentage of affected R/C ratios (outside ± 3 SDS) per individual, in reference to TBI and age at SCT see Figure 14.

Table 13. Subgroup of 39 stem cell transplant (SCT) recipients with advanced tooth development: Mean numbers of root-crown (R/C) ratios studied and teeth with disturbed R/C ratios in relation to limits of ± 3 standard deviation scores (SDS). Number (percentage) of patients with more than half their teeth affected is given for different groups.

Group	Patients, N	Mean number of R/C ratios measured / patient	Mean number of R/C ratios outside ± 3 SDS / patient	Significance	> 50% of R/C ratios outside ± 3 SDS; N (%), patients	Significance
All	39	21.6	12.1		25 (64)	
TBI	29	20.9	14.1	$p = 0.001$	24 (83)	$p < 0.001$
Non-TBI	10	23.7	6.3		1 (10)	
Y) ≤ 3.0 yrs	11	19.6	10.5	Differences between age groups: NS $p = 0.013$ $p = 0.306$ $p = 0.045$	8 (73)	Differences between age groups: NS $p = 0.006$ $p = 0.045$ $p = 0.088$
M) 3.1-5.0 yrs	12	20.2	15.2		10 (83)	
O) ≥ 5.1 yrs	16	24.1	10.9		7 (44)	
Y) TBI/non-TBI	8/3	18.1/23.3	13.6/2.0		8/0 (100/0)	
M) TBI/non-TBI	9/3	19.4/22.3	16.1/12.3		9/1 (100/33)	
O) TBI/non-TBI	12/4	23.8/25.0	12.9/5.0		7/0 (58/0)	

Mann-Whitney U and chi-square (Fischer's exact) tests for statistical analyses.

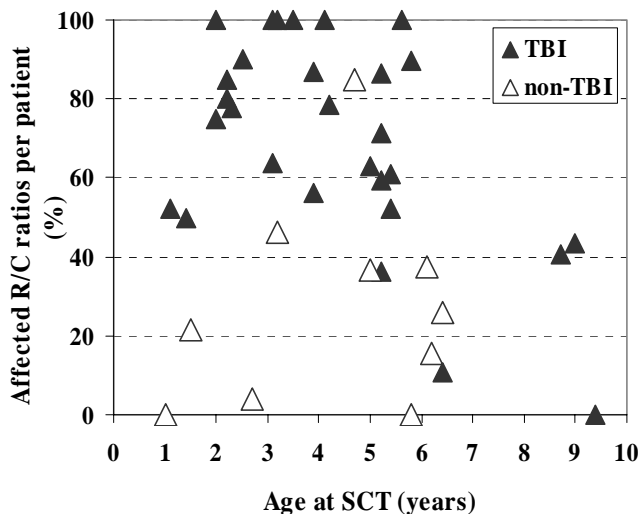


Figure 14. Percentage of teeth (of all fully developed teeth) with root-crown (R/C) ratios deviating more than ± 3 standard deviation scores (SDS) in individual patients with advanced tooth development (N = 39), in reference to total body irradiation (TBI) and age at stem cell transplantation (SCT). According to ± 3 SDS limits, 6 of 39 patients (15%) had all of their fully developed teeth affected.

11.3.2. Root-crown ratios at tooth level (IV)

All 945 fully developed permanent teeth of 52 SCT recipients were included in the R/C ratio analysis (microdontic teeth and third molars excluded, Table 6, page 59). The mean SDS of all fully developed permanent teeth in SCT recipients was -3.4 . In teeth exposed to HDC and TBI, the mean SDS deviated more than in teeth exposed to HDC only: Mean SDS of R/C ratios were -4.0 and -1.8 , respectively ($p < 0.001$) (Table 14).

Mean SDSs at the time of the examination were -3.1 , -4.4 , and -3.0 in the three age groups Y, M, and O, respectively. Group M differed significantly from the others ($p < 0.001$) (Table 14). Especially in the TBI patients were dental roots poorly developed. The difference in mean SDSs of R/C ratios between the TBI and non-TBI patients in all age groups was highly significant. Furthermore, inside the TBI and non-TBI groups, all age groups differed from each other (Table 14). The most seriously affected mean SDSs of R/C ratios were in the teeth of the patients who were 3.1 to 5.0 at SCT. The mildest changes were in the teeth of the oldest age group if TBI was given, but in the youngest age group if the preparative regimen prior to SCT was only HDC (non-TBI) (Table 14; Figure 15). Median SDSs of R/C ratios with upper and lower quartiles and minimum and maximum values are shown in Figure 15.

Table 14. Mean standard deviation scores (SDS) of root-crown (R/C) ratios in 945 fully developed permanent teeth of 52 patients studied, divided into subgroups according to total body irradiation (TBI) and age at stem cell transplantation (SCT). Percentages of teeth with deviating R/C ratios (outside ± 2 and ± 3 SDSs) are also presented.

