THE ORAL MICROFLORA OF CHILDREN UNDERGOING BONE MARROW TRANSPLANTATION

A study of the changes in the oral microbial flora, gingival health and mucosal immunity before, during and after transplantation

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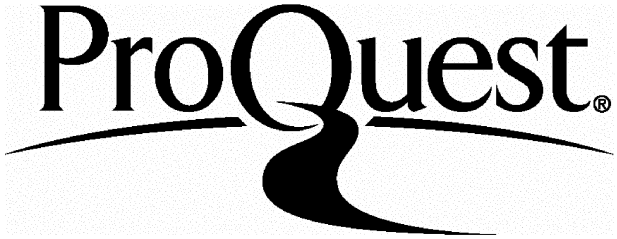

ABSTRACT

Changes in the oral flora and mucosal immunity of 2 groups of children undergoing bone marrow transplantation have been investigated.

Patients and methods: Twenty three children received a conditioning regimen of fractionated total body irradiation and chemotherapy (TBI group). A further 11 children received a conditioning regimen of chemotherapy only (CTO group). Both groups were matched with healthy children.

Dental indices: Dental caries, plaque and gingivitis scores were recorded. There were significant increases in the scores for dental plaque and gingivitis at 7 days post - transplantation in both the TBI and CTO groups ($p < 0.03$).

Microbiological methods: Saliva was collected on 4 occasions during the peri - transplantation period. Standard microbiological techniques were used for enumeration and speciation of viridans streptococci, *Candida*, *Enterobacteriaceae* and enterococci.
The most significant changes occurred at 7 days post - transplantation. There were decreases in the mean total aerobic and anaerobic bacterial counts in the TBI group ($p < 0.0003$ and $p < 0.0002$) and the CTO group ($p < 0.03$ and $p< 0.009$). The proportion of the '*oralis* group' of the viridans streptococci as a percentage of the total anaerobic count was increased in the TBI group ($p < 0.001$) with simultaneous decreases in the isolation frequency of *S. parasanguis* ($p < 0.008$), *S. sanguis* ($p < 0.03$) and *S. salivarius* ($p < 0.00001$).

Immunological methods: Total salivary IgA, secretory IgA, total IgG, IgA and antibodies to *S. mitis* and *S. oralis* were estimated with ELISA. The main findings were increased salivary IgG at 7 days post - transplantation in both the TBI ($p < 0.01$) and CTO groups ($p < 0.02$).

Conclusions: The oral flora of children undergoing bone marrow transplantation changed so that they were at increased risk of opportunistic infection from the '*oralis* group' during the period of intense immunosuppression.

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ABBREVIATIONS

ALL	Acute Lymphoblastic Leukaemia
BMT	Bone Marrow Transplant
CTO Group	Chemotherapy Only Group
dmfs	Decayed, missing, filled deciduous tooth surfaces
dmft	Decayed, missing, filled deciduous teeth
DMFS	Decayed, missing, filled permanent tooth surfaces
DMFT	Decayed, missing, filled permanent teeth
E.U.	E.L.I.S.A. Units
GOS	Great Ormond Street Hospital For Children
GvHD	Graft versus Host Disease
O.D.	Optical Density
'*oralis* group'	*S. mitis* and *S. oralis* collectively
RMH	Royal Marsden Hospital
S - IgA	Secretory IgA
TBI Group	Chemotherapy and Total Body Irradiation Group

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Felix qui potuit rerum cognoscere causas.

Lucky is he who has been able to understand the causes of things.

Virgil, Georgics no. 2, 1. 490 (of Lucretius)

CHAPTER 1

INTRODUCTION

This study concerns changes in the oral microflora of children undergoing bone marrow transplantation. The main emphasis is on the changing population of the viridans or oral streptococci relative to the overall microbial balance of the oral cavity. These changes have been recorded at different pathophysiological stages in the peri - transplantation period and related to mucosal and gingival health.

Bone marrow transplantation has become increasingly effective in the treatment of haematologic, immunologic, metabolic and neoplastic diseases (Seto et al. 1985; Champlin 1990; Mattsson 1991). The purpose of this procedure is to provide patients with haematopoietic stem cells that will proliferate to form a normal haematopoietic and immune system to replace their own which has become impaired by disease, or to replace a missing enzyme (Soutar and King 1995). Before infusion of the donor haematopoietic stem cells, the recipient undergoes an intensive course of treatment. This is the conditioning regimen, the aim of which is to eradicate existing disease and suppress the immune system, thus allowing the new bone marrow to establish itself and grow. Before the new bone marrow engrafts there is a period of pancytopenia during which the recipient has no defence against infection and is at risk from opportunistic pathogens.

The most serious complications associated with transplantation are graft versus host disease and infection. The patients are susceptible to bacterial and fungal infections until the granulocyte count rises above 0.5 x 10^9/l . Almost all recipients develop pyrexias although only 50 percent of the blood cultures are positive (Rogers1989). Organisms from the gastro-intestinal tract, generally enterobacteria and *Pseudomonas aeruginosa,* have been previously reported to be the major pathogens and are associated with high rates of mortality. However, during the last decade there has been an increasing number of reports of septicaemias associated with staphylococci and streptococci, particularly the oral or viridans streptococci, in immunosuppressed patients (Greenberg et al. 1982; Heimdahl et al. 1989; Villablanca et al. 1990; Wahlin B. 1990; Holzel and de Saxe, 1992; Bochud et al. 1994a; Richard et al. 1995). Furthermore, the viridans streptococci have been increasingly isolated from bronchial aspirates and washings of neutropenic patients with respiratory distress syndrome (Bochud et al. 1994a) and from patients suffering from infective endocarditis (Bayliss et al. 1983). These

streptococci are oral commensals and are of little systemic pathological consequence in the healthy individual, although certain species are associated with the aetiology of dental caries and dental abscess formation (Loesche 1986; Marsh and Martin 1992). The viridans streptococci comprise a predominant part of the oral flora (Marsh and Martin 1992) and, with *Candida*, exhibit the greatest potential to behave as pathogenic organisms originating from the mouth.

The cytotoxic drugs and irradiation which comprise the conditioning regimen prior to bone marrow transplantation damage the oral mucosa, causing mucositis and xerostomia which may be severe during the neutropenic period. This leads to changes in local immunity and increased adherence of bacteria and yeasts to the oral mucosa (Norhagen et al. 1990). Potential microbial pathogens, particularly the oral streptococci, may enter the bloodstream via the damaged mucosa (Heimdahl et al. 1989). In addition, the increased levels of plaque and gingivitis present at this time facilitate the entry of oral organisms into the systemic circulation through the gingival margin (Roberts et al. 1997). Many of the streptococcal isolates from blood cultures have been reported only as viridans streptococci. Others have been further characterised using a commercial system and identified as *S. mitis* or *S. sanguis* (Classen et al. 1990; Burden et al. 1991). There have been several recent and important advances in the taxonomy of the viridans streptococci which are not necessarily reflected in the commercial systems. Subsequent identification schemes include many other taxonomic changes which enable more accurate and reliable characterisation of the organisms of this group (Kilian et al. 1989; Beighton et al. 1991a) The oral streptococci, particularly *S. mitis* and *S. oralis,* collectively the '*oralis* group' of the viridans streptococci, have been frequently isolated from blood cultures of neutropenic patients (Burden et al. 1991; Bochud et al. 1994b).
In addition to the microbial ecology of the peri - transplantation period, changes in bacterial plaque, gingivitis and local mucosal immunity have also been examined and recorded in the present study.

The general hypotheses underlying this study may be enumerated as follows:
1. The oral microflora and mucosal immunity in children undergoing bone marrow transplantation may be different from matched control children at baseline, before starting the conditioning regimen.

2. Changes in the oral microflora and mucosal immunity may be apparent very soon after transplantation. These changes may be a result of the intense immunosuppressive

effect of the conditioning regimen, antibiotic therapy and transfer to parenteral nutrition.

3. There may be differences in the incidence and prevalence of oral mucosal lesions between the children receiving a conditioning regimen of fractionated total body irradiation with chemotherapy and those receiving chemotherapy only, during the period of intense immunosuppression.

Accordingly, the aims and objectives of the study were:

(1) To establish data for the oral microflora in children receiving intensive immunosuppressive treatment for bone marrow transplantation.

(2) To use this data to identify the species of oral bacteria that predominate at the different stages of treatment.

(3) To use this information to develop effective preventive mouthcare regimens.

In detail, the following investigations were carried out:
(1) identification and quantification of the main oral pathogens, with particular reference to the viridans streptococci, *Candida* species, *Enterobacteriaceae* and enterococci
(2) estimation of salivary levels of IgA, secretory IgA, antibodies to *S. mitis* and *S. oralis* in 2 groups of bone marrow transplant children and their matched controls.

The patients investigated at the stated intervals were:

(a) TBI group: conditioning regimen of fractionated total body irradiation and chemotherapy
(b) CTO group: conditioning regimen of chemotherapy only

Investigations for the TBI and CTO groups were carried out at the following specified times:

(i) Baseline, before the conditioning regimen
(ii) 7 days post - transplantation, when the peripheral neutrophil count was $< 10^8/l$
(iii) After the peripheral neutrophil count had risen above $0.5 \times 10^9/l$ on 2 consecutive days
(iv) 120 days post - transplantation

The matched controls were sampled on 2 occasions 120 days apart

REVIEW OF THE LITERATURE

Bone Marrow Transplantation

One of the first attempts at bone marrow transplantation was carried out in 1891 by Brown-Sequard and d'Arsonaval (Quine 1896), a patient suffering from leukaemia being unsuccessfully treated by oral administration of bone marrow. Subsequent attempts were made using intramuscular injection of bone marrow (Schretzenmayr 1937), intramedullary infusion of allogeneic bone marrow (Migdalska 1958)and intravenous injection (Morrison and Samwick 1940). All these attempts failed and it was not until 1957 that intravenous infusion of bone marrow to treat end stage leukaemia demonstrated evidence of engraftment (Thomas et al. 1957). The first long-term survivor with evidence of complete engraftment was reported in 1963 (Mathe et al. 1963).

The conditions which may be treated in this way include the following:

(i) haematological malignancies, e.g. acute lymphoblastic leukaemia (ALL)

(ii) certain non-malignant haematological diseases e.g. aplastic anaemia

(iii) haemoglobinopathies including some cases of thalassaemia and sickle cell anaemia

(iv) disorders of the immune system, e.g. severe combined immunodeficiency, which is characterised by defective cell-mediated and humoral immunity. These patients lack resistance to all types of infection (Forfar and Arneil 1993).

Certain conditions such as some childhood solid tumours in which the marrow is not primarily involved are treated with irradiation or high dose chemotherapy, or a combination of both modalities. These treatments suppress the marrow for prolonged periods and autologous bone marrow rescue is required (Table 1).

Haematopoietic stem cells for transplantation can be derived from a number of sources but most frequently from bone marrow (Table 1). Before infusion of the bone marrow (or other source of haematopoietic stem cells) the patient undergoes a course of intensive treatment. This is the conditioning regimen and the drugs used for this depend on the disease being treated (Appendix 2). For some of the solid childhood tumours, such as neuroblastoma, high dose chemotherapy will be used for conditioning followed by autologous bone marrow transplantation (Table 1). Prior to allogeneic transplantation for haematological malignancies, such as acute lymphoblastic leukaemia, chemotherapy is combined with fractionated total body irradiation (Table 1). The main aim of this is eradication of residual disease and also immunosuppression, which will allow the new bone marrow to grow. This necessarily involves

Table 1 : Sources of Haematopoietic Stem Cells

(1) Bone Marrow
(a) Autologous
Patient's own bone marrow, which is harvested and stored for re-infusion
(b) Allogeneic
Identical twin - syngeneic HLA matched sibling Other family member who is not HLA identical Unrelated donor, but compatible
(2) Peripheral Blood Stem Cells
Haematopoietic stem cells are stimulated by administration of recombinant growth factors, causing these cells to move from the bone narrow into the peripheral blood, where they are collected by a cell separator. They are then stored and re-infused at a later date
(3) Umbilical Cord Blood Stem Cells
This is a relatively small source of stem cells

ablation of the patient's own bone marrow. The donor bone marrow can then be infused and the stem cells settle in the bone marrow spaces where reconstitution of the haematopoietic system takes place.

Types of Bone Marrow Transplant

Autologous Bone Marrow Transplant

The patient's own bone marrow is harvested, stored and then re-infused after the conditioning regimen. This type of transplant is used in the treatment of some solid childhood tumours, for example neuroblastoma and rhabdomyosarcoma, when the treatment necessary to eradicate the disease also destroys the bone marrow. This procedure is also referred to as bone marrow rescue. Autologous bone marrow rescue is occasionally used following treatment for some leukaemias. However, there is a risk that the leukaemia may not have been completely eliminated and some malignant cells may be re-infused (Gale and Butturini 1989). Although the recipients are profoundly immunosuppressed as a result of the conditioning regimen, there are no compatibility problems or graft versus host disease with an autologous transplant.

Allogeneic Bone Marrow Transplant

An allogeneic transplant is usually derived from a matched sibling donor. This may be referred to as allogeneic bone marrow rescue, following intensive treatment for the acute leukaemias, immune disorders and some haemoglobinopathies. The situation may arise where a transplant is the only effective form of treatment but no histo - compatible sibling donor, or any other suitable family member is available. A search of national and international bone marrow registries is then carried out to find an unrelated but compatible donor.

Peripheral Stem Cell Transplant

This type of transplant is rapidly increasing in use and has now largely replaced autologous bone marrow transplantation. An advantage is that engraftment takes place more rapidly, which reduces the patient's time in hospital and consequently the risk of nosocomial infection.

Umbilical Cord Blood Stem Cells

Although there are haematopoietic stem cells in blood from the umbilical cord, the number is small and transplants using these stem cells are limited to babies and small children.

Graft Versus Host Disease

One of the main problems with allogeneic transplants is graft versus host disease (GvHD), which may occur even though the donor is HLA compatible (Mitsuyasu et al. 1986; Powles 1990). Symptoms vary from a mild itchy rash on the hands and feet to skin desquamation and severe diarrhoea and liver failure. The risk is minimised by the use of immunosuppressive drugs. These include methotrexate on designated days immediately following transplantation and cyclosporin A, which is continued for about 6 -12 months post - transplantation, depending on the underlying medical condition and type of transplant.
The phenomenon of GvHD appears to have an anti - leukaemic effect (Champlin 1990; Soutar and King, 1995). In part, this may be because the graft recognises any residual tumour cells as 'non-self' and destroys them. The problem of graft rejection and GvHD is more severe for the recipient of bone marrow from an unrelated donor (Mattsson 1991). To minimise GvHD, the patient's T- cells are depleted *in vivo* before the conditioning regimen and the donor marrow is T- cell depleted *in vitro*, before infusion.

Clinical Considerations

Bone marrow transplantation is a serious undertaking and is used when it is the only effective form of treatment available. The antecedent conditioning regimen profoundly suppresses the bone marrow and the recipient rapidly becomes neutropenic and immunosuppressed. The new bone marrow is then introduced and the patients are nursed in isolation cubicles with laminar air flow until there is evidence of reconstitution of the haematopoietic system. This usually takes place within 2 to 6 weeks of transplantation and is indicated by recovery of the neutrophils which appear in increasing numbers in the peripheral circulation. When the neutrophil count rises above 0.5 x 10^9 /l the isolation procedures are gradually relaxed. The use of granulocyte colony stimulating factor reduces the length of the neutropenic period (Hann 1994).

The estimated number of paediatric bone marrow transplants in the United Kingdom for 1994 was 300 (J. Cornish, Bristol Children's Hospital, personal communication). This includes the increasing number of peripheral blood stem cell transplants.

Dental Disease

Dental Caries, Bacterial Dental Plaque and Gingivitis

Dental caries is a disease of the mineralised tissues of the teeth. The main aetiological factors are a susceptible tooth surface, the ingestion of fermentable carbohydrate at frequent intervals and bacteria interacting over a period of time. Dental caries will only develop in the presence of all 4 factors (Newbrun 1969).

Bacterial dental plaque is a bio - film consisting of bacteria, metabolites and host - derived macromolecules which form on all tooth surfaces. The genesis of plaque is an amorphous organic pellicle which forms on the clean enamel surface. The main components of the pellicle are salivary glycoproteins which provide a site for the attachment of bacteria to the tooth surface. The early bacteria are mainly cocci , particularly streptococci, and actinomyces which multiply and produce extracellular material to which other bacteria adhere. Within a few days the plaque is thicker and now contains gram - negative rods and filamentous bacteria. The accumulation of dental plaque is the main aetiological factor in the development of gingivitis. There have been many reports indicating a decrease in gingivitis and periodontitis when effective plaque removal is instituted (Axelsson and Lindhe 1977).

Chronically infected, untreated or inadequately restored teeth can lead to serious systemic sequelae, for example opportunistic infection, in immunosuppressed patients especially during a period of immunosuppression as a result of chemotherapy (Peterson et al. 1987; Karr and Kramer 1992; Cheatham and Henry 1994). Although there are few data for dental disease in bone marrow transplant children, there are a number of reports for children in remission from ALL. Although children suffering from ALL are not directly comparable with children undergoing bone marrow transplantation, also suffering from ALL, the children initially will have undergone similar chemotherapy regimens to achieve remission. There was no difference in dental caries experience in 52 children in remission from cancer and their siblings (Nunn et al. 1991). There was also no difference in dental caries in another group of 68 children with ALL who were treated with 3 different modalities compared with the normal population (Sonis et al. 1995). The dental caries status of 45 Finnish children suffering from ALL were compared with age and gender matched controls (Pajari et al. 1995). Twenty two of the children were treated with combination chemotherapy (CT group) and 4 others with bone marrow transplantation (BMT group). There were no differences in the decayed, missing and filled permanent tooth surfaces (DMFS) and decayed, missing or filled permanent teeth (DMFT) between the CT and BMT groups and their respective controls. These indices were significantly greater in the 19 children who were treated with combination chemotherapy and cranial irradiation (CNS group) compared with the controls. As a whole, the caries experience of the ALL children was significantly greater than that of the controls. Caries experience in the anterior teeth of 12 year olds was also found to be significantly greater in the leukaemia group and that of the BMT children greater than the CT and CNS groups. The results of the BMT children should be interpreted with caution because of the very small group size.

The DMFT was also increased in a group of 14 - 17 year old adolescents who had been treated with chemotherapy for cancer and survived for a median period of 9 years (Dens et al. 1995). The investigators felt that this was not due to the effects of cytotoxic treatment or neglected oral hygiene but to lack of professional dental care during the chemotherapy and poor follow up. A group of 41 American children suffering from a variety of haematological and immunological disorders, haemoglobinopathies and solid tumours, undergoing bone marrow transplantation were examined before the conditioning regimen (Berkowitz et al. 1987). Thirteen children presented with dental caries. There was a total of 15 deciduous and 1 permanent non-vital teeth and 7 large cavities in permanent molar teeth. As there was no control group, it is not possible to make an objective comparison. Fifteen children from a further group of 43 children with diagnoses of haematological malignancy or solid tumours

were found to have untreated caries before starting chemotherapy (Fayle and Curzon 1991). Again, there was no control group for comparison. A different group of children in remission from ALL were found to have a significantly lower number of decayed deciduous teeth than the controls, but no differences in the missing or filled values (Fleming and Kinirons 1993). There was also a significantly greater number of extracted permanent teeth in the ALL group, but no difference in the decayed or filled indices. Thirty children were followed at monthly intervals between 6 months and 1 year during chemotherapy for ALL. No carious lesions developed during this time (O'Sullivan et al. 1994). Seven children from a group of 22 developed new carious lesions 1 year after bone marrow transplantation but there was no significant difference between those who underwent pre-transplant conditioning with TBI and chemotherapy and chemotherapy only (Dahllof et al. 1988a). Nineteen children who were conditioned with TBI and chemotherapy for bone marrow transplantation and a further 57 children treated with chemotherapy were followed up 3 years post - transplantation and post-treatment. There were no significant differences in the caries experience for both deciduous and permanent teeth (Nasman et al. 1994).