Group (N, teeth)	Mean SDS (SD)				R/C outside ± 2 SDS / teeth studied (%)	R/C outside ± 3 SDS / teeth studied (%)
	All	TBI	Non-TBI	TBI / non-TBI		
All (945)	-3.4 (2.10)				77	56
TBI (674)	-4.0 (1.87)				85	68
Non-TBI (271)	-1.8 (1.83)				55	25
TBI / non-TBI	$p < 0.001$				$p < 0.001$	$p < 0.001$
Age at SCT (N, teeth)	Mean SDS (SD) of R/C ratio			TBI / non-TBI	R/C outside ± 2 SDS / teeth studied (%)	R/C outside ± 3 SDS / teeth studied (%)
	All	TBI	Non-TBI			
Y) ≤ 3.0 yrs (226)	-3.1 (2.69)	-4.3 (1.81)	-0.3 (2.14)	$p < 0.001$	71	56
Sig. Y/M	$p < 0.001$	$p = 0.012$	$p < 0.001$		$p < 0.001$	$p < 0.001$
M) 3.1-5.0 yrs (262)	-4.4 (1.98)	-5.0 (1.94)	-3.0 (1.27)	$p < 0.001$	91	73
Sig. M/O	$p < 0.001$	$p < 0.001$	$p < 0.001$		$p < 0.001$	$p < 0.001$
O) ≥ 5.1 yrs (457)	-3.0 (1.60)	-3.3 (1.58)	-2.0 (1.22)	$p < 0.001$	72	46
Sig. O/Y	$p = 0.180$	$p < 0.001$	$p < 0.001$		$p = 0.788$	$p = 0.023$

Mann-Whitney U Test was used for statistical analyses.

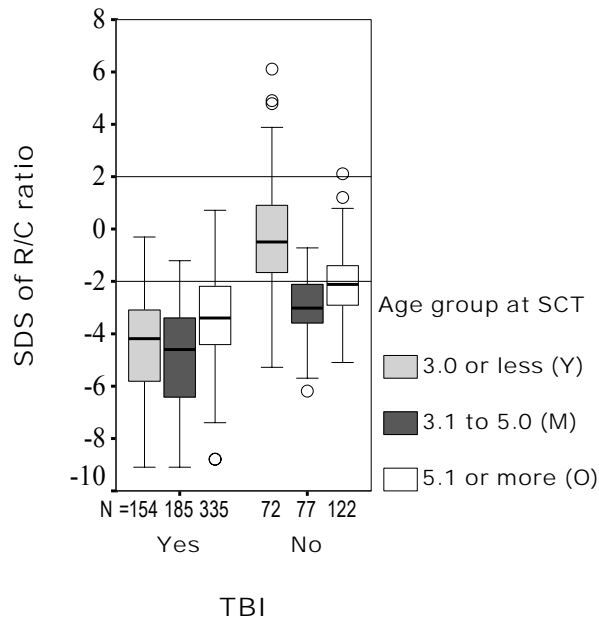


Figure 15. Median standard deviation score (SDS), upper and lower quartiles, and minimum/maximum values for root-crown (R/C) ratios of 945 permanent teeth in 52 stem cell transplant (SCT) recipients, in relation to age at SCT and total body irradiation (TBI). Each box represents interquartile range, containing 50% of the values. Line across box indicates median SDS of R/C ratio. Whiskers show highest and lowest SDS values, excluding outliers (circles). N is the number of teeth studied in subgroups. Mean values of R/C ratios and significances between groups are in Table 14.

In more than three-quarters (77%) of the teeth assessed, the R/C ratio fell outside the ± 2 SDSs limits, and in more than half (56%) of the teeth it deviated more than ± 3 SDSs (Table 14). Significantly more R/C ratios of permanent teeth in the TBI group than in the non-TBI group fell outside ± 2 SDSs and ± 3 SDSs limits (Table 14).

Teeth in the age groups Y and O were equally affected, if the percentage of teeth with R/C ratios out of ± 2 SDS limits was considered, and were more affected in group Y with ± 3 SDS limits employed ($p = 0.023$). The highest percentages of R/C ratios outside the ± 2 SDSs (91%) and ± 3 SDSs (73%) appeared in Group M (Table 14). Subdividing the age groups further according to TBI revealed the highest percentage of the R/C ratios as outside ± 2 SDSs, 97%, in the teeth of the TBI patients who were 3.1 to 5.0 years at SCT. In each of the three age groups Y, M, and O, the number of R/C ratios, deviating more than ± 2 SDSs or ± 3 SDSs was significantly higher in the TBI than in non-TBI groups ($p < 0.001$) (Figure 16).

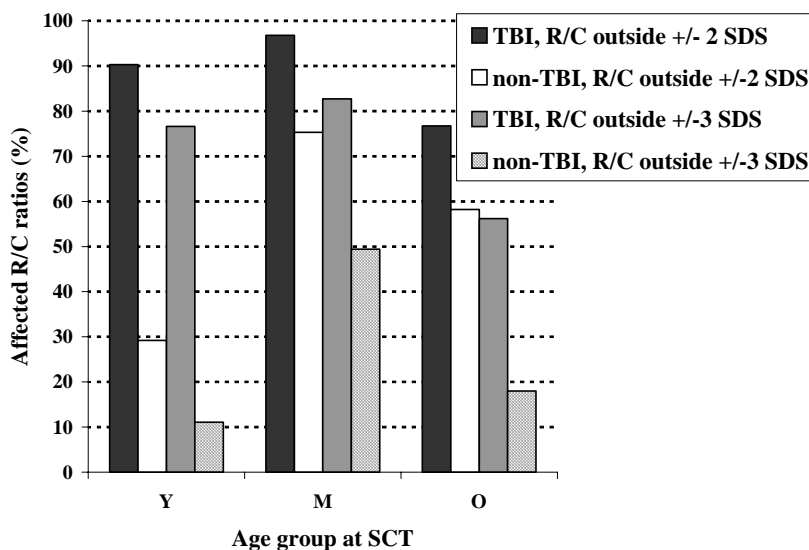


Figure 16. Percentages of root-crown (R/C) ratios outside ± 2 SD- and ± 3 SD scores (SDS) in 945 permanent teeth of 52 stem cell transplant (SCT) recipients. Results are divided according to total body irradiation (TBI) and presented for the three age groups (Y: ≤ 3.0 years at SCT; M: 3.1-5.0 years at SCT; O: ≥ 5.1 years at SCT). In all comparisons, differences between TBI and non-TBI groups were significant ($p < 0.001$).

11.4. Discussion of root-crown ratios and agreement with previous studies

11.4.1. Theory and practice of root development

Tooth development is governed via signal molecules repeatedly functioning throughout tooth morphogenesis (Section 1.3.; Figure 2). After crown development is complete, a double-layered HERS directs root morphogenesis (reviewed by Ten Cate, 1996). Little is known of the molecular mechanisms of root development, for instance, how is root length determined, or what mechanism makes the apex close. Signaling occurs between epithelial and mesenchymal tissues, and mesenchyme probably directs the size, shape, and number of roots. Strong *Dlx-2* expression in HERS appeared during formation of acellular cementum, indicating that *Dlx-2* might be involved in the control of epithelial cell differentiation in the root area, even if its specific role remained unclear (Lezot et al., 2000). Furthermore, after detecting *Msx2* expression in the root sheath, it was suggested to be involved in root morphogenesis (Yamashiro et al., 2003). After all, even if root development probably shares signaling molecules and regulatory genes active in crown development, the two processes are not equal (Yamashiro et al., 2003). Molecular mechanisms of clinically evident disturbances of root development in human teeth following anticancer therapy are obscure. It is likely that chemotherapeutic agents, for instance, interfere with the basic mechanisms of root development involving epithelial-mesenchymal interactions. However, direct cell toxicity is another probable mechanism, at least when cells of HERS are subject to high doses of irradiation, CT or both.

All the SCT recipients of the present study had alterations in the root development of their permanent teeth. TBI had a clear impairing effect on R/C ratios and on the number of injured teeth when compared to effects of treatment with HDC alone. This result differed from the radiation effects on tooth agenesis and microdontia, in which the TBI and non-TBI groups only occasionally differed from each other. There is no unequivocal answer why root development seems to be more vulnerable to TBI than are the initial phases of crown development. One possibility is that the molecular processes—differing at least to some extent from those of crown development—are more easily injured in the root area. Another, perhaps more probable explanation is that root development is a long process compared to the phases during which the presence or absence of a tooth or the crown size is determined. The possibility that TBI affects tooth agenesis or microdontia is diminished when the “critical time window” is narrow. In root development, the vulnerable period is very long, several years, as new cells differentiate along with root growth, and for all this time TBI is able to disturb root development. The clinical consequences were seen in practice: In all age groups, the TBI effect on R/C ratios was significant. Animal experiments (Section 5.1.1.) confirm the effect of irradiation (without toxic chemotherapy) on root development in several animal species (Burstone, 1950; Bruce, 1950b; Gowgiel, 1961; Donohue and Perreault, 1964).

CCT and conditioning therapy prior to SCT, i.e., HDC with or without TBI, were responsible for current study patients’ aberrant root development. When TBI was not included in the preparative regimen, fewer teeth were injured, and the damage was less severe. Still, in more than half the teeth of the non-TBI patients, R/C ratios deviated from ± 2 SDSs. This indicates the ability of chemotherapeutic agents, which disturb tooth development in animals and in vitro studies (Section 5.1.2.), to disturb tooth development also in SCT recipients exposed to anticancer CT. This was a new finding, as in earlier studies on SCT patients almost all patients had received TBI, making the separate effect of CT on root development impossible to study (Dahlöf et al., 1988; Näsman et al., 1994; Näsman et al., 1997a; Duggal, 2003).

Age at SCT was an important factor as regards consequences of treatment for dental roots. Root development begins, or is at its initial phases, between 3 and 5 years in several teeth, and if the most intensive treatment occurred at that period, the R/C ratios were severely compromised. If TBI preceded SCT at that age (Group M), root development was injured in practically all teeth (Figure 16). When environmental insults affect root development at its beginning, it remains disturbed ever since, and there seems a considerable possibility that these treatment-related disturbances may result in clinically meaningful consequences during years to come.

11.4.2. Agreement with previous studies

Deficient root development has been reported after CCT in several studies. The common problem in most of them is their subjective methods, not described at all or not in detail (Table 3, pages 49-52). The pioneering work found that 22% of patients had tooth abnormalities after CT, but microdontia was also included in this figure (Jaffe et al., 1984). Several other reports detected deficient root development in about 10 to 30 % of patients (Maguire et al., 1987; Nunn et al., 1991; Näsman et al., 1994, 1997a; Kaste et al., 1997, 1998). Dental root development was disturbed in all ALL patients < 5 years at diagnosis, and

in all ALL patients (children) > 5 years at diagnosis and receiving CRI in addition to CCT (Sonis et al., 1990). That study used a rating scale to quantify developmental disturbances of teeth in different study groups, but rating criteria were subjective. High disturbance percentages (63-84%) were also reported by Rosenberg et al. (1987), who compared length of premolars to length of permanent first molars. In the present study, all SCT recipients in the non-TBI group had permanent teeth showing deviant R/C ratios. Our number of affected patients is higher than presented earlier, although the results of Sonis et al. (1990) were close to ours. HDC of the SCT recipients, in addition to CCT, likely led to an increased number of patients affected. Another probable explanation is that our objective measurement method recognized smaller alterations than did the earlier methods.