Several studies have found that both adults and children conditioned with TBI have reduced saliva flow for variable periods of time (Heimdahl et al. 1985; Dahllof et al. 1988a; Dahllof et al. 1988b; Jones et al. 1992; Nasman et al. 1994; Chaushu et al. 1995), which increases susceptibility to dental caries (Scully and Cawson 1982; Carl 1995). In 2 groups of adult patients, the parotid saliva flow rate was less than the pre-conditioning values for up to 5 months post - transplantation (Chaushu et al. 1995) and was found to recover slowly over time in the other group (Jones et al. 1992). In contrast, the salivary secretion of children who had survived more than 3 years post - transplantation was still significantly lower than matched controls (Nasman et al. 1994), strongly indicating long-term if not some permanent damage to the salivary glands.

One group of investigators reported that no differences were found in the plaque and gingival index of ALL children and those suffering from other malignancies in remission. (Fleming and Kinirons 1993; Dens et al. 1995). Other workers found no difference in the gingival health but an increased plaque score in children in remission (Nunn et al. 1991). Children below the age of 5 years receiving 2400 cGy of cranial irradiation for treatment of ALL had higher plaque and periodontal index scores than those treated with chemotherapy alone or with chemotherapy and 1800 cGy of cranial irradiation. They were therefore considered to be at greater risk of developing periodontal disease (Sonis et al. 1995). In children and adults under

treatment with cytotoxic drugs using chlorhexidine or a placebo mouthrinse, the plaque and gingival indices were no different between the 2 groups at the start or at the midpoint and improved towards the end of treatment (Weisdorf et al. 1989). The patients were given good oral care throughout, which reduces oral bacterial loading. Adults using chlorhexidine mouth rinse were compared with those using placebo for 60 days from the first day of the chemoradiotherapy conditioning regimen (Ferretti et al. 1987). There were significant reductions in the plaque and gingivitis scores of the chlorhexidine group. Thirty days after discontinuation of chlorhexidine plaque and gingivitis scores approached baseline levels. Adults undergoing chemotherapy and rinsing with chlorhexidine were found to have better dental health than adults not using chlorhexidine (McGaw and Belch 1985). During a 2 year prospective follow-up of 214 children suffering from cancer (Childers et al. 1993) the children with leukaemia were found to have a significantly greater gingivitis score than those with sarcomas or other solid tumours.

Effects of the Conditioning Regimen on the Oral Mucosa

The effects of chemotherapy, particularly methotrexate or chemotherapy and irradiation together are well documented (Lockhart and Sonis 1981; Carl and Higby 1985; Seto et al. 1985; Barratt, 1987; Dahllof et al. 1988b; Dahllof et al. 1989; Bergmann et al. 1990; Wahlin 1990; Fayle and Curzon 1991; Childers et al. 1993; Carl, 1995). These cytotoxic effects lead to changes in local immunity and increased accumulation of micro - organisms on the mucosal surface (Norhagen et al. 1990). The clinical changes range from small areas of erythema on the mucosa to swelling of the salivary glands and lips, ulceration of the lips, tongue, mucosa, anterior pillar of fauces and the pharynx and pseudomembrane formation. Although patients who are herpes simplex virus positive are treated with prophylactic acyclovir, occasionally there is re-activation of the virus and the appearance of lesions in and around the mouth. The oral complications are most severe during the period of intense immunosuppression and neutropenia following the conditioning regimen. It is during this time that the child is most at risk from opportunistic infection. The oro - pharyngeal mucosa is inflamed and because of pain and discomfort oral hygiene procedures are difficult for many children and their carers to manage effectively. The integrity of the surface layer of the mucosa may be impaired and this together with increased levels of bacterial plaque and gingival inflammation facilitates entry of endogenous bacteria into the systemic circulation.

Oral Flora

The mouth has a resident microflora with a characteristic composition which, in the healthy individual, is in balance with the rest of the body (Marsh 1989). Under certain circumstances, for example during periods of intense immunosuppression, the components of the oral microflora can become opportunistic pathogens. Bacteraemias and septicaemias of endogenous origin are well recognised in these patients. An increasing number of these episodes are associated with gram - positive bacteria, particularly the viridans streptococci. Other taxa, for example staphylococci, *Candida* species, *Enterobacteriaceae* and enterococci also cause infections in immunosuppressed patients, but the main emphasis of this work is on the potential role of the viridans streptococci.

Viridans Streptococci

The first micro - organisms to colonise the mouth of the neonate are the pioneer species which become the components of the pioneer microbial community (Marsh and Martin 1992). The major components of the oral flora of the neonate are streptococci although there are fewer species in the neonate mouth than in the adult mouth and the bacterial numbers are less. A recent study reported that *S. mitis* biovar I and *S. oralis* together, comprised 55% of the pioneer species, *S. salivarius* formed 25.3% with a combination of *S. anginosus*, *S. mitis* biovar II and *S. sanguis* making up another 11.4% (Pearce et al. 1995). The environment is gradually modified by the metabolic activities of the pioneer species and by tooth eruption which facilitate the colonisation of the oral cavity by other bacterial taxa.

Identification and Classification of the Viridans Streptococci

The human species of viridans streptococci were so named because many, but not all, produce greenish discoloration of blood agar (alpha haemolysis). These streptococci have recently been divided into four phylogenetic groups on the basis of 16S rRNA sequence analysis (Kawamura et al. 1995). These have been designated the mutans group (*S. mutans* and *S. sobrinus*); the mitis group (*S. mitis, S. oralis, S. sanguis, S. parasanguis* and *S. gordonii*); the salivarius group (*S. salivarius* and *S. vestibularis*) and the anginosus group (*S. intermedius, S. constellatus* and *S. anginosus*). A short biochemical scheme differentiating the currently recognised species of viridans streptococci is shown in Table 2.

Many attempts have been made to identify and classify the human viridans streptococci (Sherman et al. 1943; Carlsson 1968; Colman 1968; Colman and Williams 1972; Kral and Daneo - Moore 1981; Bridge and Sneath 1983; Kilian et al. 1989; Beighton et al. 1991a). An

identification scheme was proposed for the oral viridans streptococci based on their physiological characteristics (Colman and Williams 1972), recognising 5 species *S. mutans, S. mitior, S. sanguis, S. salivarius* and *S. milleri. S. pneumoniae* was also included although these strains may be easily differentiated from the viridans streptococci and are rarely isolated from the oral cavity. Almost the same physiological tests were used by a different group of investigators (Hardie and Bowden 1972) who concluded that these 5 oral species could be presumptively identified by 7 phenotypic characteristics. These were acid formation in mannitol and sorbitol broth; hydrolysis of aesculin and arginine; production of acetoin from glucose; hydrogen peroxide production on chocolate agar; polymer production from sucrose. A further scheme was proposed in America, using clinical isolates and type strains, based on serology, a series of carbohydrate fermentation tests, hydrolysis of aesculin and arginine and other biochemical tests (Facklam 1977). The viridans streptococci were described as *S. mutans, S. mitis, S. sanguis* I, *S. sanguis* II, *S. salivarius, S.* MG - *intermedius* and *S. anginosus* - *constellatus*. The characteristics of *S. uberis* and *S. acidominimus* which are species rarely encountered in humans, were also included in this scheme. Three main groups of investigators were in general agreement concerning the identification of *S. mutans, S. salivarius* and what is referred to as typical *S. sanguis.* (Carlsson, 1968; Colman and Williams 1972; Facklam 1977). Strains identified as *S. sanguis* and *S. mitior* by Colman and Williams (1972) were described as *S. sanguis* I, II or *S. mitis* by Facklam (1977). The 1980 Approved Lists of Bacterial Names (Skerman et al. 1980) included 7 species of oral viridans streptococci. These were *S. mutans, S. mitis, S. sanguis, S. salivarius, S. anginosus, S. constellatus* and S. *intermedius.*

Mutans Group (*S. mutans* and *S. sobrinus*)

The mutans streptococci are the principle micro - organisms implicated in the initiation of caries in humans (Loesche 1986). Although seven separate species have been described within the mutans group (Beighton et al. 1991b) only 2 of these are isolated from human saliva and dental plaque. These species are *S. mutans* and *S. sobrinus.* Several biochemical schemes have been proposed to differentiate the various species of mutans streptococci and related species (Hardie and Bowden 1972; Coykendall,1974; Kral and Daneo - Moore 1981). These schemes have been based on small numbers of representative strains. *S. mutans* strains have been divided into 2 biotypes according to their ability to ferment melibiose. Melibiose - positive *S. mutans* hydrolysed α - glucoside, β - glucoside and α - galactoside and fermented a wide range of carbohydrates (Beighton et al. 1991b). Melibiose - negative fermenters hydrolysed

α - glucoside and α - galactoside and fermented amygdalin and raffinose less frequently. Aesulin hydrolysis was also less frequent. *S. sobrinus* demonstrated a poor overall ability to ferment the same range of carbohydrates; very few strains hydrolysed β - glucoside and none hydrolysed α - galactoside.

Mitis Group (*S. oralis, S. mitis, S. sanguis, S. parasanguis* and *S. gordonii*)
S. oralis was first designated as a distinct species in 1982 (Bridge and Sneath 1982) and the description first amended in 1985 (Kilpper - Balz et al. 1985). As a result of DNA homology, chemical cell wall analysis and physiological tests it was proposed that some strains which had previously been assigned to *S. sanguis* II (Facklam, 1977) and *S. mitior* (Colman and Williams 1972) should be re - assigned to the taxon *S. oralis* The description of *S. oralis* was emended again in 1989 following an intensive investigation of biochemical and physiological characteristics and serological reactivity (Kilian et al. 1989). Twenty one strains were assigned to the taxon *S. oralis* including the type strain NCTC 11427 and a number of other reference strains which were formerly described as *S. sanguis* II or *S. mitior*. Most of the strains produced extracellular polysaccharide, IgA protease and neuraminidase. Aesculin and arginine were not hydrolysed and inulin was not fermented. DNA - DNA hybridisation data for 6 strains (Kilian et al. 1989) confirmed that *S. oralis* was genetically homogeneous and well separated from other species of the viridans streptococci, including *S. mitis*. Further work demonstrated that *S. oralis* species, unlike some other viridans streptococci were unable to bind salivary α - amylase (Kilian and Nyvad 1990).

S. oralis is able to bind glycoproteins and mucins in the salivary pellicle (Hsu et al. 1994) and is thus an important early coloniser of enamel surfaces (Nyvad and Kilian 1990). *S. oralis* produces IgA protease (Kilian et al. 1989) and a range of glysoside activities including neuraminidase, β - *N* - acetylglucosaminidase and β - galactosidase (Kilian et al. 1989; Beighton et al. 1991a). This species is able to liberate carbohydrates from the oligosaccharide side chains of mucin - like glycoproteins (Beighton et al. 1995) and can survive on this source of carbon. *S. oralis* and *S. mitis* (biovars I and II) were formerly identified as a single species, *S. mitior* (Colman and Williams 1972). One of the reasons for this was probably because the cell walls of *S. mitis* and *S.oralis* have a similar structure. They both contain a ribitol teichoic acid and lack a significant amount of rhamnose and gylcerol teichoic acid (Colman and Williams 1965; Colman and Williams 1972; Rosan 1978). Subsequent investigation of several strains, which had been classified as *S. oralis* and *S. mitis* (biovar I)

Table 2: Differential Phenotypic Characteristics of the Viridans Streptococci Adapted from Beighton et al. (1991a)

Enzyme Activity	Species of Viridans Streptococci												
	A	B	C	D	E	F	G	H	I	J	K	L	M
β- Fucosidase	d	d	-	-	-	-	-	d	+	-	-	-	-
β-*N*-Acetylgal-dase	-	+	d	+	+	-	-	-	+	-	-	-	-
α-Neuraminadase	-	-	-	-	+	d	-	-	+	-	-	-	-
α-L-Fucosidase	-	d	+	+	-	-	-	-	-	-	-	-	-
β-*N*-Acetlyglu-dase	d	+	+	+	+	-	-	-	+	-	-	-	-
α-Glucosidase	-	+	d	-	+	+	d	d	+	+	-	+	+
β-Glucosidase	d	d	+	-	-	-	-	d	d	-	d	+	-
α-Arabinosidase	-	d	-	-	-	-	+	+	-	-	-	-	-
Acid Produced From:													
Amygdalin	d	d	+	-	-	-	d	d	d	d	+	+	-
Inulin	d	-	+	-	-	d	-	d	-	-	-	+	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	+	+
N-acetylglucosamine	+	+	+	+	+	+	+	+	+	d	d	+	-
Raffinose	d	d	-	-	d	+	-	d	-	-	d	+	-
Hydrolysis of:													
Arginine	+	+	+	d	-	-	-	-	+	+	+	-	-

A, *S. sanguis*; **B**. *S. parasanguis*; **C**, *S. gordonii*; **D**, *S. crista;* **E**, *S.oralis*; **F**, *S. mitis*; **G**, *S. vestibularis*; **H**, *S. salivarius*; **I**, *S. intermedius*; **J**, *S. constellatus;* **K**, *S. anginosus*; **L**, *S. mutans*; **M**. *S. sobrinus*

(+, > 85% of strains positive; d, 15-85% of strains positive; < 15% of strains positive)

β-N-Acetylgal-dase = β-N-Acetylgalactosaminidase; β-N-Acetylglu-dase = β-N-Acetylglucosaminidase

demonstrated that these 2 species exhibited only 40 - 60% DNA homology and were well separated both biochemically and serologically (Kilian et al. 1989). More recent work based on 16S rRNA sequencing demonstrated 99.93 % homology between *S mitis* and *S. oralis* and also *S. pneumoniae* (Kawamura et al. 1995) indicating that the 3 species are closely related.

The species *S. crista* has been described as *S. sanguis* with tufts of fibrils (Handley et al. 1985), the 'CR' group (Douglas et al. 1990) or 'tufted fibril group' (Beighton et al. 1991a). The results of DNA - DNA hybridisation tests on 4 strains demonstrated a relatively close relationship between *S. sanguis* and the tufted strains. However the high level of base sequence homology within the 4 strains and the phenotypic characteristics demonstrated that this group is a separate species of viridans streptococcus (Handley et al. 1991). *S. crista* hydrolyses arginine but not aesculin. β - *N* - acetylglucosaminidase and β - *N* - acetylgalactosaminidase are produced and some strains produce β - galactosidase. Salivary α - amylase is bound. This species is probably a member of Mitis group.

The name *S. sanguis* was proposed by White and Niven (1946) for a viridans type of streptococcus which hydrolyzed arginine and usually aesculin, fermented inulin, silacin, lactose and trehalose and produced extracellular dextran from glucose. This particular streptococcal strain was isolated from the blood of patients with subacute bacterial endocarditis and was formerly referred to as 'Streptococcus S.B.E.' Both biochemical and serological studies (Carlsson 1968; Colman and Williams 1972; Rosan, 1978; Kilian et al. 1989) and electrophoretic patterns (Whiley et al. 1982) suggested that *S. sanguis* was a heterogenous species. Two separate genotypes were demonstrated by Coykendall and Specht (1975) as a result of DNA base sequence homology. They suggested that one group should be named '*S. sanguis* subsp. *sanguis*' and the second group '*S. sanguis* subsp. *carlsonii*'. Further work (Kilian et al. 1989) identified 2 major clusters which contained a number of strains representing the genotypes described by Coykendall and Specht (1975). The first cluster of *S. sanguis* relates to '*S. sanguis* subsp. *carlsonii*' and was divided into 4 biovars differentiated by several biochemical and serological characteristics. The type strain, NTCT 7863 was assigned to biovar 1 and was different from other *S. sanguis* strains because it was unable to ferment several carbohydrates typical of the species. The second cluster of organisms, which was described as *S. gordonii* sp.nov. includes strains formerly grouped together as '*S. sanguis* subsp. *sanguis*' and also the type strain for *S. mitis,* NTCT 3165 (Skerman et al. 1980). *S. gordonii* was later defined as a separate species and 3 biotypes of *S. sanguis* described (Beighton et al. 1991a).

S. parasanguis was recognised as an atypical viridans streptococcus isolated from clinical samples (Whiley et al. 1990a). All *S. parasanguis* strains produced β - *N* - acetylgalactosaminide, α - glucoside and some produced α - *L* - fucoside (Beighton et al. 1991a). None of the strains tested fermented inulin. By using these biochemical tests, *S. parasanguis* can be differentiated from *S. sanguis* and *S. gordonii.*

The taxon *S. gordonii* comprises the '*S. sanguis* subsp. *sanguis*' and the previous type strain for *S. mitis,* NCTC 3165, that were excluded from the true *S. sanguis* group. *S. gordonii* was described as a homogeneous taxon which clearly differed from *S. sanguis* on the basis of DNA base composition and homology. *S. gordonii* strains ferment inulin, hydrolyse arginine and aesculin and produce extracellular polysaccharide. Other differences from *S. sanguis* were absence of IgA1 protease activity and the production of β - glucosaminidase, β - mannosidase, α -*L*- fucosidase and strong alkaline phosphatase activity. Later work with several strains of *S. sanguis* and *S. gordonii* demonstrated very clear differences between the 2 species. All strains of *S. gordonii* fermented amygdalin, arbutin and inulin and exhibited β - *N* - acetylgalactosaminidase, β - *N* - acetylglucosaminidase, α - glucosidase and β - galatosidase activity (Beighton et al. 1991a). 16S rRNA sequence comparisons demonstrated that *S. gordonii* was more closely related to *S. oralis, S. mitis* and *S. pneumoniae* than to *S. sanguis* and *S. parasanguis* (Kawamura et al. 1995).

Salivarius Group (*S. salivarius* and *S. vestibularis*)

S. salivarius was first described in 1906 (Andrewes and Horder, 1906)and comprises 25.3% of the pioneer streptococci in neonates (Pearce et al. 1995). *S. salivarius* is a relatively homogeneous group which is well separated from other species and can be clearly identified (Carlsson, 1968; Colman and Williams, 1972; Bridge and Sneath, 1983; Kilian et al. 1989). The majority of strains produce the extracellular polysaccharide, fructan from sucrose. Most strains of *S. salivarius* hydrolyse 4 - methylumbelliferyl - linked α - arabinoside, many ferment raffinose and a few strains also ferment melibiose. A similar new species, *S. vestibularis*, was described in 1988 (Whiley and Hardie, 1988) and although *S. salivarius* is closely related to *S. vestibularis* DNA - DNA hybridization demonstrated that they were sufficiently different to justify classification as separate species. All strains of *S. vestibularis* produced α - arabinosidase but differed from *S. salivarius* with absence of β - *D* - fucosidase or β - glucosidase production and the inability to ferment inulin, melibiose or raffinose (Beighton et al. 1991a).

Anginosus Group (*S. anginosus, S. constellatus* and *S. intermedius*)

The anginosus group of streptococci includes the *S. milleri* strains originally proposed by Guthof (1956) Strains identified as *S. milleri* by Colman and Williams (1972) were divided into 2 further groups by Facklam (1977) on the basis of lactose fermentation. The lactose positive strains were designated *S.* MG - *intermedius* and those which were lactose negative, *S. anginosus* - *constellatus.* The major difference between the American (Facklam, 1977) and British (Colman and Williams 1972) identification systems regarding *S. milleri* was in describing the beta-haemolytic and non-haemolytic strains as a single species. More recently, 3 DNA homology groups were identified following extensive DNA - DNA hybridisation studies (Whiley and Hardie, 1989) and phenotypic characterisation on a representative group of clinical and reference strains of the *S. milleri* group (Whiley et al. 1990b). As a result of this work, *S. anginosus, S. constellatus* and *S. intermedius* were recognised as separate species and their descriptions emended (Beighton et al. 1991a).