Dentitions of the SCT recipients were more severely damaged due to treatment than were dentitions following CCT. All patients with TBI, and 1 of 3 patients without TBI showed disturbances in root development (Dahllöf et al., 1988), and 94% and 95% of the SCT patients were affected in two other studies (Näsman et al., 1994, 1997a); these results are in good accordance with present results, with all the TBI patients affected. A surprisingly low prevalence, 33%, of “root hypoplasia” appeared in the group of 27 SCT patients (Uderzo et al., 1997). Composition of that study group may explain this low figure: some patients were “old” at treatment as regards root growth, and others were still young at the examination, with short follow-up periods. In the “old” patients, alterations of root development were probably not seen, as tooth development was (almost) finished by the time of the treatment and, in the young ones, root development was only in the beginning, and the results were not yet to be detected.

Young age at SCT is detrimental to root development (Dahllöf et al., 1988; Näsman et al., 1994, 1997a). It has been concluded that the younger the patient at SCT, the more detrimental the effects of anticancer therapy on root development (Näsman et al., 1997a). The present results do not support this view, because in our SCT group the worst disturbances of R/C ratios were in the patients aged 3.1 to 5.0 years at SCT, and patients < 3 years were less affected (Table 14; Figures 15 and 16). Several possible reasons exist for this dissimilarity. Different distributions of diagnoses in the previous and current studies must have resulted in different CCT protocols, with differing toxicity to teeth. The conditioning for SCT in part differed: the present study also included non-TBI patients, whereas all patients in the earlier report received TBI. This, however, did not explain the differing results concerning the most vulnerable age for root development. According to our study, the R/C ratios were always most severely affected in the 3.1 to 5-year-olds, no matter whether TBI was included in the preparative regimen (Table 14). In addition, direct comparison of results was difficult due to differing study methods: Previously, areas of mandibular teeth were measured to calculate crown-root ratios (Näsman et al., 1997a), whereas in the current study lengths (heights) of roots and crowns of all fully developed permanent teeth were measured for the assessment of the R/C ratios.

11.5. Conclusions on root-crown ratios

Healthy population

1. R/C ratios of the permanent mandibular teeth were larger than were the R/C ratios of the corresponding maxillary teeth.
2. R/C ratios of the permanent central incisors, maxillary lateral incisors, and maxillary first and second molars were significantly larger in males than in females.

SCT recipients

1. Disturbances in root development were evident in all SCT patients.
2. In 64% (83% of TBI patients and 10% of non-TBI patients) of the 39 SCT recipients with advanced tooth development (more than half of the teeth fully developed), the R/C ratios in more than 50% of the teeth studied deviated more than ± 3 SDSs.
3. In 21% of the patients, all R/C ratios fell outside ± 2 SDSs. All had received TBI.
4. In 85% of the teeth exposed to TBI, R/C ratios were outside ± 2 SDSs. In the non-TBI teeth the percentage was 55%.
5. The mean SDSs of R/C ratios were significantly lower (worse) in those SCT recipients whose preparatory regimen included TBI than in the non-TBI patients.
6. The most seriously affected mean SDSs of R/C ratios were in the teeth of patients who were 3.1 to 5.0 years at SCT.
7. The number of deviating R/C ratios was highest (97%) in teeth exposed to TBI at a patient age from 3.1 to 5.0 years.

12. Defect Index (I) and Individual Defect Index (thesis)

Defect Index (DeI) and Individual Defect Index (IDeI) combine tooth agenesis, microdontia, and disturbed dental root development into one figure expressing the whole magnitude of the damage to the dentition (for details, see Section 7.5.). No effort was made to exclude any possible genetic aberrations in tooth development; consequently, they are included in the index figures. The only difference between the two indices was in the classification of the R/C ratios (Section 7.5.).

12.1. Defect scores calculated with Defect Index and Individual Defect Index

DeI was created and used in a group of NBL patients (I) to determine overall dental damage. The mean DeI score for TBI patients was higher (70, SD 30.3, range 28-117) than for the non-TBI patients (15.3, SD 9.3, range 4-34) ($p < 0.001$) when the third molars were included. The two groups significantly differed from the healthy test group (1.8, SD 3.9, range 0-15) ($p < 0.001$).

DeI was tested in a group of 18 healthy adolescents with 464 assessable teeth (I). In 7 subjects the defect score differed from zero, varying between 1 and 15, with third molars included. Without third molars, the DeI ranged from 1 to 8 in 5 subjects, while in 13 the score was zero. One individual had peg-shaped maxillary lateral incisors (8 defect points), and one

lacked a maxillary second premolar (5 defect points). Three other subjects each scored 1 point, each of them having one R/C ratio considered mildly disturbed. When IDEI was tested in the same group of healthy adolescents, the same defect points 8 and 5 were recorded for the two subjects. All others scored zero, as the 3 teeth classified as with mild disturbance in DeI were also inside ± 2 SDS in IDEI (values were -1.3 , -1.8 and -1.7 SDSs). The low DeI and IDEI index scores showed that both indices, but especially IDEI, recognized R/C ratios in the dentitions tested to be normal.

DeI and IDEI were calculated for the group of 55 SCT recipients (third molars excluded). DeI gave significantly lower scores than did IDEI (means 30.5 vs. 43.5). This was according to expectations, because the classification of R/C ratios in IDEI was changed to a more accurate system in which the maximum number of defect points had risen from 3 to 4. The two indices arranged the scores of dental disturbances—in proportion to those of other patients—similarly. This is illustrated in the group of 36 SCT recipients with advanced tooth development in Figure 17, where the IDEI line rises higher than the DeI line, but the lines rise and fall almost in parallel.

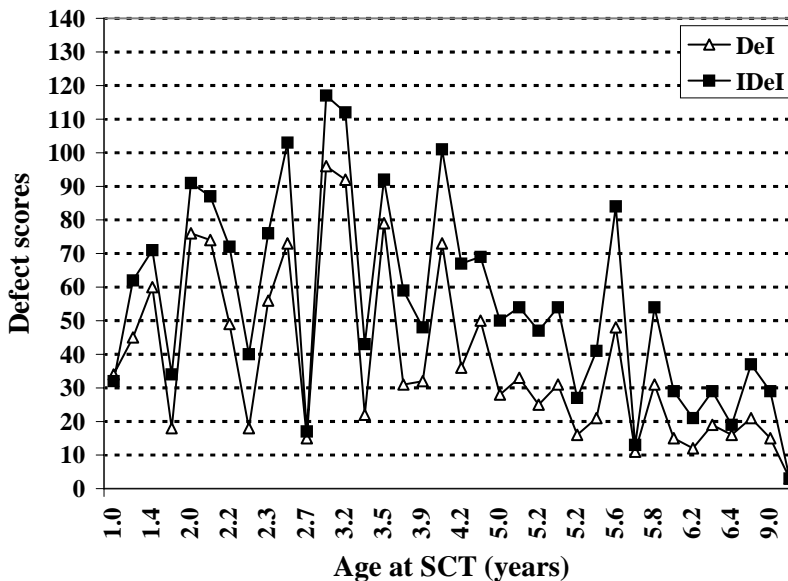


Figure 17. Comparison of defect scores assessed with Defect Index (DeI) and Individual Defect Index (IDEI) in 36 SCT recipients with advanced tooth development (≥ 20 fully developed teeth). Scores for tooth agenesis, microdontia, and R/C ratios of permanent teeth (third molars excluded) are combined in the indices. Theoretical maximum score is 140.

Some patients in the study group were so young that only a few teeth could be included in the indices, which in the future may markedly rise. For index scores that would be final or close to final at patient level, we separated out a subgroup of 36 patients in whom at minimum 20 teeth of 28 were included in the index (Table 6, page 59). The results regarding IDEI, as presented in this thesis, are calculated for this group with advanced tooth development (Table 15). IDEI scores for individual patients are shown in Table 8 (pages 69-70).

The TBI patients had significantly higher mean IDeI score than did patients with HDC only. The highest mean IDeI score was for patients aged 3.1 to 5.0 years at SCT (Group M), but difference was not significant when compared to Group Y, also severely affected. In Group O, the dental late effects of anticancer therapy were significantly milder. The SCT recipients from 2.0 to 4.1 years at SCT most often presented with high IDeI scores (Figure 18).

Table 15. Mean Individual Defect Index (IDeI) scores and other key figures for the group of 36 stem cell transplant (SCT) recipients with advanced tooth development and for subgroups divided according to total body irradiation (TBI) and age at SCT (third molars excluded).

Group	Patients, N	Mean IDeI score (median)	Range of IDeI score	Significance
All	36	55.1 (52.0)	3-117	
TBI	27	62.9 (59.0)	3-117	TBI vs. non-TBI: p = 0.003
Non-TBI	9	31.9 (29.0)	13-69	
Y) ≤ 3.0 yrs	11	62.3 (71.0)	17-103	Y vs. M: p = 0.387
M) 3.1-5.0 yrs	10	75.8 (68.0)	43-117	M vs. O: p = 0.001
O) ≥ 5.1 yrs	15	36.1 (29.0)	3-84	O vs. Y: p = 0.018
Y) TBI/non-TBI	8/3	75.3 (74.0) / 27.7 (32.0)	40-103 / 17-34	p = 0.012
M) TBI/non-TBI	8/2	80.8 (79.5) / 56.0 (56.0)	48-117 / 43-69	p = 0.400
O) TBI/non-TBI	11/4	40.8 (41.0) / 23.8 (25.0)	3-84 / 13-29	p = 0.138

Composition of the IDeI score differed between age groups Y, M, and O. The agenesis score in Group Y was proportionally higher (35%) than in Group M (14%), and made no contribution to the IDeI score in Group O (Figure 19A). The microdontia score had its greatest proportion in Group M, and the R/C score was almost alone (96%) responsible for the IDeI score in Group O, when third molars were excluded. The worst absolute values for R/C ratio scores were recorded in those individuals 3.0 to 5.5 years at SCT. All IDeI components contributed almost equally in the TBI and non-TBI groups (Figure 19B). Proportions of the agenesis, microdontia, and R/C ratio scores were highly variable among individual patients, but the proportion of the R/C ratio score did increase with increasing age (Figure 20). Of the 3 patients (cases 27-29 in Figure 20) with microdontia, 2 were transplanted for a recurrent disease. Considering their age at SCT, their microdontia was probably due to the earlier CCT. The remaining patient (case 28) had peg-shaped maxillary lateral incisors that could not be attributed to anticancer therapy.

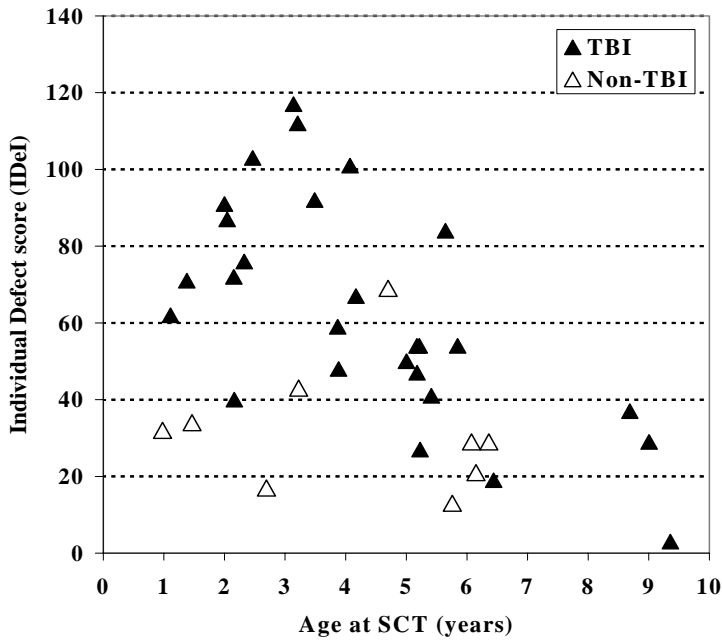


Figure 18. Individual defect scores (third molars excluded) in 36 stem cell transplant patients, according to age at stem cell transplantation (SCT). Different symbols separate patients with and without total body irradiation (TBI and non-TBI).