Opportunistic Infection

One of the most serious problems for immunocompromised patients is opportunistic bacterial, fungal or viral infection. For bone marrow transplant patients the risk is greatest during the period of intense immunosuppression which occurs as a result of the conditioning regimen, until there are signs of engraftment indicated by recovery of the peripheral neutrophil count above 0.5 x 10^9/l. *Enterobacteriaceae* and *Pseudomonas aeruginosa* are 2 of the major organisms which cause septicaemia in neutropenic patients and are associated with high mortality rates (Rogers 1989). Gram positive bacteria, particularly the viridans streptococci, have become increasingly important in bacteraemias and septicaemias in neutropenic patients (Bochud et al. 1994a).

Gram - Negative Bacteria and Enterococci

The importance of environmental and nosocomial infections are well recognised and the association between hospitalisation and oro - pharyngeal carriage of gram - negative bacteria widely reported (LeFrock et al. 1979; Shanson 1989). Particular groups of patients are considered to be at high risk of oral colonisation, for example immunosuppressed patients, head and neck cancer patients receiving post-operative irradiation and those who are generally debilitated (Spijkervet 1989). In a study undertaken in America, the aerobic faecal and oro - pharyngeal bacterial flora were examined in three groups of individuals (LeFrock et al. 1979). The first group was hospitalised (hospitalised group), the second group was hospitalised and

treated with antibiotics (antibiotic group) and the third group were non-hospitalised controls. A normal throat flora was predominant in all 3 groups at the initial examination. After 21 days, components of the normal oro - pharyngeal flora were present in all the patients in the hospitalised group but 1 or more species of gram - negative bacteria were predominant in 22.7%. In the antibiotic group, gram negative organisms predominated in 47.1% of the patients and there were no demonstrable components of the normal oro - pharyngeal flora in 17.1%. The throat flora of the controls was predominately normal after 21 days. This suggests that hospitalisation alone may be associated with oro -pharyngeal acquisition of gram - negative bacteria and the gastro - intestinal tract is the probable source. However, the acquisition of gram - negative bacteria is both endogenous and exogenous. Exogenous sources include food, water, soap, the hands of hospital staff and hospital equipment (Minah et al. 1986; Shanson 1989). The sharing of utensils and equipment between immunosuppressed patients who are not cared for in isolation facilitates the spread of these bacteria.

The oral flora of 12 patients undergoing cytotoxic treatment was compared with a healthy control group. Carriage of *E. coli* and *Klebsiella* species and *E. faecalis* was found to increase during the course of treatment (Main et al. 1984). It was conjectured that these organisms may constitute a potential reservoir of opportunistic infection. Three further studies reported increases in oral colonisation with gram - negative bacteria in immunosuppressed patients during cytotoxic therapy for cancer (Samaranayake et al. 1984; Minah et al. 1986) and haematological malignancies (Bergman 1988). Oral flora changes were examined in a group of 20 adult leukaemia patients before, during and after cytotoxic treatment (Wahlin and Holm 1988). Before induction therapy, oral gram - negative bacteria were isolated from 5 patients, 4 of whom had been transferred from other hospitals. During the whole treatment period gram - negative bacteria were isolated from each of 14 patients on at least 1 occasion. There were no differences in isolation of oral gram - negative bacteria from these patients and from comparable control subjects, not suffering from leukaemia, who were also hospitalised. This again strongly suggests that it is the hospital environment, rather than the clinical condition that predisposes to the oral carriage of gram - negative bacteria. It was also found that patients who mouthrinsed with chlorhexidine had a tendency towards increased numbers of enterococci, *Enterobacteriaceae* and / or *Pseudomonas* (Wahlin 1989). Further work was carried out to assess the effect of antibacterial treatment on the prevalence of oral *Enterobacteriaceae* and *Pseudomonas* species (Wahlin et al. 1991). The conclusion was that resistance to antibacterial drugs was not an important factor in facilitating the establishment of oral gram - negative bacteria in hospitalised leukaemia patients. These studies reported the presence of gram - negative bacteria in the mouth but do not mention them as a cause of infection. Under these

circumstances they are most likely to be transient oral colonisers. Contrary to this, other workers state that the presence of oral gram - negative bacteria is not transient, but opportunistic colonisation associated with 'abnormal' carriage which could contribute towards morbidity and mortality (Martin and van Saene 1992).

The presence of gram-negative bacteria may complicate oral healing and the endotoxins released by the dead bacteria may give rise to a variety of both systemic and local effects on the host. Oral infection caused by gram - negative bacteria has been reported. In a group of adult leukaemia patients 25% of oral infections were caused by gram - negative bacteria. The patients affected had periodontal disease and the infections occurred in the associated pockets (Dreizen et al. 1982). Although most reported investigations are of adult patients there are a few studies which have concentrated on children with leukaemia. In one study children in first remission from leukaemia did not have the high prevalence of oral gram - negative bacteria that had been reported in hospitalised patients on chemotherapy (Scully and MacFarlane 1983), a finding which again supports the acquisition of bacteria within the hospital environment. In a later study (O'Sullivan et al. 1993) oral flora changes in 34 children were investigated during cytotoxic treatment for acute leukaemia and gram – negative bacteria were isolated from the mouths of just 7 children. Gram - negative bacteria were isolated from 3 children during treatment phases, from 2 more during an infection and from a further 2 during the maintenance period. This was considered to be a low incidence of oral colonisation with gram - negative bacteria.

Fungal Species

Fungal infections are an important cause of morbidity and mortality in neutropenic patients suffering from haematological malignancy (Ellis et al. 1994) or following bone marrow transplantation (Ellis et al. 1994; Hunter et al. 1995) with a proven incidence of 10% at autopsy (Tollemar et al. 1993). *Candida* species are mainly commensals with an oral carriage rate of 20% to 60% (Epstein et al. 1980) and gut carriage of approximately 70% (Odds 1988) in the normal population. The oral carriage in 6 to 12 year old healthy children was found be 65% (Berdicevsky et al. 1984) and 41.1% in a different group of 7 to 17 year olds (Arendorf and Crawford 1989). *Candida* species, particularly *C. albicans* account for most of the serious fungal infections in immunocompromised patients (Haron et al. 1987). Routine microbiological screening was carried out on 153 adults undergoing chemotherapy or bone marrow transplantation for haematological disease. The patients were all treated with prophylactic antifungal agents. Sixty five percent of the yeasts isolated from the oral samples

were *C. albicans*. Mixed candidal populations including *C. glabarata* and *C. tropicalis* and C. *albicans* biotypes were isolated from 25 of the oral samples (Odds et al. 1989). A review of 37 reports describing 1,591 cases of systemic *Candida* infection in oncology patients over a 40 year period revealed that 46% of cases were caused by non - *albicans* species (Wingard 1994). Other species included *C. tropicalis, C. krusei, C. parasopolis* , and *C. glabrata.* In a different group of 665 children and adults who underwent bone marrow transplantation during an 8 year period, *Candida* infection was diagnosed in 76 individuals during the first year post - transplantation (Verfaillie et al. 1991). Sixty patients from whom *Candida* species were isolated died and 19 of the deaths could be directly attributed to *Candida* infection. Colonisation with one species or a combination of two or more species was reported and the main species isolated were *C. albicans* 57%, *C. glabrata* 17%, *C. tropicalis* 5.4%, *C. parasopolis* and *C. lusitaniae.* The incidence of *Candida* infection was significantly greater in patients who were treated with combination GvHD prophylaxis including methotrexate, antithymocyte globulin, prednisone, T cell-depleted bone marrow graft compared to methotrexate alone. There was a lower incidence of *Candida* infection in the methotrexate group, which was attributed to the younger age of the recipients, median 10.6 years. *Candida* carriage in two groups of children suffering from ALL and a healthy control group was monitored (Arendorf and Crawford 1989). *Candida* was detected in the mouths of all the children under treatment with chemotherapy and the prevalence was greater in the remission group than the controls group, although not significantly.
In a series of 393 children treated with autologous bone marrow rescue over a 12 year period (Besnard et al. 1993), 14 developed disseminated *Candida* infection within 3 months. Eleven children recovered which was considered to be a higher survival rate than that found in adults. This was reported as a favourable outcome related to the young age of the patients, absence of GvHD, no irradiation in the conditioning regimen and the use of anti-fungal prophylaxis. There was no mention of oral *Candida* isolates. Most of the infections were caused by *C. albicans*, but *C. tropicalis* was isolated from 6 children.

It is common practice to administer antifungal prophylaxis as the newer oral azoles are very effective. An example is fluconazole which is a triazole and has a broad spectrum activity against fungal pathogens (Ellis et al. 1994; Epstein et al. 1996). The widespread use of fluconazole has led to increasing concern regarding the potential for selection of resistant strains of *C. albicans* or other *Candida* species, for example *C. krusei* (Goodman et al. 1992). It has been suggested that itraconazole be substituted for fluconazole and other antifungal

prophylaxis for patients who are likely to be neutropenic for more than 20 days and as prophylaxis against *Aspergillus* species (Working Party Report 1993).

The Viridans Streptococci and Systemic Infection

The viridans streptococci were first identified as the causes of septicaemia in cases of childhood cancer in 1978 (Hoecker et al. 1978). During the almost 2 decades since this report, there has been an increase in the number of reports of bacteraemias caused by gram - positive cocci in febrile neutropenic patients. This has been attributed to a number of factors including the use of indwelling venous catheters, such as Hickman lines (Cohen et al. 1983; Villablanca et al. 1990; Donnelly 1995), oro - pharyngeal mucositis related to antineoplastic treatment including irradiation, methotrexate and cytosine arabinoside (Ringden et al. 1983; Kamp 1986; Heimdahl et al. 1989; Classen et al. 1990; van der Lelie et al. 1991; Rossetti et al. 1997), prolonged neutropenia (Burden et al. 1991; Martino et al. 1995; Richard et al. 1995) and use of co-trimoxazole and quinolone antimicrobials (Henslee et al. 1984; Classen et al. 1990; Richard et al. 1995). A possible route of entry into the systemic circulation is through the oral mucosa which is damaged by the effects of chemotherapy, particularly, methotrexate and irradiation (Cohen et al. 1983; Bostrom and Weisdorf, 1984; Heimdahl et al. 1989; Donnelly 1995). A review of bacteraemia due to viridans streptococci in neutropenic patients (Bochud et al. 1994a) notes the presence of oral mucositis or gingivitis in most studies. In the six studies referred to in the review mucositis and / or oral inflammation are discussed. The broader interpretation of oral inflammation may include gingivitis, in which case there is no mention of methods of recording gingivitis or the significance of a bacteraemia through the gingival crevice. The general view is that the viridans streptococci gain entry to the systemic circulation through the oral and / or pharyngeal ulcerations. However, a significant association between mucositis and viridans streptococcal bacteraemias is not always proven (Valteau et al. 1991). The investigators in a study which demonstrated the similarity between oral and blood isolates of viridans stated that the results supported the view that the oral mucosa was the portal of entry for viridans streptococci causing bacteraemias. A variety of everyday procedures, for example toothbrushing, cause a bacteraemia through the gingival margin and over half the cases have been identified as due to viridans streptococci (Roberts et al. 1997). It was also shown that 38% of positive blood cultures occurred when there was bleeding at the operative site, whereas only 16% of blood cultures were positive when there was no discernible gingival bleeding. The proportion of viridans streptococcal bacteraemias was similar to that found in the main study. (G.J. Roberts, The Great Ormond Street Hospital For Children and Guy's Dental Hospital, personal communication). In the studies discussed, the association between

these bacteraemias and the presence of mucositis is not definitely linked. If it is presumed that the inflamed oral mucosa includes the gingival margin, then the gingival tissues are also an important site of entry of bacteria into the systemic circulation.

Bacteraemia with viridans streptococci can have serious sequelae. These include pulmonary infiltrates which can lead to adult or acute respiratory distress syndrome (Mc Whinney et al. 1991; Bochud et al. 1994b), interstitial pneumonitis (Valteau et al. 1991) which in general has a poor outcome (Soutar and King 1995) and viridans streptococcal shock (Villablanca et al. 1990; Elting et al. 1992; Engel et al. 1996). The association between the viridans streptococci and infective endocarditis has been known for many years (Bahn et al. 1978; Bayliss et al. 1983).

Isolation of the oral streptococci from the blood cultures of febrile neutropenic patients suffering from a variety of haematological and solid organ malignancies, particularly members of the '*oralis* group' of viridans streptococci, has been frequently reported (Henslee et al. 1984; Classen et al. 1990; Burden et al. 1991; van der Lelie et al. 1991; Elting et al. 1992; Holzel and de Saxe, 1992; Bochud et al. 1994a; Martino et al. 1995; Richard et al. 1995; Sriskandan et al. 1995). These account for some 4% to 47% of septicaemias in bone marrow transplant adults (Heimdahl et al. 1989; Villablanca et al. 1990; Valteau et al. 1991; Martino et al. 1995). Many of these streptococcal isolates from neutropenic patients have been reported only as ' viridans streptococci' (Martino et al. 1995) whereas others have been further characterised with a commercial system e.g API - 20 STREP (Bio-merieux) and identified as *S. mitis* or *S. sanguis* (Classen et al. 1990; Burden et al. 1991). There have been several recent and important changes in the taxonomy of the viridans streptococci. These include emended descriptions of *S. sanguis, S. oralis, S. mitis, S. anginosus, S. intermedius* and *S. constellatus* and the identification of new species: *S. gordonii, S. crista, S. vestibularis* and *S. parasanguis*. These taxonomic changes are not necessarily reflected in the commercial systems. However, subsequent identification schemes include many of these taxonomic changes which enable more accurate and reliable characterisation of the viridans streptococci (Kilian et al. 1989; Beighton et al. 1991a).

Other workers have further identified species of viridans streptococci but have not necessarily described the system that has been used for this. From a group of adult bone marrow transplant patients the following species were isolated from 24 patients; *S. mitior* 29%, (re - designated *S. mitis* and *S. oralis*) *S. milleri* 20.8%, (re - designated *S. anginosus,*

S. constellatus and *S. intermedius*) *S. sanguis* 19% *and S. salivarius* 6.5% and 20.8% of isolates which were not further identified (Heimdahl et al. 1989). A commercial system Vitek AutoMicrobic Systems, St. Louis M.O., was used to help identify the predominant species in another group of children as *S. mitis* 45% (adults 47%) of isolates, *S. sanguis* 31% (adults 21%) and approximately 10% were not identified further than as viridans streptococci (Villablanca et al. 1990). In a different paediatric study no details of the speciation was given but 26.5% of septicaemic organisms were *S. mitis* 26.5% and a further 26.5% *S. sanguis*. From a group of 64 neutropenic bacteraemias in adults 41% were caused by viridans streptococci. The most prevalent species isolated from the blood cultures were *S. mitis* 15 episodes (58%) and *S. sanguis* biovar II, eleven episodes (42%). Three of the *S. sanguis* II isolates were later reclassified as *S. oralis*. From another group of 47 neutropenic patients 39 isolates of viridans streptococci (83%) were identified as *S. oralis*, 11% as *S. mitis*, 2% as *S. parasanguis*, and 2 other isolates were not further identified (McWhinney et al. 1993).

Twenty three isolates of viridans streptococci from pyrexial neutropenic patients suffering from a variety of malignant diseases were identified (Beighton et al. 1994) using both the API - 20 STREP system and a comprehensive system taking account of the recent developments in taxonomy (Beighton et al. 1991a). The identifications given by the API system were similar to several of the studies discussed. Most of the isolates were *S. mitis* (10) or *S. sanguis* II, 2 isolates were *S. salivarius*, one of these with low discrimination and the second with an unacceptable profile. With the more comprehensive scheme most of the isolates were *S. oralis* (14) or *S. mitis* (5) and 2 were *S. salivarius*. Similar results were reported for 104 blood isolates which were identified using the API - 20 STREP, the Rapid ID 32 STREP system and the comprehensive system of Beighton et al. (1991a). In this study, 39 of the isolates were recovered from patients with haematological disorders. Of these 74% were identified as *S. oralis* and 18% as *S. mitis* which had originally be identified as *S. mitis* or *S. sanguis* II. All the patients had clinical evidence of mucositis. The remaining 65 isolates were from the general hospital population and the species most frequently identified was *S. milleri*. The S. *milleri* strains were further identified as *S. anginosus, S. constellatus* and *S. intermedius* (Jacobs et al. 1995). The API - STREP 32 system was found to agree well with the comprehensive system of Beighton et al. (1991a) but not with the API 20 system. Although *S. oralis, S. mitis* and *S. sanguis* are related they are separate species and some isolates which have been identified in earlier studies as *S. sanguis* II may well be re - designated as *S. oralis*, and those formerly identified as *S. mitior* re - designated *S. oralis* and *S. mitis*, using the comprehensive system.

There have been a number of recent reports of penicillin resistant viridans streptococci in neutropenic patients (Guiot et al. 1994; Carratala et al. 1995; Rolston et al. 1995). The prevalence of these penicillin-resistant streptococci was found to be greater in adult leukaemia patients than in children. Healthy control children were also found to have a greater prevalence than the adult leukaemia patients and similar prevalence to the leukaemia children (Guiot et al. 1994).

Mucosal Immunity

The micro-organisms which colonise the oral cavity are commensals and may become pathogenic if there is a change in the host defences. There are a number of factors which are responsible for maintaining oral health which include integrity of the mucosa, saliva and their humoral and cellular immune components (Lehner 1992). The humoral components may enter the oral cavity through the gingival crevice if there is gingival inflammation and an increase in gingival crevicular fluid . The humoral immune components are the immunoglobulins, of which the principal isotype in the saliva is IgA. IgA is synthesised locally by the plasma cells associated with the major and minor salivary glands. The locally produced IgA is dimeric, unlike the serum IgA, which is monomeric. The secretory epithelial cells of the salivary acini synthethize a secretory component (Brandtzaeg et al. 1970) which complexes with the dimeric IgA, thus forming secretory IgA (S - IgA). S - IgA concentrations are undetectable at birth but soon appear in saliva reaching adult levels by 2 to 4 years in stimulated saliva and 6 to 8 years in unstimulated saliva (Burgio et al. 1980). Lesser quantities of monomeric IgA secreted by B-lymphocytes from the gut - associated lymphoid tissue, IgG and IgM which are secreted from plasma cells generated from lymph nodes, spleen and blood, reach the oral cavity through the gingival crevicular fluid. The approximate concentration of whole saliva IgA is 19.4 mg/100ml and for IgG is 1.4 mg/100ml and the ratio between IgG and IgA is 0.07 in adults (Lehner 1992). Total IgA levels in healthy children have been reported as 45.6 μg/ml, mean age 8 years 4 months (Lenander - Lumikari et al. 1992) and 43.9 μg/ml mean age 1.5 years (Tappuni and Challacombe 1994).

A measure of immunocompetence is the presence of functional antibody. Salivary antibody to *E. coli* antigens can be detected early in life (Mellander et al. 1984; Smith and Taubman, 1993) and reach adult concentrations at the same time as S - IgA (Smith and Taubman 1993). In a group of neonates, the saliva of most of the infants contained IgA antibody reactive with between 2 and 6 antigens of *S. mitis*, at 5 weeks of age. A lower frequency of salivary IgA

antibody to *S. salivarius* was also detected. It was suggested that the antibody specificities were likely to be mucosally derived responses (Smith and Taubman 1993). The investigators also found that salivary IgA antibody to mutans streptococci was sometimes detected before colonisation of the mouth was apparent. Antibodies to *S. mitis, S. salivarius* and *S. sanguis* extracellular components were detected only after infection with the respective species.