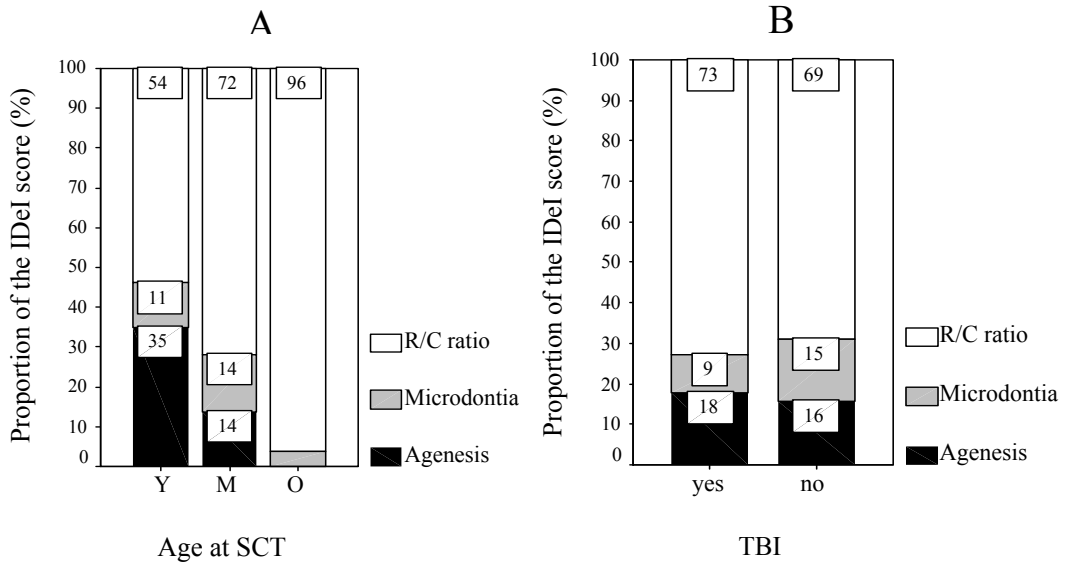


Figure 19. A.) Proportional contribution of agnesis, microdontia, and root-crown (R/C) ratio scores to total Individual Defect Index (IDeI) score in age groups Y (≤ 3.0 years at SCT; N = 11), M (3.1-5.0 at SCT; N = 10), and O (≥ 5.1 years at SCT; N = 15), and **B)** in the total body irradiation (TBI; N = 27) and non-TBI groups (N = 9).

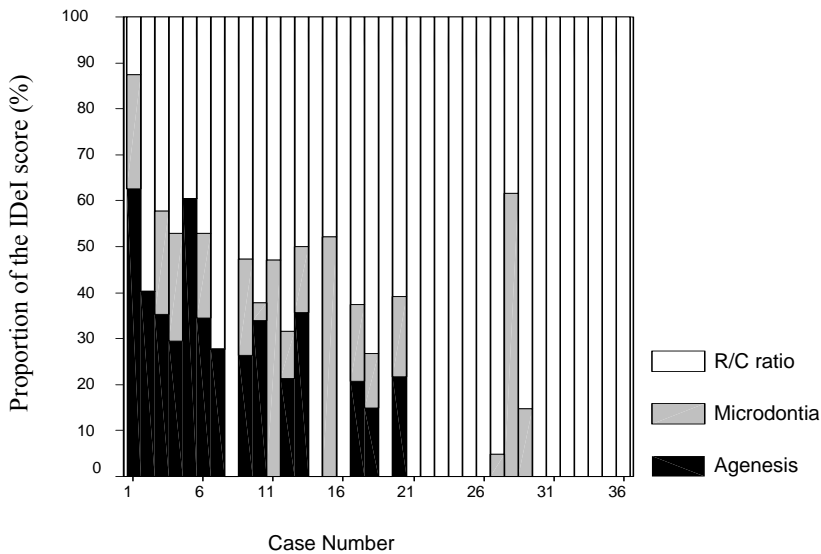


Figure 20. The proportional contribution of agenesia, microdontia, and root-crown (R/C) ratio scores to the total Individual Defect Index (IDeI) score in the 36 stem cell transplant (SCT) recipients with advanced tooth development. Cases 1-11 belong to age group Y (≤ 3.0 years at SCT), 12-21 to age group M (3.1-5.0 at SCT), and 22-36 to age group O (≥ 5.1 years at SCT).