The salivary immunoglobulins were recorded in a group of 40 adults with cancer who suffered from chemotherapy related mucositis (Jankovic et al. 1995). Serum and unstimulated salivary IgA and IgG levels were estimated by radial immunodiffusion. Serum IgA, IgG and the mean serum IgG:IgA ratios were normal. There was an increased salivary IgG and decreased salivary IgA level and the mean salivary IgG:IgA ratio was increased when compared with healthy controls. It was conjectured that the increased IgG levels were due to capillary leakage and increased transudation across the inflamed oral mucosa. The plaque and gingivitis scores for these patients were also increased, which would also lead to increased transudation of IgG through the gingival crevice. IgM, IgG and IgA concentrations in parotid saliva were estimated by ELISA in a group of 30 patients including both children and adults, most of whom received allografts (Chaushu et al. 1994a). Following the conditioning regimen of chemotherapy and irradiation there was a gradual decrease in salivary immunoglobulins. An increase in the salivary concentrations occurred from day 4 post - transplantation and continued, reaching a plateau within 2 or 3 weeks. After 3 weeks the concentrations decreased and persisted at low levels for variable periods of time. A second increase in immunoglobulin concentrations occurred at approximately 2 months post - transplantation and persisted for prolonged periods of time. This suggests that the immunolgobulin was secreted by the donor B - lymphocytes and plasma cells transferred with the graft. This is supported by results from 7 donors who were immunised with tetanus toxoid 4 - 7 days before harvesting (Chaushu et al. 1994b). Anti - tetanus toxoid IgA was present in the parotid saliva of only 1 recipient prior to transplantation. The donor bone marrow was T cell depleted and, transient high titres of anti - tetanus toxoid IgA were detected in the recipients 7 - 28 days post - transplantation. No significant titres were detected in the saliva of patients grafted with non-immunised bone marrow. It was concluded that the antibody-producing cells activated in the donor are passively transferred with the graft to the recipient. Recent work found that recipient IgG producing cells were not eradicated by the conditioning regimen and persisted in a high number of paediatric graft recipients. The hypothesis was that the lack of GvHD in most of the children resulted in the persistence of recipient IgG producing cells (van Tol et al. 1996).

One of the important features of GvHD is the recognition and destruction of host leukaemia or other abnormal cells. A small amount of GvHD has an important anti - leukaemia function. Significantly decreased levels of all classes of immunoglobulins were found in saliva and serum in 85 patients before and up to 5 years after bone marrow transplantation (Norhagen et al. 1994). Salivary IgG levels were increased before bone marrow transplantation in patients who died shortly afterwards. Patients suffering from malignant disease had greater S - IgA levels than those with non-malignant conditions. There were higher levels of S - IgA in patients treated with autologous bone marrow rescue than patients receiving allografts. Lower levels of IgG were found in serum and saliva of patients receiving an increased bone marrow cell dose at transplantation.

A recent investigation of 24 adult autograft and allograft recipients found that there were significant decreases in S - IgA and IgG between 3 and 4 weeks post - transplantation. Although the concentrations were still reduced 70 and 100 days post - transplantation , this difference was not significant (Dens et al. 1996).

CHAPTER 2

PATIENT RECRUITMENT

Ethical Approval and Selection Criteria

Ethical approval was granted by the Great Ormond Street Hospital For Children NHS Trust, The Royal Marsden NHS Trust and Merton and Sutton Area Health Authority.

The selection criteria were:-

(1) Willingness of the child and parents to participate in the study

(2) The ability of the child to co-operate fully during the oral examination and saliva sampling procedure.

An information sheet was provided prior to obtaining written consent from the parents and verbal consent from each child (Appendix 1)

Subjects and Controls

Subjects

Thirty six children were recruited from the Great Ormond Street Hospital for Children NHS Trust and the Children's Unit, The Royal Marsden Hospital NHS Trust. These were divided into 2 groups:-

Chemotherapy and Fractionated Total Body Irradiation (TBI Group)

Twenty four children were treated with cyclophosphamide 60 mg/kg with mesna on 2 consecutive days followed by between 12 and 14.4 Gy of fractionated total body irradiation over a period of 3 to 5 days (Appendix 2). Allograft rejection was minimised with methotrexate[a] on designated days post - transplantation (n = 25) with folinic acid rescue (n = 20) and cyclosporin A[b] (n = 36) In addition, five children in this group who received matched but unrelated allografts were T-cell depleted, *in vivo*, with Campath - IG[c] which is a rat anti - human monoclonal antibody. The donor bone marrow was also T - cell depleted, *in vitro*, before infusion to further reduce the risk of graft rejection. One child withdrew consent after the first examination. The data for this child and the matched control have not been included in the analysis. Seven subjects in the TBI group died before 100 days post - transplantation. One child died as result of drug toxicity, two others relapsed and a fourth child had severe graft versus host disease (GvHD). Overwhelming pulmonary complications occurred in 2 children, one of which was infection with *Aspergillus* species and the seventh child succumbed to multi-

[a] David Bull Laboratories, Spartan Close, Tachbrook Park, Warwick CV34 6RS
[b] Sandoz Products Limited, Sandoz House, 98 the Centre, Feltham, Middx TQ13 4EP
[c] Campath, University of Cambridge Laboratories, Cambridge

organ failure. One child who failed to engraft after 6 weeks was discharged home with no further treatment. Two months later donor cells were found in the bone marrow and the peripheral neutrophil count had risen above 0.5 x 10^9/l and he re - joined the study for the final clinical assessment.

Chemotherapy Only (CTO Group)

There were 12 children who received a conditioning regimen of chemotherapy only. The drug regimens included busulphan[d] 4mg/kg /4 days and cyclosphosphamide[e] 50/mg/kg/4 days with mesna or busulphan 4mg/kg/4 days and melphalan[b] 160mg/m^2 as a bolus, or a single high dose bolus of melphalan 200mg/m^2 alone (Appendix 2). These illustrate the wide range of drugs used, the details of which were subject to variation for some children. Busulphan, melphalan and cyclophosphamide are alkylating agents which act by disrupting DNA, thus interfering with cell replication. One of the side effects of cyclophosphamide is damage to the urinary tract resulting in haemorrhagic cystitis. For this reason it is given with mesna, which reacts specifically to prevent such damage. One child withdrew consent after the first examination. The data for this child and the matched control have not been included in the paired analysis but the data for the matched control have been used in other analyses.

The diagnoses and types of transplant are summarised in Tables 3 and 4.

Table 3: Diagnoses of Bone Marrow Transplant Children

TBI Group	N	CTO Group	N
Acute Myeloid Leukaemia	3	Acute Myeloid Leukaemia	3
Acute Lymphoblastic Leukaemia	18	Wiskott-Aldrich Syndrome	2
Mylelodysplasia	1	Thalassaemia	2
Kostmann Syndrome	1	Neuroblastoma	1
		Non-Hodgkin's lymphoma	1
		Rhabdomyosarcoma	2

N = number of children

[d] GlaxoWellcome Ltd., Stockley Park West, Uxbridge, Middx UB11 1BT
[e] Allen and Hanbury Ltd., Stockley Park West, Uxbridge, Middx UB11 1BT

Table 4: Types of Transplants

Transplant	TBI Group	CTO Group
	Number	Number
Autograft	0	6
HLA Matched Sibling Allograft	18	4
Matched but Unrelated Allograft	5	0
Peripheral Blood Stem Cell Transplant	0	1

Pre -Transplant Investigations

As part of the preparation for BMT, each child underwent a series of medical investigations and a full oral examination. Most of the children had good oral health before starting the conditioning regimen although a marked increase in plaque and gingivitis was noticed during the course of their treatment.

Antimicrobial Therapy

All the subjects received prophylaxis against *Pneumocystis carinii.* This comprised co-trimoxazole[f] 1.5mg/kg/day until 1 day pre-transplantation (n = 22) or pentamidime[g] 4mg/kg fortnightly throughout the transplantation period (n = 12). Cotrimoxazole, at an age and weight related dose, was recommenced by all subjects when the neutrophil count had risen above 1.0 x 10^9/l. Acyclovir[f] 250 mg/m^2 was started 5 days prior to transplantation as prophylaxis against herpes simplex virus. Pyrexial episodes were treated empirically with first line antibiotic regimens which cover the main groups of gram-positive and gram-negative bacteria. These were either a combination of ceftazidime[e] 150mg/kg/day and amikacin[h] 20 mg/kg/day which was changed to ciprofloxacin[i] 10mg/kg/day and amikacin 20 mg/kg/day, or gentamicin[j] 2mg/kg/day and piperacillin[j] 300 mg/kg/day and flucloxacillin[h] 100mg/day, or tazobactam[k] 4.5g 8 hourly. The antibiotics were changed according to the sensitivity of any

[f] The Wellcome Foundation Limited, Crewe Hall, Crewe, Cheshire CW1 1UB
[g] Rhone-Poulenc-Rorer Ltd., 52 St. Leonards Road, Eastbourne, East Sussex BN21 3YG
[h] Bristol Myers Squibb Pharmaceuticals Ltd., 141-149 Staines Road, Hounslow, Middx TW3 3JA
[i] NHS Supplies, South East Division, Guy's Hospital, St. Thomas's Street, London SE1 9RT
[j] Lederle Laboratories, Fareham Road, Gosport, Hants PO13 OAS
[k] Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead, Berks SL6 OPH

bacteria isolated from blood cultures, for example vancomycin[j] 10 mg/kg 6 hourly or teicoplanin[h] 10mg/kg/12 hourly for 3 doses followed by 10mg/kg/day , or imipenen[l] 15mg/kg/6 hourly. The dosage was adjusted and monitored for each child. Pyrexias persisting for five days with no positive blood culture were treated with ambisome,[m] a colloidal form of amphotericin,[n] in a dosage of 1-3mg/kg/day

Mouth Care Regimen

On admission to hospital each subject commenced a mouth care regimen. This comprised anti-fungal prophylaxis with either itraconazole[o] 2.5mg/day (GOS) or a combination of nystatin[i] 100,000 units, and amphotericin 10mg 4 times daily (RMH). Children unable to tolerate itraconazole were prescribed fluconazole 3mg/kg/day starting 1 day before the transplant. In addition, the Great Ormond Street subjects were instructed to rinse the mouth 4 times daily with 0.2% chlorhexidine[p] in order to minimise the level of bacterial dental plaque.

General Care

As the neutrophil counts decreased towards the end of the conditioning regimen the children were kept out of close contact with other people. On completion of the conditioning regimen each child was nursed by 1 parent in an isolation cubicle with filtered laminar air flow until there was evidence of engraftment and reconstitution of the haematopoietic system. This was indicated by the recovery of the neutrophil count which occurred within 2 to 6 weeks after transplantation. The introduction of granulocyte colony stimulating factor had encouraged this recovery, thus shortening the length of the neutropenic period. When the neutrophil count had risen above 1 x 10^9/l on two consecutive days, the isolation procedures were gradually relaxed. It is during this period of intense immunosuppression that the bone marrow recipients are most at risk from opportunistic infection.

During this period of isolation strict isolation procedures were maintained for all personnel entering and leaving the cubicle. Clothes and bedding were changed daily; food items were restricted and prepared 'clean'; all crockery was sterilised and conventional soap was not used in the cubicles

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[l] Merck, Sharp and Dohme Ltd., Hertford Road, Hoddesdon, Herts EN11 9BU
[m] Vestar Limited, Hills Road, Cambridge, CB2 1NS
[n] Unimed Pharmaceuticals Limited, 24 Steynton Avenue, Bexley, Kent DA5 3HP
[o] Pfizer Limited, Sandwich, Kent CT13 9NJ
[p] Hibitane Dental, ICI Macclesfield, U.K.

Controls

Thirty three children were drawn from schools in the Merton and Sutton Area Health Authority and matched for age, gender, ethnicity and social class with the transplant children.
Two children withdrew consent after the first examination so two others were recruited. Two further control children were each an excellent match for one child in each treatment group. Since the 2 treatment children were included in the study within few weeks of each other, a two way match was statistically acceptable. Each matched control subject was recruited within 3 months of the corresponding BMT child.

Protocol For Oral Examination

All oral examinations were carried out in the same manner using a disposable mirror and a Duracell junior torch as light source. Indices were recorded for dental caries using the World Health Organisation criteria (1984), and for bacterial dental plaque and gingivitis using a modification of the method of O'Leary (Franco et al. 1996). The presence of mucositis (Seto et al. 1985) and any other lesions was also recorded.

Dental Caries

All the teeth were examined and indices recorded as the dmfs / dmft index (the number of decayed, missing and filled surfaces of deciduous teeth) and the DMFS / DMFT index (the number of decayed, missing and filled surfaces of permanent teeth).

Bacterial Dental Plaque

Four gingivally related quadrisections of each tooth (mesiobuccal, distobuccal, distolingual and mesiolingual) were visually examined for bacterial dental plaque deposits to give the plaque score. The proportion of tooth quadrisections which had discernible deposits of bacterial dental plaque was calculated as a percentage of the total number of deciduous and permanent tooth quadrisections present.

Gingivitis

The gingivae were visually examined for gingivitis, determined by the presence or absence of reddened gingival tissue were examined, using a simplified gingival index based on the number of tooth quadrisections with associated gingivitis. The proportion of tooth quadrisections with associated gingivitis was calculated as a percentage of the total number of deciduous and permanent tooth quadrisections present.

It was not possible to use more detailed plaque and gingival indices. Very young children, particularly when unwell, are unable to co-operate for the period of time necessary for more detailed examinations. In addition, examination of the gingival margin and crevice with a probe, albeit a blunt probe, was **not** permitted in immunocompromised and thrombocytopenic patients because of the risk of bleeding and associated bacteraemia.

Collection of Oral Rinses

To collect the saliva, each child was asked to rinse the mouth with 5 ml of sterile 0.9% (w/v) normal saline for 30 seconds. This method was chosen following a pilot study which had shown that the smaller children could not produce sufficient saliva during the period of intense immunosuppression during the transplantation period. The rinse was found to be quick and acceptable since it was similar to that used in the mouth care regimen.

Timing of Oral Examinations

Oral examinations were carried out and saliva samples were collected at specific times during the transplantation period (Table 5).

Analysis of Data

All data were tested for normality using the Shapiro - Wilks test. Since neither the raw data nor the $\log_{10}$ transformed data were normally distributed, the statistical analysis was completed on the raw data using non - parametric tests. The statistics package was SPSS for Windows Release 7.0.

Table 5: Times for Oral Examinations and Collection of Oral Rinses

Data for Bone Marrow Transplant Patients at Four Treatment Related Times				
	Baseline: (before conditioning regimen)	**7 Days post-BMT**	**Neutrophil count > 0.5 x 10 ^{9}/l**	**120 days post-BMT**
1	Dental Caries	-	-	-
2	Plaque	Plaque	-	Plaque
3	Gingivitis	Gingivitis	-	Gingivitis
4	Mucositis	Mucositis	-	Mucositis
5	Other lesions	Other lesions	-	Other lesions
6	Oral rinse	Oral rinse	Oral rinse	Oral rinse
	Data for Controls as for BMT patients	***No data for Controls***	***No data for Controls***	**Data for Controls as for BMT**

COLLECTION OF SPECIMENS AND MICROBIOLOGICAL TECHNIQUES

Collection of Saliva Samples

At each of the sampling times every child rinsed with 5ml of sterile aqueous 0.9% (w/v) sodium chloride for 30 seconds. The oral rinses were transported in a sterile container on ice to the laboratory for processing within 3 hours of collection. Each sample was vortexed. One millilitre of the rinse was removed, mixed with 1 ml of glycerol and stored in a sterile cryotube[a] at -70°C for immunoglobulin analysis.

Media For Primary Bacterial Culture

Non-Selective Media

Fastidious anaerobe agar[b] (FAA) supplemented with 5% (v/v) defibrinated horse blood[c] was prepared to determine the total aerobic and anaerobic bacterial counts. The plates for the aerobic counts were incubated in air supplemented with 5% CO_2 at 37° C for 3 days. The other plates were incubated anaerobically in a chamber with an atmosphere consisting initially of 90% nitrogen, 5% hydrogen and 5% carbon dioxide, at 37° C for 7 days.

Selective Media For Primary Isolation of Specific Organisms

(1) Mitis salivarius agar[d] (MSA) supplemented with 0.1% potassium tellurite[d] was prepared for growth of the viridans streptococci. The presence of potassium tellurite suppresses the growth of bacteria other than the viridans streptococci. The plates were incubated anaerobically at 37° C for 3 days

(2) Mitis-salivarius agar supplemented with 0.1% potassium tellurite, bacitracin[e] 0.2 units/ml and Sucrose 15% w/v (BMSA) was prepared for growth of mutans streptococci (*S. mutans* and *S. sobrinus).* The bacitracin solution was filter sterilised using a 0.2μm filter[a] The combination of sucrose and bacitracin inhibits the growth of most human viridans streptococci except for the mutans streptococci (Gold et al. 1973). The plates were incubated anaerobically at 37° C for 3 days.

(3) Sabouraud dextrose agar[d] (SDA), was prepared for growth of yeasts, primarily *Candida* species. The plates were incubated aerobically at 37° C for 24 hours.

(4) MacConkey agar[d] was prepared for the growth of *Enterobacteria* and enterococci. The plates were incubated aerobically at 37° C for 24 hours.

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[a] Gelman Sciences, Brookmills Business Park, Caswell Road, Northampton NN4 7EZ
[b] Lab M, Topley House, P.O. box 19, bury, Lancs. BL9 6AV
[c] TCS Microbiology, Botolph, Claydon, Buckingham MK18 2LR
[d] Oxoid Unipath Ltd., Basingstoke, Hampshire RG24 OPW
[e] Sigma - Aldrich Company Ltd., Fancy Road, Poole, Dorset DH12 4QN

All media were autoclaved at 121° C for 15 minutes, supplements being added after cooling to about 50° C. Plates were poured in a dust free environment, dried for 30 minutes and stored at 4° C ready for use within 2 or 3 days.

Processing of Oral Rinses

Tenfold serial dilutions (10^{-1} to 10^{-5}) of the oral rinse were prepared in Fastidious anaerobe broth[b] and 100 µl aliquots of the appropriate dilutions inoculated onto both non-selective and selective media (Table 6).

Table 6: Dilutions of Oral Rinse

	Media	Dilutions
Non-selective	Fastidious anaerobe agar	10^{-3} to 10^{-5}
	Fastidious anaerobe agar	10^{-3} to 10^{-5}
Selective	Sabouraud dextrose agar	*neat, 10^{-1}
	MacConkey agar	10^{-1}, 10^{-2}
	BMSA	*neat, 10^{-1}, 10^{-2}
	MSA	10^{-3} to 10^{-5}

* undiluted oral rinse

Enumeration of Micro-organisms

Aerobic and anaerobic bacteria

FAA plates with between 30 and 200 colonies were selected for enumeration. All the colonies were counted and the total aerobic and total anaerobic counts calculated per ml of oral rinse.

Viridans Streptococci

S. mutans colonies, appearing as dark blue, crumbly colonies embedded in the BMSA plates, were counted and recorded. The number of each different colony type on the MSA plates was counted and recorded. Between 1 and 12 different colonial morphologies were found on the plates for each rinse.

Yeasts and Candida Species

Each raised, smooth, round and white-yellow yeast - like colony type was counted and recorded from the SDA plates. Other probable yeasts which had a crinkled surface or were brownish in colour were also counted.

Enterobacteriaceae and Enterococci

E. coli and *Klebsiella* species and other gram - negative bacteria which ferment lactose, appearing as pink colonies on MacConkey agar after 24 hours incubation, were counted and recorded for each oral rinse. Possible *Pseudomonas* species which did not ferment lactose, were also counted. Enterococci, which grow as small round magenta colonies, were counted and recorded.

Reproducibility of Isolation and Enumeration of Micro - organisms from Oral Rinses

Three studies were undertaken to assess the reproducibility of sample processing, colony counting and identification.

Study One: Reproducibility of Sample Processing

Oral rinses were collected from 20 children attending the Department of Orthodontics and Paediatric Dentistry, Guy's Hospital. Each rinse was divided into 2 and processed as 2 separate samples and inoculated on both selective and non - selective media. The colonies were counted from the different media and compared.

Study Two: Reproducibility of Sampling Procedure

Two oral rinses were collected from each of 20 subjects 30 minutes apart, and processed in the same manner as in Study One. The colonies were counted from the different media and compared.

Study Three: Inter-Examiner Variability

Thirty seven different media plates which had already been inoculated were counted and described by the investigator and one other person. The results were compared

Identification of Micro-organisms

Micro-organisms were characterised initially by gram - staining to determine the gram reaction and cellular morphology of each colony type. This was followed by specific tests for each group of bacteria.

Identification of Viridans Streptococci

Two of each different colony type were subcultured into 4 ml of sterile Todd-Hewitt broth[d] and incubated at 37°C for 48 hours. These cultures were checked for purity by gram - staining the broth and by inoculation of each broth onto Columbia base agar[d] supplemented with 5% defibrinated horse blood (CBA). The CBA plates were incubated anaerobically at 37° C for 24 hours. Any mixed or contaminated cultures were subcultured until pure.

Carbohydrate Fermentation Tests

The broths were subjected to a range of carbohydrate fermentation tests using a sterile microtitre plate format (Beighton et al. 1991a). These carbohydrates were amygdalin, arbutin, inulin, lactose, mannitol, melibiose, *N* - actetylglucosamine, raffinose and sorbitol, which were prepared using thioglycollate medium and purple broth base (Table 7).

Each carbohydrate was aliquoted in 125 µl volumes in the 8 wells of the same column on every microtitre tray. Forty five microlitres of each broth culture was added to a single horizontal row of wells (Figs 1 and 2). The last row in each series of plates was the control and contained carbohydrate only. The plates were incubated anaerobically at 37°C for 24 hours. A change in colour from purple to yellow was recorded as a positive result, indicating that the carbohydrate had been fermented and the final pH was < 5.2. Tests that remained purple were recorded as negative.

Table 7: Preparation of Carbohydrates

<table>
<tr><th colspan="3">Amygdalin, Arbutin, Inulin, Lactose, Mannitol, Melibiose
N - acetylglucosamine, Raffinose, Sorbitol</th></tr>
<tr><td colspan="3">2.4 g Thioglycollate Medium[f] (without dextrose or indicator)
1.6 g Purple Broth Base[f]
1.0 g Carbohydrate[e]
100 ml Distilled Water</td></tr>
<tr><th>Aesculin</th><th>Arginine</th><th>Salt A</th></tr>
<tr><td>1.0g Tryptone
0.5g Yeast Extract
1.0 g Sodium Acetate
0.05 g Ferric Ammonium Citrate
0.5 g Aesculin
0.1 ml Tween 80
0.5 ml Salt A
0.5 ml Salt B
100 ml Distilled Water</td><td>0.5 g Peptone
0.3 g Yeast Extract
0.3 g Glucose
1.0g Sodium Acetate
0.3 g L - Arginine
0.1 ml Tween 80
0.5 ml Salt A
0.5 ml Salt B
100 ml Distilled Water</td><td>0.16 g $CaCl_2$ an
0.16 g $MgS0_4$
400 ml Distilled Water
Salt B
0.8 g K_2HPO_4
8.0g $NaHC0_3$
1.6 g NaCl
0.8 g KH_2P0_4
400 ml Distilled Water</td></tr>
</table>

After (Kral and Daneo - Moore 1981)

[f] Difco Laboratories Ltd. P.O. Box 14B, Central Avenue, West Molesey, Surrey KT8 2SE

Hydrolysis of Aesculin and Arginine

Aesculin and arginine were aliquoted in 145 µl volumes into the next 2 columns following the carbohydrates, on the sterile microtitre plates (Figs 1 and 2). The broths were inoculated in the method described for the carbohydrate fermentation tests. Aesculin hydrolysis was noted by a change in colour to black. Arginine hydrolysis was indicated by a change to orange on the addition of Nessler's reagent[g] (Figs 1 and 2) Any results that were inconclusive were repeated.

Detection of Pre-formed Enzyme Activity

To test for the presence of enzyme activity, each broth was cultured on CBA and incubated anaerobically for 24 to 48 hours. Colonies of each isolate were removed from the CBA plates with a sterile cotton bud and suspended in 1.5ml of 50mM N - tris (hydroxymethyl) 2-aminoethanesulfonic acid[e] (TES) buffer pH 7.5 at an optical density (O.D.) of 0.1 at 620 nm. This suspension was used to determine the ability of each isolate to hydrolyse a series of 4-methylumbelliferyl (MU -) linked substrates[e] (Beighton et al. 1991a). These substrates were: MU - β fucoside, MU - β - *N* - acetylgalactosaminide, MU - α - neuraminate, MU - α fucoside, MU - β - *N* - acetlyglucosaminide, MU - α - glucoside, MU - β - glucoside, MU - α - galactoside, MU - α - arabinoside and MU - β - galactoside. Each enzyme substrate was prepared from powder which was initially dissolved in 0.5 ml of dimethyl sulphoxide. This was added to 9.5ml of TES buffer to make a 1mg/ml stock solution. The working concentration of 100 µg/ml was prepared by adding 1ml of the stock solution to 9 ml of TES buffer. The stock solutions were stored at - 20°C and the working solutions at 4°C.

To detect enzyme activity, 20 µl of enzyme substrate and 45 µl of bacterial suspension were added to a well in a single non-sterile, flat bottomed microtitre tray, in the same manner as the carbohydrates. The plates were incubated aerobically at 37°C for 3 hours. Substrate hydrolysis was determined by measuring the fluorescence on a Perkin Elmer fluorimeter[h] using excitation emission at wavelengths of 380 nm and 460 nm respectively. Presence of enzyme activity was positive if there was an increase in fluorescence of 50 arbitrary units above the control. This increase in fluorescence of the fluorophore methylumbellyferone, resulted in the liberation of approximately 20 nmoles of substrate being degraded. The last row in each series of plates was the control and contained enzyme substrate only.

[g] BDH Laboratory Supplies, Poole, Dorset BH18 1TD
[h] Perkin Elmer Ltd., Post Office Lane, Beacons field, Buck HP9 1GA

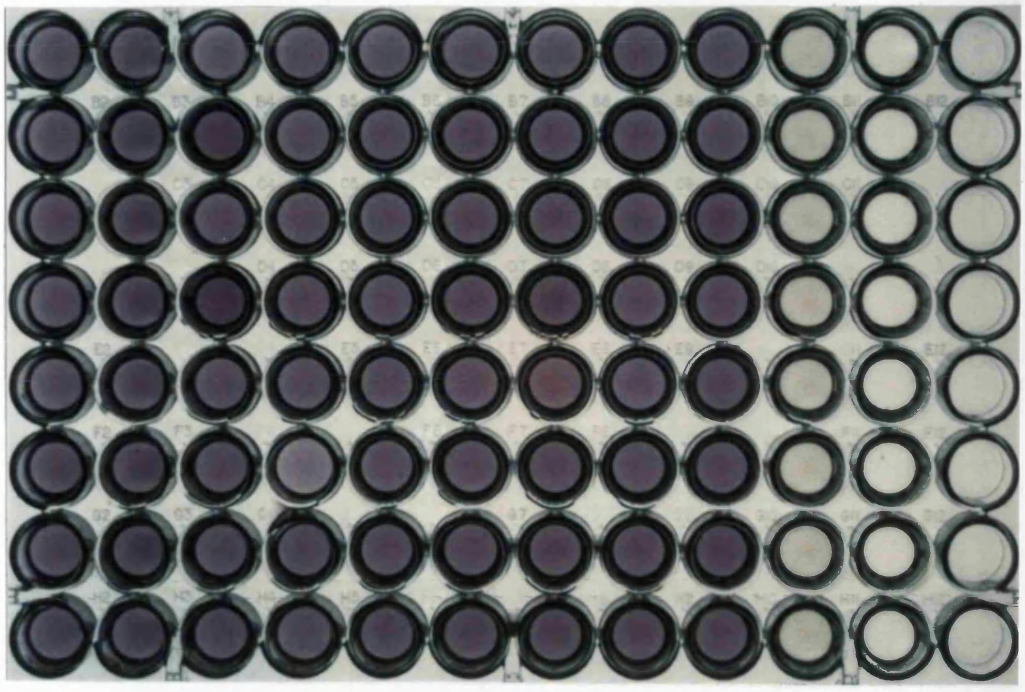

Fig 1: Microtitre tray containing carbohydrates (purple), aesculin and arginine (columns 10 and 11) before inoculation with broth cultures

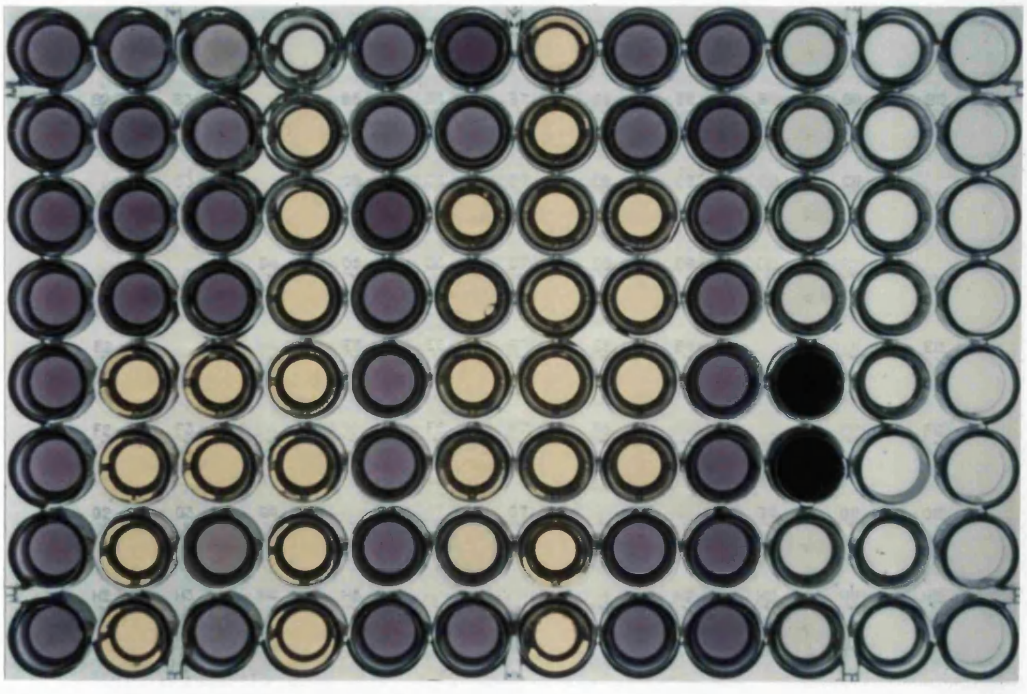

Fig 2: Microtitre tray in Fig 1 following anaerobic incubation for 24 hours at 37° showing carbohydrate fermentation (yellow) and aesculin hydrolysis (black)

The combined results of the carbohydrate fermentation tests and the tests for presence of pre-formed enzymes were used to identify the viridans streptococci isolates, by comparing them with those reported in the literature (Whiley and Hardie, 1988; Beighton et al. 1991a; Whiley et al. 1995) and with type strains of the organisms. Any organism with an inconclusive profile was subjected to repeated testing.

Regular quality testing was carried out by culturing type strains in the same manner (Tables 8 and 9) and also by incubating media plates which had not been inoculated to ensure that there were no contaminants. The type strains used were: *S. mutans* NCTC 10449, *S. sobrinus* NCTC 12279, *S. mitis* NCTC 551, *S. oralis* NCTC 11427, *S. sanguis* NCTC 7863, *S. parasanguis* NCTC 5898, *S. salivarius* NCTC 8618, *S. vestibularis* MM1, *S. gordonii* NCTC 7865, *S. cristae* CR 311, *S. anginosus* NCTC 10713, *S. constellatus* NCDO 2226 and *S. intermedius* NCDO 2227. Most of the type strains were obtained from the National Collection of Type Strains, Colindale; *S. cristae* CR 311 and *S. vestibularis* MM1 were provided by Dr. R.A. Whiley.

Identification of *Candida* Species

Two of each different colony type were each emulsified in 45 µl of TES buffer. These were added to 2 separate wells in the tray with 20 µl of 4 - methyumbelliferyl - linked ß - N - acetylgalactosamidine for rapid identification of *C. albicans* (Perry and Miller 1987). Two control wells were prepared containing the enzyme substrate and 45 µl TES buffer only. The tray was incubated aerobically for 2 hours at 37°C and then placed on a UV transilluminator[i] Blue fluorescence indicated substrate hydrolysis and the isolate was recorded as *C. albicans.* All isolates, including those with a positive result using the rapid identification test, were subcultured and their identity confirmed by inoculation into the API ID 32 C Identification System for Yeasts[j] .

Identification of *Enterobacteriaceae*

Gram-negative bacteria which fermented lactose and were oxidase negative (Cowan and Steele 1975), were grown in pure culture and further identified using a commercial system, API 20 E Identification System for *Enterbacteriaceae* and Other Gram - Negative Rods. Possible non-lactose fermenting *Pseudomonas* species which were oxidase positive, were identified by a similar API 20 NE Identification System for Non - Enteric Gram - Negative Rods.

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[i] Ultra Violet Products Incorporated, San Gabriel, California, USA

[j] BioMeriuex UK Ltd., Grafton House, Grafton Way, Basingstoke, Hampshire RG22 6HY

Table 8: Carbohydrate Fermentation and Substrate Hydrolysis Patterns of the Type Strains of Viridans Streptococci[a]

Type Strains - Viridans Streptococci													
	Mutans Group		Mitis Group						Salivarius Group		Anginosus Group		
Carbohydrate Fermentation	**mutans**	**sobrinus**	**mitis**	**oralis**	**sanguis**	**parasanguis**	**gordonii**	**crista**	**salivarius**	**vestibularis**	**anginosus**	**constellatus**	**intermedius**
Amygdalin	+	-	-	-	-	+	+	-	+	+	+	+	+
Arbutin	+	-	-	-	+	+	+	+	+	+	+	-	+
Inulin	+	+	-	-	+		+	-	+	-	-	-	-
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	-	-	-	-	-	-	-	-	-	-	-
Melibiose	+	-	+	-	-	-	-	-	-	-	+	+	+
N - acteylglucosamine	+	-	+	+	+	+	+	+	-	+	+	-	-
Raffinose	+	-	+	-	-	+	-	+	+	-	+	-	-
Sorbitol	+	-	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis of:													
Aesculin	+	-	-	-	+	+	+	-	+	+	+	-	+
Arginine	-	-	-	-	+	+	+	+	-	-	+	+	+

[a] Type strains for each species are listed on page 60

Table 9: Preformed Enzyme Hydrolysis Activity of the Type Strains of Viridans Streptococci[a]

Type Strains - Viridans Streptococci													
	Mutans Group		Mitis Group						Salivarius Group		Anginosus Group		
Enzyme Activity	**mutans**	**sobrinus**	**mitis**	**oralis**	**sanguis**	**parasanguis**	**gordonii**	**crista**	**salivarius**	**vestibularis**	**anginosus**	**constellatus**	**intermedius**
β- fucosidase	-	-	-	-	+	+	-	-	-	-	-	-	+
β-*N*-acetylgal-dase[b]	-	-	-	+	-	+	+	+	-	-	-	-	+
α-neuraminadase	-	-	-	+	-	-	-	-	-	-	-	-	+
α- fucosidase	-	-	-	-	-	-	+	+	-	-	-	-	-
β-*N*-acetlyglu-dase[c]	-	-	-	+	-	+	+	+	-	-	-	-	+
α-glucosidase	+	+	+	+	-	+	-	-	+	+	+	+	+
β-glucosidase	+	-	-	-	+	+	+	-	+	-	+	-	+
α-galactosidase	-	-	-	-	+	-	-	-	-	-	-	-	-
α-arabinosidase	-	-	-	-	-	-	-	-	-	-	-	-	-
β-galactosidase	-	-	+	+	+	+	+	+	-	+	+	-	-

[a] Type strains for each species are listed on page 60

[b] β - *N* - acetylgalactosaminidase

[c] β - *N* - acetlyglucosaminidase

Identification of Enterococci

Magenta colonies on the MacConkey agar, which were probable enterococci, were grown in pure culture on CBA. Each culture was inoculated in to 4 ml volume of sterile Todd Hewitt broth, incubated for 24 hours and subjected to the same carbohydrate fermentation tests as the viridans streptococci. Isolates which were aesculin positive were subcultured onto CBA. A bacterial suspension was prepared and incubated with the 4 - methylumbelliferyl-linked substrates leucine[e] and pyroglutamyl - *B* -napthylamide[e] (Murray et al. 1995). Isolates with positive activity with both enzyme substrates were further identified either as *E. faecium* or *E. faecalis*, using a commercial kit, API Rapid ID 32 STREP.

Analysis of Data

All raw and $\log_{10}$ data were tested for normality using the Shapiro - Wilks test and very few of the data sets were normally distributed. All analyses were completed on the raw data using appropriate non - parametric tests. These included the Wilcoxon Matched Pairs test for comparison of bacterial counts within each patient group and the Mann - Whitney test for comparisons between the transplant and control groups. The isolation frequency of bacteria was compared within each group using the McNemar test and between patient groups with the Chi -Square test. The statistics package was SPSS for Windows Release 7.0.

Proportion of Streptococcal Species

The proportion of each streptococcal species was expressed as a percentage of the total streptococcal count and as a percentage of the total anaerobic count.

Detection Limits of Bacteria

The detection limit for the viridans streptococci, *Candida* species, *Enterobacteriaceae,* enterococci and the total aerobic and anaerobic bacterial counts was 10 colony forming units per ml of oral rinse. The detection limit for the percentages of the individual streptococcal species, except for mutans streptococci on BMSA, was approximately 2% of the total colony count. This assumes an average count of 50 colony forming units per plate. Bacteria which were not cultured above the detection limits were considered not to be present.

The medians are presented as $\log_{10}$ transformed. The mean values and standard deviation were also calculated using the $\log_{10}$ transformed data.

IMMUNOLOGICAL PROCEDURES

Assays

The oral rinses which had been frozen with glycerol for storage (see Chapter2), were centrifuged at 11500g for 10 minutes and aliquoted into 100 µl volumes. These were maintained at -70°C until needed.

Salivary IgA antibody against *S. oralis* and *S. mitis* was estimated by the indirect or sandwich ELISA (Engvall and Perlmann, 1972) as modified by Czerkinsky et al. (1983). The basic principles behind the indirect ELISA are firstly, passive binding of the antigen to a solid surface, usually a microtitre tray. Unbound antigen is removed by washing. The test saliva samples are added and specific antibodies in the saliva bind to this solid phase. Antibody that has not bound is removed by washing. An anti - immunoglobulin antibody, in this case mouse anti - human, is added, and attaches to any antibody bound in the sample. The bound antibody in the sample is detected by a second anti-species enzyme labelled antibody, for example rabbit anti - mouse IgG (Kemeny and Chantler, 1988).