12.2. Discussion of defect indices

Defect points for the indices were subjectively selected, keeping in mind the clinical importance of the defect to the patient. There was no doubt about the highest score going to tooth agenesia. Microdontia was more difficult. In some cases teeth were very small, resembling grains; in other cases their appearance was normal and their size nearly half the normal. From the clinical point of view, microdontic teeth of differing sizes should have been classified by different scores to increase the “clinical accuracy” of the indices. However, a subjective method was used in microdontia assessment, and separation of different classes would have been unreliable—as also would have been the measurement of horizontal distances in PRGs, which is not considered as reliable as measurement of the vertical distances (Rejebian, 1979; McDavid et al., 1985; Larheim and Svanaes, 1986; Welander et al., 1989). Setting limits for the R/C ratio defect scores was also by clinical judgment. Although the classification based on SDSs (IDeI) was easy after the limits were set, no evidence exists that these limits, from the clinical point of view, are valid. This will be revealed only by long-term follow-up.

Earlier, on a severity rating scale, the mean severity rating for developmental dental disturbances was calculated for groups of ALL patients with various treatment modalities (Sonis et al., 1990). That rating scale was based on the number of patients affected and thus did not identify the number of teeth affected or severity of disturbances at patient level. The scale separated patient groups, and the conclusion was that those groups in which patients were < 5 years at diagnosis and received CRI (18 or 24 Gy) had the highest scores in the severity rating scale. Although the methods differed, these main findings were confirmed in the present study, where irradiation (TBI) and young age at SCT (< 5 years) led to a higher

IDeI score (Table 15). Variables used in the severity rating scale (Sonis et al., 1990) and in the IDeI score were not identical. On the earlier study, anomalies in root number and the presence of enamel hypoplasiae—diagnosed as notches on proximal tooth surfaces—were checked, whereas the current study ignored them. Scales used to assess root development also differed: subjective in the earlier vs. objective in the present study.

Defect indices that sum up the late effects of anticancer therapy in the dentition at patient level have not been used earlier. Although genetic developmental dental disturbances are included in the indices, these probably have a minor effect on ultimate index scores. This is assumed because the prevalences of tooth agenesis (8%), peg-shaped maxillary lateral incisors (1.3-2.3%) as representing the most common form of microdontia, and short root anomaly (1.3%) in a Finnish population are low (Haavikko, 1971; Alvesalo and Portin, 1969; Apajalahti et al., 2002) when compared to those of SCT recipients.

The current age group division (Y, M, O) was originally based on the schedule of tooth development. Calcification of permanent teeth, except the third molars, has usually begun at 3 years (Logan and Kronfeldt, 1933; Schour and Massler, 1940). Accordingly, our assumption was that the proportion of the tooth agenesis score would be highest for patients ≤ 3.0 years at SCT. This held true, as shown in Figure 19A for 36 SCT patients with advanced tooth development. Occasionally tooth agenesis was also seen later: the maximum age in patients with tooth agenesis, excluding third molars, was 4.1 years at SCT and 3.6 years at diagnosis (Case 27 in Table 8, pages 69-70). At that age, missing maxillary lateral incisors cannot be related to treatment. Absence of a mandibular second premolar may also have had a genetic cause. Based on the present study, anticancer therapy initiated after the age 3.5 years was very unlikely to have resulted in tooth agenesis (except in third molars).

Between the ages of 3 and 5 years, root development starts in the early-developing teeth, and crown development is in process in the late-developing teeth. Treatment during this period was thought to result in disturbances in both root and crown (microdontia) development. Findings of the current study were not so clear-cut as regards microdontia, which contributed to the defect score almost equally in age groups Y and M (Figure 19A). If microdontia was present after the SCT age of 4.1 years, it could be explained by earlier CCT, or was considered genetic. It was concluded that if anticancer therapy was initiated after the age of 4, risk for microdontia would be minimal (except in third molars). The proportion of the R/C ratio score from Group Y to Group M increased, fulfilling our expectations.

In children > 5 years at SCT, we were waiting to see disturbed root development and agenesis or microdontia of the third molars. Based on the schedule of tooth development and earlier studies on children with cancer, we expected these children to be less affected than were younger SCT patients. Our results mainly confirmed our assumptions. The proportion of the R/C ratio score of the IDeI increased and was virtually alone responsible for the defect score (Figures 19, 20). In patients > 6 years at SCT and > 5 years at diagnosis, the highest defect points for the R/C ratio (= 4) were never reached. Usually second premolars, second molars, and in a few cases canines scored higher defect points than did the other teeth. Microdontia of the third molars was surprisingly infrequent, but agenesis, as expected, was common.

We had no preconceptions concerning the age at which risk for the most severe developmental dental aberrations, as to combined agenesis, microdontia, and R/C ratios, would be at its highest. We received no support from the earlier studies, which had not

applied individual defect scores. In the present novel result, the highest IDeI scores (= the most severe disturbances) appeared for patients aged 2 to 4 years at SCT (Figure 18).

12.3. Conclusions on defect indices

1. Defect indices enable comparison of the quantity of overall developmental dental defects between patient groups and between individuals.
2. IDeI, in which defect scores of R/C ratios are based on the SDSs, is more objective than DeI and this is recommended for studies including R/C ratios.
3. In all 55 SCT patients, the IDeI score differed from zero, meaning that all patients had some developmental dental disturbances.
4. Individual variation in IDeI score was great (3-117) for the entire study group and for the subgroups.
5. TBI patients had higher mean IDeI scores (62.9) than did non-TBI (HDC) patients (31.9). TBI caused the greatest increase in IDeI score in the children ≤ 3.0 years at SCT, when compared to non-TBI patients.
6. Patients who were > 5 years old at SCT had significantly lower (better) mean IDeI scores (36.1) than did younger patients. Thus, young age (< 5 years) at SCT was a risk for high defect scores.
7. At the age group level, the highest mean defect score (75.8) was recorded in children from 3.1 to 5.0 years at SCT.
8. At an individual level, the highest defect scores were in children aged 2.0 to 4.1 years at SCT.
9. For the total IDeI score, proportions for tooth agenesis, microdontia, and R/C ratio scores varied among age groups.