The concentrations of total salivary IgA, secretory IgA (S - IgA) and IgG were measured by capture ELISA. (Engvall and Perlmann, 1972). A class specific anti-immunoglobulin antibody binds to a solid surface which then binds or 'captures' the immunoglobulin in the test saliva sample. An anti - immunoglobulin antibody, again mouse anti - human, is then added which binds to immunoglobulin from the sample. This in turn is detected by anti-species enzyme labelled antibody, for example rabbit anti - mouse IgG (Kemeny and Chantler, 1988)

The solid phase in all the assays was Immunolon 11[a], flat bottom 96 well microtitre plates. Standard ELISA buffers were used for all assays (Appendix 3)

Stage 1: Coating Layer

Salivary IgA antibodies to S. mitis and S. oralis

The type strains used for the coating were *S. mitis* NS51 and *S. oralis* NCTC 11427.
The bacterial cell suspensions were prepared after Czerkinsky et al. (1983) (Appendix 3). The O.D. of the suspensions was estimated with a spectrophotometer[b] at a wavelength of 540nm. An O.D. value of 1.85 was equivalent to a cell concentration of 8×10^9 cells/ml. Each well was coated with 100 µl of the appropriate bacterial cell suspension for the assay. The plates were covered and incubated at 37°C for 2 hours. (Table 10)

Total IgA and Secretory IgA

Each well was coated with 100 µl of rabbit anti - human IgA[c], antibody titre 2.2 mg/l, at a

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[a] Dynatech Laboratories, Inc
[b] Perkin Elmer Ltd., Post Office Lane, Beaconsfield, Bucks. HP9 1GA
[c] Dynatech Laboratories Ltd., Dynex Technologies, Daux Road, Billingshurst, W. Sussex RN14 9SJ

dilution of 1/1000 (Dakopats A0262). This was prepared in phosphate buffered saline (PBS) + 0.02% azide. The plates were covered and stored overnight at 4°C. (Table 10)

Total IgG

Each well was coated with 100 µl of rabbit anti - human IgG[d] (M06124), antibody titre 1 - 3 mg/ml, prepared in PBS + azide at a dilution of 1/1000 was prepared in PBS and the plates were covered and stored overnight at 4°C
(Table 10).

Table 10: Coating Layers For Each Assay

Assay	Coating	Dilution	Incubation	Washing
IgA and S - IgA	Rabbit ant-human IgA	1:1000	4°C Overnight	3 x PBS+Azide
IgA antibodies	Bacterial cell suspension in 0.3% methyl glyoxal,	4 x 10^9	37°C - 2 hours	3 x PBS+Azide
IgG	Rabbit anti-human IgG	1:1000	4°C Overnight	3 x PBS+Azide

Stage 2: Blocking

The plates were washed 3 times with PBS + azide[e] to remove the unbound antigen. The non - specific binding sites on each well were blocked with 200 µl of PBS + Bovine Serum Albumin[e] (BSA) at a concentration of 0.5% + Tween 20[e] (T20) at a concentration of 0.05%. The total IgA, S - IgA and IgG plates were incubated for 2 hours at 37°C; the plates with the bacterial cell coating were stored at 4°C overnight.

Stage 3: Oral Rinses

The plates were washed 3 times with PBS. Twenty oral rinses were added to each microtitre plate at 4 doubling dilutions (Table 11)

Table 11: Oral Rinse Dilutions For Each Assay

Assay	Starting Volume	Starting Dilution of Oral Rinse	Final Volume
IgA and S - IgA	200 µl	1:50	100 µl
IgA antibodies	200 µl	1:2.5	100 µl
IgG	200 µl	1:10	100 µl

[d] Bionostic Limited,Tythe Farm, Wyboston, Beds MK44 3AT
[e] Sigma-Aldrich Company Limited, Fancy Road, Poole, Dorset BH12 4QN

Quantification of Standards

Total Salivary and Secretory IgA

Human colostrum (Sigma[e] 1-1010) was used as the standard for calculation of total salivary and secretory IgA. The 100% pure colostrum powder was dissolved in 150 nM NaCl at a final concentration of 5mg/ml. This standard was added in duplicate to the middle columns of each total IgA plate. The starting dilution was 1/3000 which contained 1.6μg/ml IgA. As human colostrum contains 96% secretory IgA (Kerr 1990), the standard value was adjusted to account for this. The starting dilution was 1/3000 which was assumed to contain 1.54 μg / ml S - IgA.

Salivary IgA Antibodies to S. mitis and S. oralis

Pooled human whole saliva was the standard for salivary antibodies to *S. mitis* and *S. oralis.* Twelve samples of whole saliva highly positive to *S. mitis* were combined and used as the standard for the antibody assays. Eleven different samples of whole saliva which were highly positive for *S. oralis* were combined and used as the standard in the *S. oralis* antibody assays. The arbitrary concentration for these standards was 2000 ELISA units (E.U.) per ml. The starting dilution for the assays was 1/5 and the concentration was therefore 400 E.U.

Total Salivary IgG

A further 6 samples of pooled human saliva were calibrated with World Health Organisation serum (ref: 67 / 99) as the standard for total salivary IgG estimations. The concentration of IgG in the WHO serum was 1200 mg / % and in the quantity in the pooled saliva, 935.0 ng /ml. The starting dilution of the standard for the assays was 1/10, which contained 9.375 ng / ml.

There were 4 blank wells on each tray, to which all the layers except the saliva were added. These were negative controls. The plates were stored overnight at 4°C.

Stage 4: Detection Layer

The plates were washed 3 times with PBS. The detection layers were as follows:-
Total IgA and I gA antibodies to *S. oralis* and *S. mitis*: mouse anti-human IgA monoclonal antibody[f] (Oxoid M26012 Clone 2DY), S- IgA: monoclonal anti-secretory component (Sigma code 1 - 6635, clone GA-1) and for IgG: mouse anti-human IgG (Bionostics M06014)
Each of these was prepared in PBS BSA/ 20 at 1/000 dilution and 100 μl added to each well of the appropriate assay plates. The plates were incubated for 2 hours at 37°C. (Table 12)

[f] Oxoid Unipath Ltd., Basingstoke, Hampshire RG24 OPW

Table 12: Detection Layer For Each Assay

Assay	Detection Layer	Dilution	Incubation	Washing
IgA & IgA Antibodies	Mouse anti-human IgA	1:1000	37°C - 2 hours	3 x PBS
S - IgA	Monoclonal anti-secretory component	1:1000	37°C - 2 hours	3 x PBS
IgG	Mouse anti-human IgG	1:1000	37°C - 2 hours	3 x PBS

Stage 5: Conjugate

The plates were washed 3 times with PBS. Alkaline phosphatase rabbit anti-mouse IgG (whole molecule) conjugate (Sigma[e] code A-1902) was prepared in PBS/BSA/T20 at a dilution of 1/500 for the total, secretory and specific IgA and 1/1000 for the IgG assays.
One hundred microlitres was added to each well. The plates were incubated at 37° for I hour.

Stage 6: Substrate and Developing

The plates were washed twice with PBS and once with distilled water. The substrate, p - Nitrophenyl phosphate disodium[e] dissolved in diethanolamine buffer (5 ml/5mg tablet of substrate) and 100 µl added to each well. The plates were developed at room temperature to approximately 1.5 O.D. of the most concentrated standard. The developing time for total IgA and Secretory IgA assays was 6 minutes; Salivary IgA antibody assays were 16 minutes and 12 minutes for IgG. The reaction was stopped by adding 50 µl of 3 M sodium hydroxide to each well. The plates were read by an ELISA reader at a wavelength of 405nm.

Optimisation of Assays

Studies were carried out to determine the optimum concentration of the coating layer, oral rinses, standards, detection layers and developing times.

Coefficient of Variation

All the samples were run in a single assay using the same buffers, reagents and antibodies. Regular quality testing was carried out by including oral rinses from the same 4 'test' children in each assay, at the same concentrations as the sample oral rinses. The coefficient of variation was calculated for each assay and variations both within and between each assay were monitored.

Analysis of Data

All raw data were tested for normality using the Shapiro - Wilks test and very few of the data sets were normally distributed. Results which were greater than 2 standard deviations from the

mean were excluded from the analyses together with the matched pairs for statistical comparisons between the transplant and control groups. All analyses were completed on the raw data using appropriate non - parametric tests.

CHAPTER 3: CLINICAL, MICROBIOLOGICAL AND IMMUNOLOGICAL RESULTS

DENTAL DISEASE

Reproducibility of Dental Indices

Studies were completed to assess the reproducibility of recording indices for caries, bacterial dental plaque and gingivitis and the Kappa value calculated (Altman 1992).

Dental Caries

Ten full arch toothblocks were examined by the main examiner (VSL) and a paediatric dentist using the World Health Organisation criteria to assess inter-examiner agreement (1984). Both examiners had been calibrated on previous occasions for district and regional dental health surveys. The same toothblocks were examined again after a 2 week period by the main examiner to assess intra-examiner agreement. The results are as follows:-

Inter - examiner agreement = 96.43%	**Kappa = 0.9448**
Intra - examiner agreement = 97.47%	**Kappa = 0.9609**

Bacterial Dental Plaque

A visual plaque score was carried out on 10 children by both examiners.

Inter - examiner agreement = 90.81%	**Kappa = 0.8322**

Gingivitis

Gingivitis was recorded for 10 children by the 2 examiners.

Inter - examiner agreement = 90.12%	**Kappa = 0.8114**

Mean Ages of Subjects and Matched Controls

Each transplant child was closely matched for age with a control child (Table 13).

The age range for the TBI group was 5 years 1 month to 13 years, and for the CTO group 4 years 11 months to 15 years and 10 months.

Table 13: Mean Ages of Bone Marrow Transplant and Matched Control Groups

TBI Group			Controls			
Male						
No.	**Mean**	**sd**	**No.**	**Mean**	**sd**	**Sig**
16	8.03	2.42	16	7.91	2.47	ns
Female						
No.	**Mean**	**sd**	**No.**	**Mean**	**sd**	**Sig**
7	8.61	1.28	7	8.93	1.08	ns
CTO Group			**Controls**			
Male						
No.	**Mean**	**sd**	**No.**	**Mean**	**sd**	**Sig**
8	7.99	3.57	8	8.11	3.49	ns
Female						
No.	**Mean**	**sd**	**No.**	**Mean**	**sd**	**Sig**
3	13.92	0.54	3	14.03	0.44	ns

Sig = statistical significance
ns = no significant difference

Ethnicity of Subjects and Matched Controls

The transplant children were predominantly Caucasian (n = 14). There were some children of Asian (n = 4), Middle Eastern (n = 3) and Afro-Caribbean origin (n = 2), all of whom were matched appropriately.

Oral Examinations and Collection of Oral Rinses

Oral examinations were carried out and saliva samples were collected at specific times during the transplantation period. These are shown in Table 14.

Table 14: Times of Oral Examinations and Oral Rinses: Transplant and Matched Control Groups

Baseline	**Post - BMT**	**N > 0.5 x 10^9/l**		**End of Study**	
Patient Groups		**No. of Days Post-BMT**		**No. of Days Post-BMT**	
		Mean	**Range**	**Mean**	**Range**
TBI	7 days	23..5	15 - 27	119	100 - 146
CTO	7 days	16.5	13 - 19	113	109 -125
Controls	-	-	-	117/118 days from baseline	104 - 134

N = Neutrophil count

DENTAL INDICES AND ORAL HEALTH

The dental caries score was recorded for each child at baseline and the results expressed as dmfs and dmft for the deciduous dentition and as DMFS and DMFT for the permanent dentition. The amount of untreated disease was calculated as a percentage of the total dental caries score for each group of children. The percentage of untreated disease includes caries and recurrent caries affecting existing restorations.

The plaque and gingivitis scores were recorded at baseline, 7 days post - transplantation and 113/119 days post - transplantation for the children in the treatment groups and for the matched controls at baseline and 118/119 days later.

Mucosal inflammation and ulceration (mucositis) occurred during the period of intense immunosuppression following the conditioning regimen and was most severe between approximately 5 and 12 days post - transplantation. The mucosal status on day 7 post - transplantation is reported using the notation of Seto et al (Seto et al. 1985).

WHOLE CONTROL GROUP

Dental Indices

A total of 33 matched control children were recruited from schools in Merton and Sutton Area Health Authority. The caries score was recorded at baseline only and the bacterial plaque and gingivitis scores were recorded at baseline and at the end of the study. The caries, plaque and gingivitis data have been analysed for the entire control group (n = 33)

Dental Caries

The proportion of control children who were caries free was 57.6% and the mean decayed, missing and filled scores are shown in Table 15.

Table 15: Dental Caries Indices: Whole Control Group (n = 33)

dmfs	dmft	DMFS	DMFT
2.42	1.42	0.21	0.14

Untreated Caries

The percentage of untreated caries in both the deciduous and permanent teeth is shown in Table 16.

Table 16: Percentage of Untreated Caries: Whole Control Group (n = 33)

Dentition	Mean	sd	Median	Min	Max
Deciduous	21.06	38.83	0.00	0.00	100.00
Permanent	0.00	0.00	0.00	0.00	0.00

Plaque

There were no differences between the plaque score or the proportion of tooth quadrisections covered by plaque as a percentage of the deciduous and permanent dentitions between baseline and 118/119 days later (Tables 17 and 18).

Gingivitis

There was no difference in the gingivitis scores or the proportion of quadrisections with associated gingivitis as a percentage of the total number of quadrisections in the deciduous and permanent teeth between baseline and 118/119 days later (Tables 17 and 18).

There was no oral ulceration detected in the mouths of any of the control children.

Table 17: Plaque and Gingivitis Scores at Baseline and the End of the Study: Whole Control Group (n = 33)

	Baseline					117/118 Days Later					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	4.35	6.32	0	0	21	3.58	5.10	1	0	19	ns
Gingivitis	1.62	2.94	0	0	8	1.77	2.90	0	0	12	ns
Permanent Dentition											
Plaque	8.89	8.86	7	0	36	9.36	8.78	8	0	36	ns
Gingivitis	8.07	8.96	4	0	36	9.14	8.74	8	0	30	ns

Sig = statistical significance
ns = no significant difference

Table 18: Percentage of Quadrisections Covered by Plaque and Quadrisections Associated with Gingivitis at Baseline and the End of the Study: Whole Control Group (n = 33)

	Baseline					117/118 Days Later					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	8.94	13.01	0	0	37.5	6.99	9.78	1.79	0	31.67	ns
Gingivitis	3.72	7.80	0	0	33.33	3.76	6.90	0	0	25	ns
Permanent Dentition											
Plaque	15.07	14.52	11.81	0	56.25	16.95	15.80	15.33	0	55	ns
Gingivitis	12.93	12.45	9.17	0	40	16.78	16	12.55	0	55	ns

Sig = statistical significance
ns = no significant difference

CHANGES IN THE ORAL HEALTH OF THE TBI GROUP

Dental Indices at Baseline

Dental Caries

The proportion of children who were caries free was 47.8%, which was not significantly different from the 65.2% of the matched controls. The dmfs score at baseline was greater than the matched control group ($p < 0.05$) and the dmft was also increased but not significantly (Fig 3). There were no differences in the DMFS or DMFT between the TBI and control children (Table 19).

Untreated Caries

There was a greater percentage of untreated caries in the matched control children but this was not significantly different from the TBI children. (Table 20).

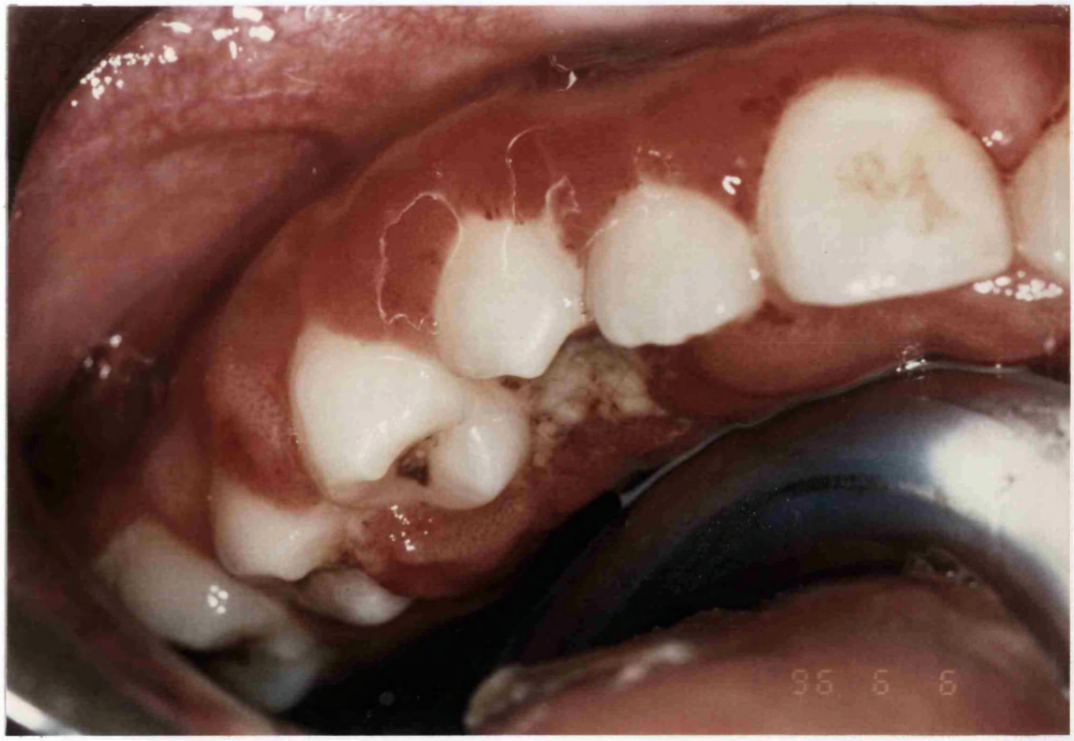


Fig 3: Dental caries in the upper right premolar and molar teeth. There is also extensive gingivitis and pseudomembrane formation

Plaque

There was no significant difference in the mean plaque score or proportion of tooth quadrisections covered by plaque as a percentage of the total number of deciduous and permanent tooth quadrisections between the TBI and matched control group at baseline (Tables 21 and 22).

Gingivitis

There was no significant difference in the mean gingivitis score or the proportion of quadrisections with associated gingivitis as a percentage of the total number of quadrisections in the deciduous and permanent teeth between the TBI and controls groups at baseline (Tables 21 and 22).

Table 19: Decayed Missing Filled Surfaces and Teeth: TBI and Matched Control Groups

	TBI Group (n = 23)					Matched Control Group (n = 23)					
	Mean	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
dmfs	7.38	10.43	4	0	40	2.00	3.56	0	0	12	**$p < 0.05$**
dmft	2.53	2.82	2	0	8	1.10	1.79	0	0	5	ns
DMFS	0.00	0.00	0	0	0	0.11	0.32	0	0	1	ns
DMFT	0.00	0.00	0	0	0	0.11	0.32	0	0	1	ns

Sig = statistical significance
ns = no significant difference

Table 20: Percentage of Untreated Dental Caries: TBI and Matched Control Groups

	TBI Group (n =23)					Matched Control Group (n = 23)					
	Mean	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Deciduous Dentition	9.54	30.08	0	0	100	20.63	39.79	0	0	100	ns
Permanent Dentition	0	0	0	0	0	0	0	0	0	0	ns

Sig = statistical significance
ns = no significant difference

Table 21: Plaque and Gingivitis Scores at Baseline: TBI and Matched Control Groups

	TBI Group (n = 23)					Matched Control Group (n =23)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	6.81	8.78	2	0	28	4.38	5.68	0	0	18	ns
Gingivitis	4.14	5.70	0	0	15	1.71	3.05	0	0	8	ns
Permanent Dentition											
Plaque	15.42	20.72	9	0	88	8.47	9.55	4	0	36	ns
Gingivitis	17.95	26.38	7	0	100	8.32	9.70	4	0	36	ns

Sig = statistical significance
ns = no significant difference

Table 22: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at Baseline: TBI and Matched Control Groups

	TBI Group (n = 23)					Matched Control Group (n = 23)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	12.55	16.07	5.56	0	56.82	9.40	12.71	0	0	37.50	ns
Gingivitis	8.58	12.60	0	0	38.89	4.13	8.43	0	0	33	ns
Permanent Dentition											
Plaque	29.16	28.76	18.75	0	88.89	14.34	13.59	10	0	40	ns
Gingivitis	28.29	29.62	17.39	0	100	14.36	13.70	10	0	40	ns

Sig = statistical significance
ns = no significant difference

Bacterial Dental Plaque and Gingivitis Seven Days Post - transplantation

Three children refused examination

Plaque

There was a significant increase in the mean plaque score from baseline for the deciduous teeth ($p < 0.003$) and the permanent teeth ($p < 0.001$) (Fig 4). The proportion of tooth quadrisections covered by plaque as a percentage of the total number of tooth quadrisections increased significantly for both the deciduous ($p < 0.003$) and permanent dentitions ($p < 0.001$) (Tables 23 and 24).