13. General notes

The current studies were focused on the effects of HDC with or without TBI and of age at SCT on tooth agenesis, microdontia, and R/C ratios of permanent teeth. One could ask why these variables were selected, and if they were the best possible for studying disturbed tooth development. In earlier studies, irradiation and age at treatment have been important factors, and their selection was evident. Age at SCT was chosen, because the most intensive treatment, considered most toxic also to teeth, occurred then. Analysis of the results showed that for some children who had a lengthy period between their first anticancer CT and their recurrent disease, requiring SCT, age at diagnosis had explained some dental aberrations better than did age at SCT. In the whole patient group, however, age at diagnosis and age at SCT explained dental defects equally well. Impaired tooth development of our patients in the TBI groups separated them from patients in the non-TBI groups in several respects, so this variable also appeared a proper choice.

Many other dental and medical aspects were not reported. Hypomineralizations, manifesting themselves as enamel opacities or hypoplastic aberrations were excluded from the defect indices, although they were increased in some studies on children with anticancer

therapy (Pajari et al., 1988b; Näsman et al., 1994). Although these decalcifications may result from anticancer therapy, it would have been very difficult or impossible to make a distinction between etiologies of opaque lesions, as they are common also in healthy children. For instance, in Finland, 13 to 19% of children have demarcated hypomineralization lesions merely in their permanent first molars (Alaluusua et al., 1996; Hölttä et al., 2001; Leppäniemi et al., 2001). Fluorosis, causing hypomineralizations with a cloudy appearance, was not included in these figures. Furthermore, in children with a history of anticancer therapy, tooth decalcifications may result from compromised oral hygiene due to sensitivity or to ulcerations of the oral mucosa, and to the possible change in eating habits during cancer therapy.

In the six cases where RT (other than TBI) was delivered to the skull area, we ignored the scattered irradiation. In connection with CRI, it has been calculated to be 3 to 6% in various oral areas (Pajari et al., 1988a), which means that three patients may have received scattered irradiation doses from about 0.4 to 1.4 Gy to some parts of their mouth. When compared to the TBI doses of these patients (10-14 Gy), this minor increase was probably of little consequence to teeth. The three other irradiation fields (for local metastases) in the skull area were even further from the teeth, with hardly any scattered irradiation delivered to the dental area. Because deviations from the reference values for absorbed radiation doses occur in individual patients and in various intraoral sites during TBI (Bågesund et al., 1998), calculation of the exact patient- and site-specific radiation doses for teeth would have been impossible. Consequently, we considered it relevant to maintain the reference doses of TBI.

The effect of different diagnoses and the required CCT and HDC protocols on tooth development would have been an interesting topic for the study. This was not possible, however, because age variations in diagnostic groups were great and subgroups were small. Differences in toxicity profiles for chemotherapeutic agents have been obvious in animal studies (Section 5.1.2.), although no research from the dental point of view has been performed on some of them. It is probable that differences in the dental toxicity occur also in humans and may have clinical consequences. To confirm this hypothesis, study groups should be large enough and adjusted for age.

Several other medical variables received no attention in the present study. For instance, infections and other morbidity during cancer therapy, other treatment-related late effects like hormonal dysfunction, or GVHD in patients with allogeneic SCT were not recorded. Their role in tooth development is obscure. However, hair loss, destruction of sweat glands, and vertical ridges of nails have been described in GVHD (reviewed by Higman and Vogelsang, 2004). This is interesting, since disturbances of these ectodermal organs and of teeth often occur together in ectodermal dysplasia syndromes (reviewed by Mikkola and Thesleff, 2003) (Section 2.1.1.). This may be a clue to GVHD effects on developing teeth, but no studies yet confirm or disprove this speculation.

14. Concluding remarks

The current study was focused on developmental dental defects after anticancer therapy and SCT in children. A great part of the work was development of study methods, which included determination of the R/C ratios of permanent teeth in a healthy population, from their PRGs. We believe that the new methods, aiming at objectivity enabled us to notice dental aberrations with good accuracy. Our methods are also described in detail, which is the prerequisite for repeatability and, possibly, for multi-center studies.

Based on the current study, the following conclusions can be presented:

1. All SCT patients had developmental defects in their permanent teeth.
2. Risk for developmental dental defects was highest in children < 5 years at SCT.
3. TBI led to an increased overall quantity of developmental dental defects, but CT without TBI also caused aberrations in teeth of all SCT patients.
4. Risk for tooth agenesis was very high—perhaps 100%—in patients ≤ 2.0 years at SCT.
5. Anticancer therapy initiated after the age of 3.5 years was very unlikely to result in tooth agenesis, and no missing teeth, except third molars, were found in patients who were 4 years or older at the start of CT and 5 years or older at SCT.
6. If the anticancer therapy was initiated after the age of 4 years, risk for microdontia was minimal (except in the third molars).
7. High defect points resulting from the R/C ratios were found at individual level between the SCT ages of about 3 to 5.5 years.
8. The highest IDEI scores, expressing overall dental damage, were for patients who were approximately 2 to 4.5 years at SCT. However, individual variation did occur.

Our results provide new and detailed information that may help dental professionals in counseling children and their guardians after cancer therapy. The data can be utilized in the prediction of future developmental dental defects, and they are of aid when dental follow-up of the SCT patients is planned. It should be noted, however, that with the changing protocols of cancer therapy and the individual differences between the patients, the results obtained from one group of patients should not be indiscriminately generalized to others.

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