Gingivitis

There was a significant increase from baseline in the mean gingivitis score for both the deciduous ($p < 0.001$) and the permanent teeth ($p < 0.001$) (Fig 4). The proportion of quadrisections associated with gingivitis as a percentage of the total number of quadrisections increased significantly for both the deciduous ($p < 0.001$) and the permanent dentitions ($p < 0.001$). Spontaneous gingival bleeding was observed in 3 children (Tables 23 and 24).

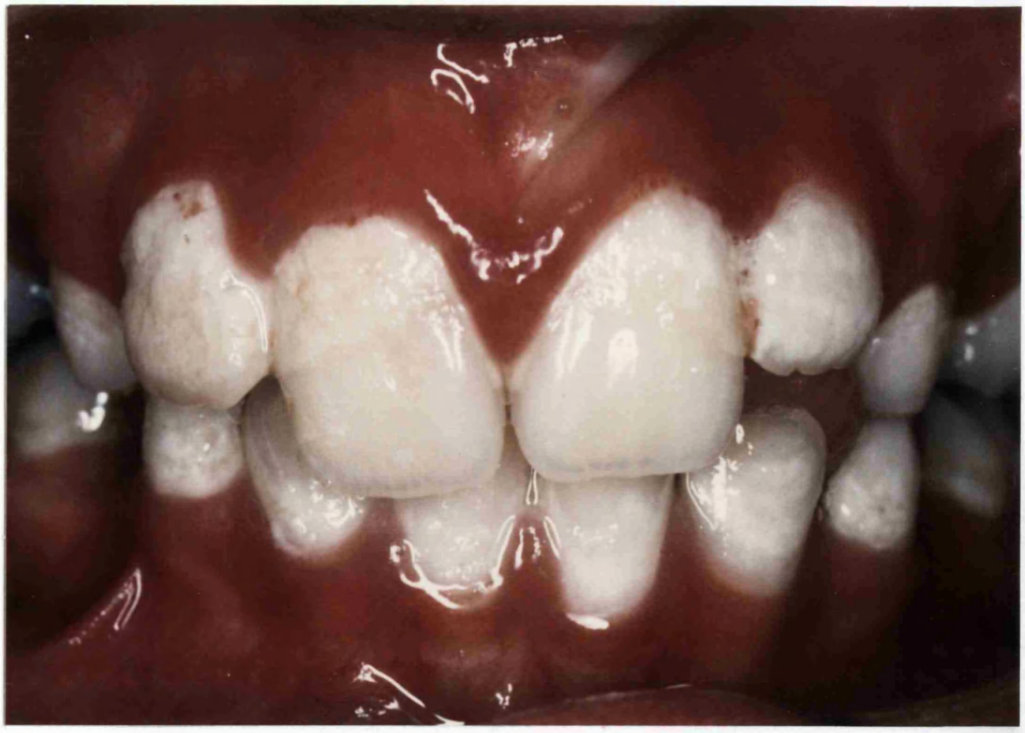


Fig 4: Extensive deposits of bacterial plaque and gingival inflammation with new blood vessel formation in the marginal gingivae

Table 23: Plaque and Gingivitis Scores at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): TBI Group

	Baseline (n = 20)					7 Days Post - Transplantation (n = 20)*					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	5.75	7.50	1	0	25	18.63	16.03	20	0	48	**p < 0.003**
Gingivitis	4.35	5.77	0	0	15	21.21	18.51	18	0	60	**p < 0.001**
Permanent Dentition											
Plaque	14.65	20.46	8	0	88	33.28	25.61	29.50	0	112	**p < 0.001**
Gingivitis	11.45	13.54	5.5	0	46	31.56	18.61	33.50	0	70	**p < 0.001**

Sig = statistical significance
ns = no significant difference
* 3 children refused

Table 24: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): TBI Group

	Baseline (n = 20)					7 Days Post -Transplantation (n = 20)*					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	11.43	15.62	2.78	0	56.82	37.17	32.50	43.75	0	100	**p < 0.003**
Gingivitis	9.01	12.77	0	0	38.89	41.12	34.34	50	0	100	**p < 0.001**
Permanent Dentition											
Plaque	27.70	28.74	18.75	0	88.89	71.87	36.99	87.85	0	100	**p < 0.001**
Gingivitis	21.88	24.53	15.63	0	88.33	70.81	35.21	81.70	0	100	**p < 0.001**

Sig = statistical significance
ns = no significant difference
* 3 children refused

Mucositis

All the children except for 3 who had no sign of mucosal inflammation, complained of a sore throat and dysphagia (Figs 5 and 6).

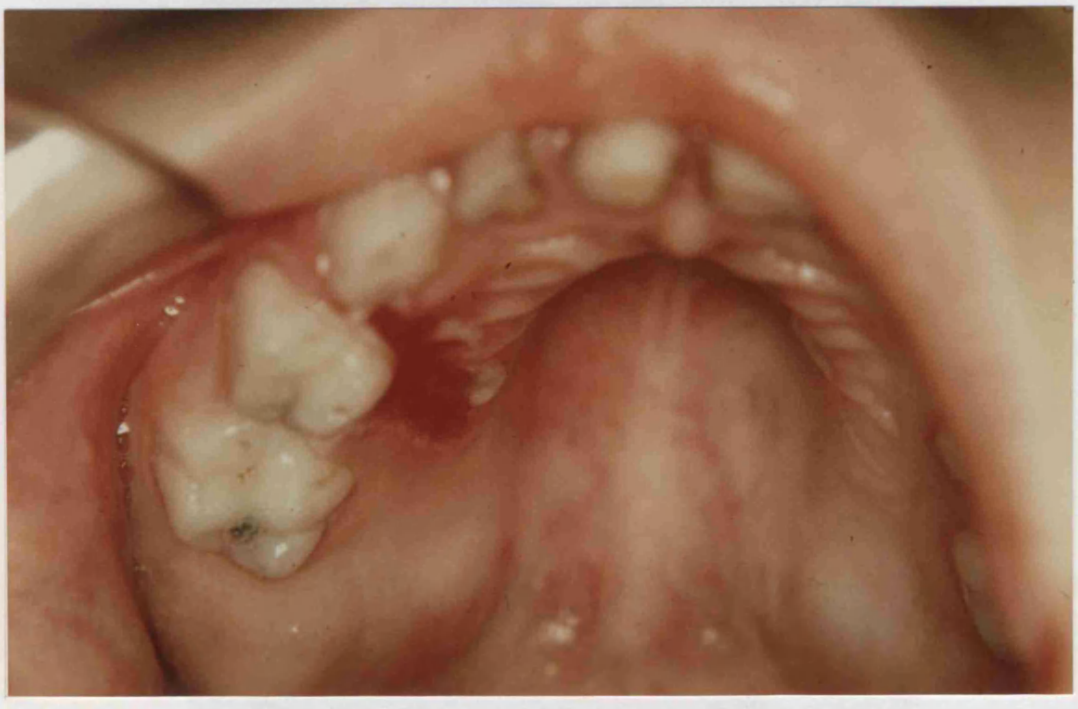


Fig 5: Ulcer on palatal mucosa adjacent to the upper right first deciduous premolar

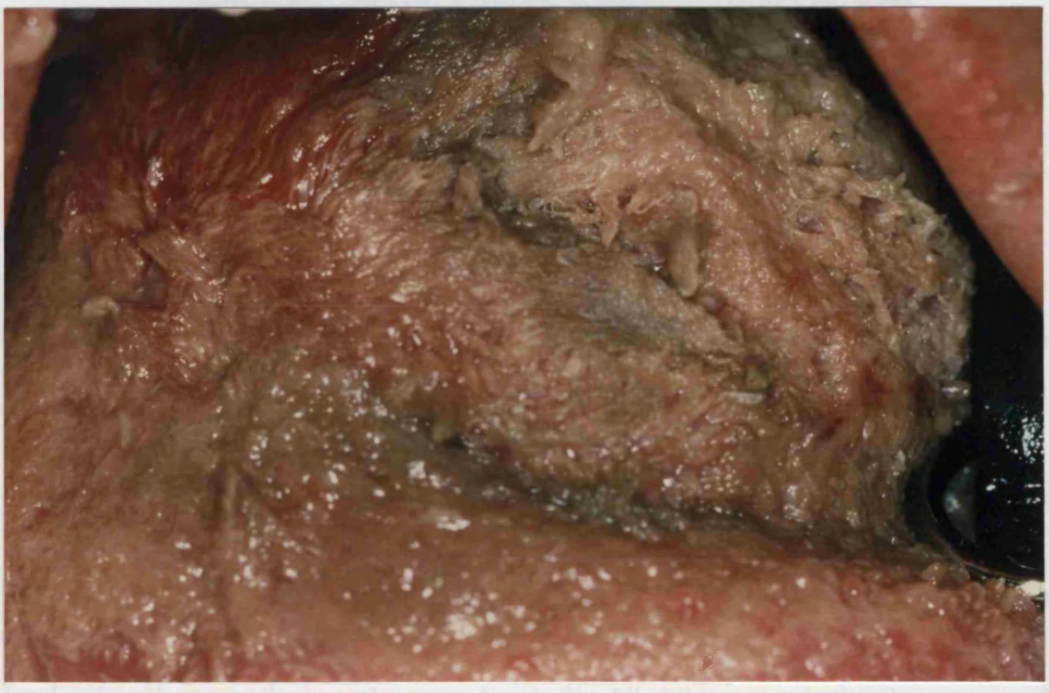


Fig 6: Brown furry tongue: Extensive involvement which indicates increased oral bacterial loading. There is also bleeding from several small breaches of the epithelium

A further 7 children had thick ropy saliva and 3 refused examination. Five children had noticeable parotid swelling and a further 10 complained of parotid discomfort (Figs 7 and 8).

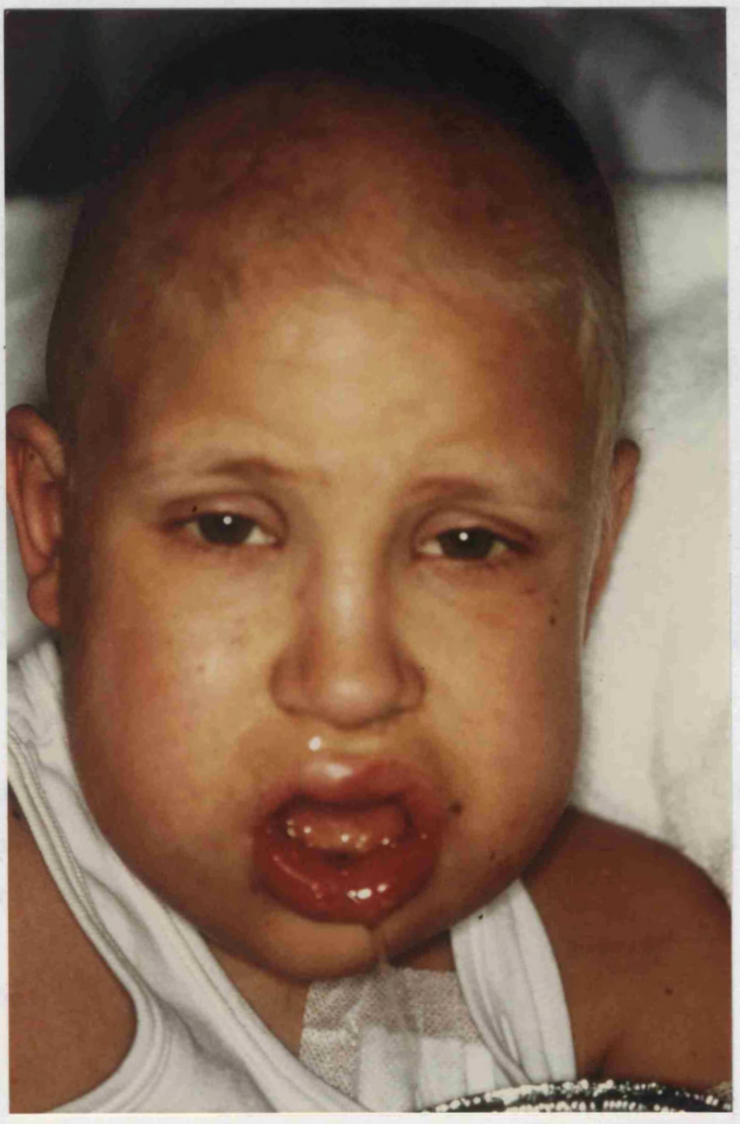


Fig 7: There is parotid swelling and the lips are also swollen and shiny. The child is drooling saliva; his throat is so sore that he is unable to swallow

Seven children had swollen or sore and cracked lips. Three children had ulcers mainly on the ventral surface and lateral borders of the tongue (grade 3) and 2 others on the hard palate, floor of the mouth and the sublingual papillae (grade 4). In 2 children the ulcers on the tongue were bleeding. Ten children had generalised mucosal inflammation (grade 3) and 2 others localised erythema on the buccal mucosa (grade 2). All children required oral or parenteral pain relief.

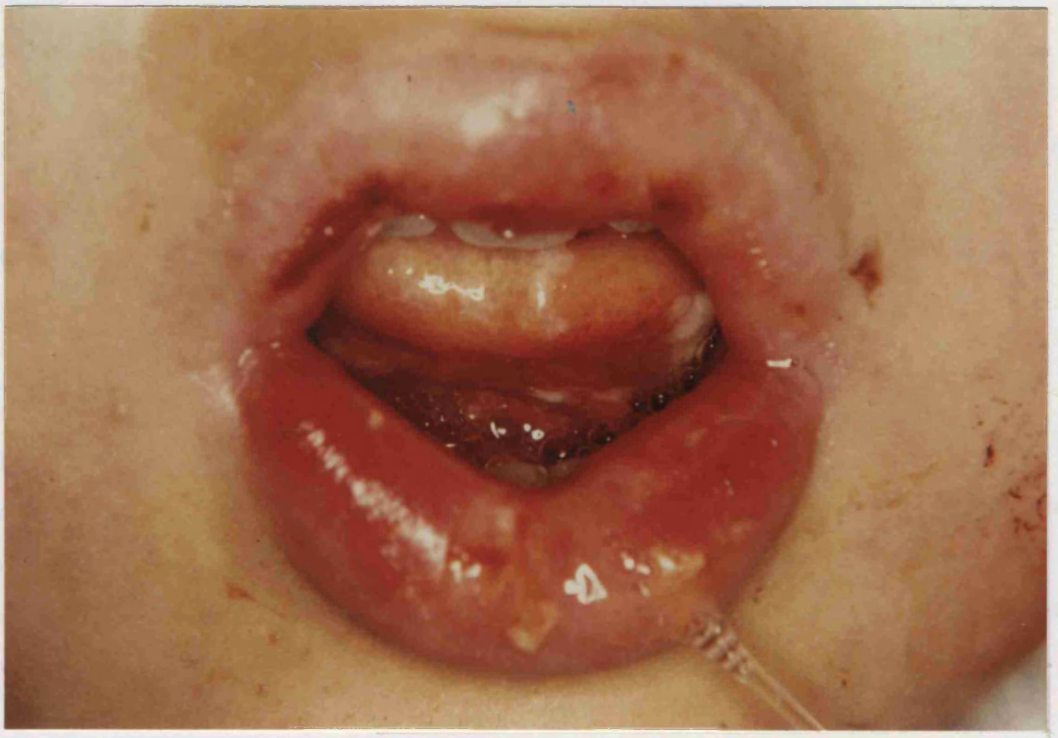


Fig 8: Close - up view of the child in Fig 7. There is crusting of the lips, pseudomembrane formation along the lateral borders and ventral surface of the tongue and small petechial haemorrhages.

Bacterial Dental Plaque and Gingivitis 119 Days Post - Transplantation

Plaque

There was no significant difference in the mean plaque score or proportion of tooth quadrisections covered by plaque as a percentage of the total number of deciduous and permanent teeth between baseline and 119 days post - transplantation (Tables 21, 22, 25 and 26).

Gingivitis

There was no significant difference in the mean gingivitis score or the proportion of quadrisections associated with gingivitis as a percentage of the total number of tooth quadrisections for either the deciduous or permanent dentitions between baseline and 119 days post - transplantation (Tables 21, 22, 25 and 26).

Comparison of Plaque and Gingivitis at the End of the Study: TBI and Matched Control Groups

Plaque and Gingivitis

There were no significant differences in the mean plaque score or the proportion of tooth quadrisections covered by plaque as a percentage of the total number of tooth quadrisections, or the mean gingivitis scores and proportion of quadrisections associated with gingivitis in the deciduous and permanent dentitions, between the TBI group and controls at the end of the study (Tables 25 and 26).

Summary of Results for Dental Disease: TBI Group

The dental indices were similar for the TBI children and the matched controls except for the dmfs which was significantly greater in the TBI children. There were no significant differences in the plaque and gingival scores or proportion of tooth quadrisections covered by plaque and quadrisections associated with gingivitis between the TBI and control groups at baseline or at the end of the study.

An important finding was a significant increase in the total plaque score and proportion of tooth quadrisections covered by plaque and total gingivitis score and proportion of quadrisections associated with gingivitis, at 7 days post - transplantation. All the children except for 3 had clinical signs of mucositis.

Table 25: Plaque and Gingivitis Scores at End of Study: TBI and Matched Control Groups

	TBI Group (n =16)					Matched Control Group (n =16)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	4.33	6.39	2	0	24	3.27	4.48	0	0	12	ns
Gingivitis	1.80	2.83	0	0	8	2.13	3.42	0	0	12	ns
Permanent Dentition											
Plaque	10.58	21.45	4	0	77	8.58	9.52	5.50	0	28	ns
Gingivitis	5	6.89	1.50	0	20	8.58	9.52	5.50	0	28	ns

Sig = statistical significance
ns = no significant difference

Table 26: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at End of Study: TBI and Matched Control Groups

	TBI Group (n = 16)					Matched control Group (n =16)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	6.39	8.74	2.50	0	30	6.82	9.52	0	0	25	ns
Gingivitis	3.27	5.55	0	0	18.18	4.87	8.61	0	0	25	ns
Permanent Dentition											
Plaque	14.14	19.08	9.40	0	68.75	15.76	12.96	16.07	0	39.29	ns
Gingivitis	8.11	10.02	3.13	0	28.13	14.46	13.17	15.33	0	39.29	ns

Sig = statistical significance
ns = no significant difference

CHANGES IN THE ORAL HEALTH OF THE CTO GROUP

Dental Indices at Baseline

Dental Caries

The proportion of children who were caries free was 54.5%, which was not significantly different from the 45.5% of the matched controls. There was no difference in the dmfs and dmft or the DMFS and DMFT or the combined total between the CTO group and the matched controls (Table 27)

Untreated Caries

There was no difference in the proportion of untreated caries for the deciduous and permanent teeth (Table 28).

Plaque

There was no significant difference in the mean plaque score or in the proportion of tooth quadrisections covered by plaque as a percentage of the total number of deciduous and permanent tooth quadrisections between the CTO and matched control groups at baseline (Tables 29 and 30)

Gingivitis

There was no significant difference in the mean gingivitis score or in the proportion of quadrisections associated with gingivitis expressed as a percentage of the total number of quadrisections in the deciduous and permanent teeth, between the CTO and controls groups at baseline (Tables 29 and 30).

Table 27: Decayed, Missing, Filled Surfaces and Teeth: CTO and Matched Control Groups

	CTO Group (n =11)					Matched Control Group (n = 11)					
	Mean	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
dmfs	10.86	17.65	0	0	6	3.00	4.00	0	0	10	ns
dmft	4.14	5.61	0	0	14	2.00	2.31	0	0	5	ns
DMFS	1.33	3.64	0	0	11	0.44	1.01	0	0	3	ns
DMFT	0.67	1.66	0	0	5	0.22	0.44	0	0	1	ns

Sig = statistical significance
ns = no significant difference

Table 28: Percentage of Untreated Dental Caries: CTO and Matched Control Groups

	CTO Group (n =11)					Matched Control Group (n =11)					
	Mean	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Deciduous Dentition	15.48	27.06	0.00	0.00	71.43	16.33	37.28	0	0	100	ns
Permanent Dentition	0.56	1.33	0.00	0.00	4.00	0	0	0	0	0	ns

Sig = statistical significance
ns = no significant difference

Table 29: Plaque and Gingivitis Scores at Baseline: CTO and Matched Control Groups

	CTO Group (n =11)					Matched Control Group (n =11)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	4.57	5.77	2	0	14	4.14	8	0	0	21	ns
Gingivitis	2	5.29	0	0	14	0.86	2.27	0	0	6	ns
Permanent Dentition											
Plaque	16.78	21	13	0	67	8.8	7.84	7	0	20	ns
Gingivitis	21.78	35.09	13	0	100	6.8	7.61	5	0	20	ns

Sig = statistical significance
ns = no significant difference

Table 30: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at Baseline: CTO and Matched Control Groups

	CTO Group (n = 11)					Matched Control Group (n =11)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	8.54	9.81	6.25	0	25	6.50	13.16	0	0	35	ns
Gingivitis	3.57	9.45	0	0	25	1.43	3.78	0	0	10	ns
Permanent Dentition											
Plaque	30.57	29.26	37.50	0	75	14.31	17.84	11.11	0	56.25	ns
Gingivitis	35.04	36.46	37.50	0	100	7.60	8.79	7.89	0	25	ns

Sig = statistical significance
ns = no significant difference

Bacterial Dental Plaque and Gingivitis Seven Days Post - Transplantation

Plaque

There was a significant increase in the mean plaque score from baseline for the deciduous teeth ($p < 0.03$) and the permanent teeth ($p < 0.01$). The proportion of tooth quadrisections covered by plaque as a percentage of the total number of tooth quadrisections was significantly increased for both the deciduous teeth ($p < 0.04$) and the permanent teeth ($p < 0.01$) (Tables 31and 32).

Gingivitis

There was a significant increase from baseline in the mean gingivitis score for both the deciduous ($p < 0.03$) and the permanent teeth ($p < 0.02$). The proportion of quadrisections associated with gingivitis as a percentage of the total number of tooth quadrisections was significantly increased for both the deciduous ($p < 0.03$) and permanent teeth ($p < 0.02$). One child had spontaneous gingival bleeding (Tables 31 and 32).

Mucositis

Nine children complained of a sore throat. Two children had ulcers on the lateral borders of the tongue one of which was bleeding and localised erythema (grade 3). All except for 3 children had localised erythema on the buccal mucosa and palate (grade 2).

Table 31: Plaque and Gingivitis Scores at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): CTO Group

	Baseline (n = 11)					7 Days Post - Transplantation (n = 11)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	4.47	5.77	2	0	14	21.43	22.38	10	0	64	**p < 0.03**
Gingivitis	2	5.29	0	0	14	14.71	11.73	10	0	32	**p < 0.03**
Permanent Dentition											
Plaque	16.78	21	13	0	67	37.78	30.47	32	0	112	**p < 0.01**
Gingivitis	21.78	35.09	13	0	100	37.78	30.47	32	0	112	**p < 0.02**

Sig = statistical significance

Table 32: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): CTO Group

	Baseline (n = 11)					7 Days Post -Transplantation (n = 11)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	8.54	9.81	6.25	0	25	35.27	33.85	25	0	100	**p < 0.04**
Gingivitis	3.57	9.45	0	0	25	24.65	16.80	25	0	46.43	**p < 0.03**
Permanent Dentition											
Plaque	30.57	29.26	37.50	0	75	58.33	36.51	58.33	0	100	**p < 0.01**
Gingivitis	35.04	36.46	37.50	0	100	58.33	36.51	58.33	0	100	**p < 0.02**

Sig = statistical significance

Bacterial Dental Plaque and Gingivitis 113 Days Post -Transplantation

Plaque

There was no significant difference in the mean plaque score or proportion of tooth quadrisections covered with plaque as a percentage of the total number of tooth quadrisections in either the deciduous or permanent teeth 113 days post - transplantation (Tables 29,30,33 and 34).

Gingivitis

There was no significant difference in the mean gingivitis score or proportion of quadrisections associated with gingivitis as a percentage of the total number of quadrisections for either the deciduous or permanent teeth between baseline and 113 days post - transplantation (Tables 29,30,33 and 34).

Comparison of Plaque and Gingivitis at the End of the Study: CTO and Matched Control Groups

Plaque and Gingivitis

There were no significant differences in the mean plaque score and the proportion of tooth quadrisections covered by plaque as a percentage of the total number of tooth quadrisections, or the gingivitis score and proportion of quadrisections associated with gingivitis as a percentage of the total quadrisections for both the deciduous and permanent dentitions between the CTO and matched control groups at the end of the study (Tables33 and 34).

Summary of Results of Dental Disease: CTO Group

The dental indices were similar for the CTO children and the matched controls. There were no significant differences in the dental caries, plaque and gingival indices between the CTO and control groups at baseline or at the end of the study.

There was a significant increase in the total plaque score and proportion of tooth quadrisections covered with plaque and total gingivitis score and proportion of quadrisections associated with gingivitis at 7 days post - transplantation.

All the children except for 3 had clinical signs of mucositis.

Table 33: Plaque and Gingivitis Scores at End of Study: CTO and Matched Control Groups

	CTO Group (n =11)					Matched Control Group (n =11					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	1.14	1.95	0	0	4	5.57	7.39	2	0	19	ns
Gingivitis	0.57	1.51	0	0	4	1.71	2.43	0	0	6	ns
Permanent Dentition											
Plaque	13	13.11	12	0	41	6.90	6.23	6	0	17	ns
Gingivitis	13	13.11	12	0	41	6.10	5.55	6	0	17	ns

Sig = statistical significance
ns = no significant difference

Table 34: Percentage of Surfaces Covered by Plaque and Quadrisections Associated with Gingivitis at End of Study: CTO and Matched Control Groups

	CTO Group (n = 11)					Matched Control Group (n =11)					
Deciduous Dentition	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**	**Sig**
Plaque	1.73	3.03	0	0	7.14	8.50	11.73	4.17	0	31.67	ns
Gingivitis	0.71	1.89	0	0	5	2.47	3.28	0	0	7.89	ns
Permanent Dentition											
Plaque	16.63	17.38	12.50	0	50	13.88	17.05	8.93	0	53.13	ns
Gingivitis	19.87	16.59	16.67	0	50	12.80	17.03	8.93	0	53.13	ns

Sig = statistical significance
ns = no significant difference

Comparison of Dental Caries, Plaque and Gingivitis Indices: TBI and CTO Groups

Dental Caries

There was no significant difference in the dmfs, dmft or amount of untreated caries between the TBI and CTO group. The DMFS and DMFT were significantly greater in the CTO group ($p < 0.04$) and the proportion of untreated caries ($p < 0.04$).

Plaque and Gingivitis

The were no significant differences in the plaque and gingivitis indices at baseline, at 7 days post - transplantation or at the end of the study between the TBI and CTO groups.

Mucositis

Although children in both the transplant groups were affected by mucositis, the clinical signs and symptoms were more severe in the TBI group.

Summary of Results of Dental Disease: TBI and CTO Groups

(1) The DMFS, DMFT and amount of untreated caries was greater in the CTO group, but there was no difference in the plaque and gingivitis indices between the 2 transplant groups.

(2) Mucositis was more severe in the TBI group.

MICROBIOLOGY RESULTS

Reproducibility of Isolation and Enumeration of Micro - organisms from Oral Rinses

Study One: Reproducibility of Sample Processing

Oral rinses from 20 healthy children were divided into halves and processed on both selective and non - selective media as separate samples. The colonies from each half were counted and compared (Table 35). There were no significant differences in the colony counts between samples and the correlation coefficient for each type of medium was statistically significant ($p < 0.0001$)

Table 35: Comparison of the Recovery of Bacteria from Aliquots of the Same Oral Rinses (n = 20)

	Sample 1		Sample2		
Media	**Mean**	**Median**	**Mean**	**Median**	**rho***
MacConkey	2.47	0	2.71	0	1.00
Sabouraud	3.45	0	3.40	0	1.00
BMSA	4.65	4.83	4.65	4.84	0.99
MSA	6.92	7.04	6.88	7.01	0.85
FAA (anaerobic)	8.28	8.38	8.25	8.36	0.95

*rho = Spearman Rank Correlation Coefficient

Study Two: Reproducibility of Sampling Procedure

Two oral rinses were collected 30 minutes apart, from 20 healthy children and processed on both selective and non-selective media, the number of colonies counted and compared between the samples (Table 36). Two samples were excluded because of contamination. There were no significant differences between the colony counts from the first and second sample. The correlation coefficients for the 2 samples for each type of media were statistically significant ($p < 0.0001$).

Table 36: Results From the Oral Rinses to Test the Reproducibility of Sample Procedure (n = 18)

	Sample 1		Sample2		
Media	**Mean**	**Median**	**Mean**	**Median**	***rho**
MacConkey	3.19	0	3.13	0	0.97
Sabouraud	3.19	0	1.61	0	0.89
BMSA	2.62	2.56	2.63	2.63	0.79
MSA	6.61	6.72	6.66	6.78	0.75
FAA (aerobic)	6.83	6.82	6.62	6.72	0.80
FAA (anaerobic)	6.78	6.95	6.88	6.98	0.79

*rho = Spearman Rank Correlation Coefficient

Study Three: Inter-examiner Variability of Colony Counting

Thirty seven different media plates were randomly selected, described and counted by the investigator and one other trained person. There were no statistical differences between the colony counts and the correlation coefficient for the 2 sets of counts was statistically significant ($p < 0.0001$). The results were as follows (Table 37):

Table 37: Results for Inter - Examiner Variability of Colony Counting

	Mean	**Median**	**rho***
Examiner 1	7.21	7.15	
Examiner 2	7.20	7.16	0.99

*rho = Spearman Rank Correlation Coefficient

Summary of Reproducibility Tests

The were no significant differences in the bacterial counts in each of the 3 studies. The correlation coefficient between each pair of samples were all statistically significant, which demonstrates a high level of agreement. The methods of sample procedure and sample processing and colony counting were all satisfactory.

THE STABILITY OF THE ORAL FLORA OF THE CONTROL GROUP

A total of 33 matched control children was recruited from schools in Merton and Sutton Area Health Authority. The bacterial counts from their oral rinses were recorded at baseline and 118 days later for those matched with the TBI children and 117 days later for those matched with CTO children. The microbiological data have been analysed for the entire control group (n = 33), the results demonstrating the stability of the oral flora in healthy children.

Total Bacterial Counts

There were no significant differences in the mean total aerobic or anaerobic bacterial counts between baseline and 117/118 days later (Table 38).

Viridans Streptococci

Isolation Frequency

A wide variety of species of viridans streptococci was isolated from the oral rinses of these children including *S. mutans, S. mitis, S.oralis, S. gordonii, S. sanguis, S. parasanguis, S. salivarius, S. vestibularis, and S. intermedius* (Table 38). There was no significant difference in the mean number of streptococcal species or in the frequency isolation of individual species from the oral rinses at baseline or 117/118 days later (Table 38). There was also no difference in the mean number of each streptococcal species isolated at baseline or the end of the study (Table 39). The most numerous and frequently isolated streptococcal species were *S. mitis*, *S. oralis* and *S. salivarius.*

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There were no significant differences between the proportion of the total streptococcal count as a percentage of the total anaerobic count at baseline and at the end of the study (Table 40).

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There were no significant differences in the proportion of each streptococcal species as a percentage of the total streptococcal count, except for *S. salivarius.* This decreased significantly from a mean value of 39.06 at baseline to 28.50 after 117/118 days ($p < 0.01$) (Table 41).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There were no differences in the proportion of each streptococcal species as a percentage of the total anaerobic count between baseline and at 117/118 days later (Table 42).

The 'oralis group' (S. mitis and S. oralis) as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between baseline and the end of the study (Table 43). The '*oralis* group' was not isolated from 2 children at baseline and not from one of them 118 days later.

Candida Species

C. albicans was the only yeast species isolated from the control children. *C. albicans* was isolated from the oral rinses of 6 children at baseline and from 2 children 118/119 days later. There was no difference in the isolation frequencies. *C. albicans* was isolated from only 1 child on both occasions. The range of number of colonies per ml of oral rinse was 20 - 200 per ml of oral rinse at baseline and 40 and 6500 colonies respectively for the 2 children after 117/118 days.

Enterobacteriaceae and Enterococci

Neither *Enterobacteriaceae* or enterococci were isolated from the oral rinses of any of the control children during the study.

Summary of Results at Baseline and the 117/118 Days Later: Control Group

These results demonstrate the stability of the oral flora in a group of healthy schoolchildren who were the matched controls for the study.

There was no significant difference in the mean values of the total aerobic and anaerobic bacterial counts between baseline and the end of the study. There was a wide variety of streptococcal species isolated from the oral rinses both at baseline and 117/118 days later. There was little change in the absolute numbers or the proportion of each species, with the exception of *S. salivarius* which decreased significantly at the end of the study ($p < 0.01$). The isolation frequency of *C. albicans* was low and neither *Enterobacteriaceae* nor enterococci were isolated from the oral rinses of these children.

Table 38: Total Bacterial Counts as Colony - Forming Units per ml Oral Rinse ($\log_{10}$) at Baseline and 117/118 Days Later: Whole Control Group (n = 33)

	Baseline						117/118 Days					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mitis	60.6	3.45	2.91	4.70	nd	7.08	72.7	4.36	2.76	5.73	nd	7.78
S. oralis	66.7	4.07	2.97	5.90	nd	7.90	78.8	4.78	2.62	6.04	nd	7.61
S. gordonii	6.1	0.34	1.38	nd	nd	6.30	6.1	0.36	1.45	nd	nd	6.23
S. salivarius	93.9	5.72	1.59	6.04	nd	7.20	93.9	5.60	1.57	5.89	nd	7.49
S. vestibularis	9.1	0.55	1.77	nd	nd	6.42	6.1	0.37	1.48	nd	nd	6.71
S. sanguis	39.4	2.45	2.86	nd	nd	6.95	45.5	2.69	3.02	nd	nd	7.20
S. parasanguis	51.5	2.78	2.77	4.48	nd	6.15	51.5	2.84	2.83	4.30	nd	6.43
S. mutans	33.3	1.10	1.67	nd	nd	4.79	24.2	0.74	1.41	nd	nd	4.81
S. intermedius	6.1	0.36	1.46	nd	nd	6.30	3.0	0.21	1.20	nd	nd	6.90
Total Aerobic Count	100	7.26	0.50	7.26	5.97	8.22	100	7.26	0.48	7.09	6.53	8.21
Total Anaerobic Count	100	7.50	0.50	7.63	6.61	8.37	100	7.53	0.50	7.61	6.53	8.38

nd = not detected

% = Percentage of children

Table 39: Mean Number of Species of Viridans Streptococci Isolated from the Oral Rinses of the Control Group (n = 33)

Baseline			117/118 Days Later			
Mean	**sd**	**Median**	**Mean**	**sd**	**Median**	**Sig**
3.67	0.92	4.00	3.85	0.97	4.00	ns

Sig = statistical significance
ns = not statistically significant

Table 40: Mean Total Streptococcal Count as a Percentage of the Total Anaerobic Count: Whole Control Group (n = 33)

Baseline			117/118 Days Later			
Mean	**sd**	**Median**	**Mean**	**sd**	**Median**	**Sig**
17.86	14.89	13.58	19.62	22.08	15.15	ns

Sig = statistical significance
ns = not statistically significant

Table 41: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline and 117/118 Days Later: Whole Control Group (n = 33)

	Baseline					117/118 Days				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mitis	14.42	20.92	3.09	nd	72.97	21.07	23.42	12.5	nd	94.37
S. oralis	22.10	23.15	15.46	nd	79.21	28.03	26.91	25.58	nd	90.41
S. gordonii	0.82	4.52	< 0.01	nd	25.97	1.42	6.96	< 0.01	nd	39.54
S. salivarius	39.06[a]	25.25	34.15	nd	97.75	28.50[a]	24.23	25.13	nd	99.99
S. vestibularis	3.06	11.94	< 0.01	nd	64.67	2.93	11.94	< 0.01	nd	57.30
S. sanguis	9.78	18.02	< 0.01	nd	81.82	10.64	15.44	< 0.01	nd	57.69
S. parasanguis	9.76	15.92	1.10	nd	57.14	7.14	11.36	2.02	nd	47.72
S. mutans	0.07	0.21	< 0.001	nd	1.14	0.03	0.15	< 0.001	nd	0.86
S. intermedius	0.92	3.76	< 0.01	nd	18.18	0.24	1.38	< 0.01	nd	7.92

nd = not detected

a = Significant decrease from baseline $p < 0.01$

Table 42: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline and 117/118 Days Later: Whole Control Group (n = 33)

	Baseline					118 Days				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mitis	3.05	5.20	0.42	nd	22.88	3.61	5.38	1.33	nd	24.90
S. oralis	5.40	9.44	1.54	nd	40.50	6.67	14.57	3.89	nd	83.67
S. gordonii	0.16	0.89	nd	nd	5.13	0.22	1.06	nd	nd	6.01
S. salivarius	6.39	6.82	4.03	nd	27.03	5.72	12.79	2.47	nd	73.13
S. vestibularis	1.35	6.88	nd	nd	39.39	0.38	1.68	nd	nd	9.20
S. sanguis	1.23	2.96	nd	nd	11.84	1.78	3.16	nd	nd	12.59
S. parasanguis	1.53	3.46	0.10	nd	14.44	1.56	3.78	0.07	nd	20.15
S. mutans	0.01	0.03	nd	nd	0.15	0.004	0.02	nd	nd	0.09
S. intermedius	0.11	0.48	nd	nd	2.63	0.10	0.58	nd	nd	3.32

nd = not detected

Table 43: The '*oralis* Group' as a Percentage of the Total Anaerobic Count: Whole Control Group (n = 33)

Baseline			117/118 Days Later			
Mean	**sd**	**Median**	**Mean**	**sd**	**Median**	**Sig**
8.45	11.54	3.10	10.26	16.37	5.85	ns

Sig = statistical significance
ns = not statistically significant

CHANGES IN THE ORAL FLORA OF THE TBI GROUP

Comparison of the Oral Flora at Baseline: TBI and Matched Control Groups

The baseline oral flora of the TBI children (n = 23) and their matched controls were compared at baseline. The results are as follows:-

Total Bacterial Counts

The mean total aerobic count was significantly greater in the TBI group than in the matched controls ($p < 0.03$). There were no significant differences in the mean total anaerobic counts (Table 44).

Viridans Streptococci

Isolation Frequency

A similar range of species of the viridans streptococci was isolated from the oral rinses of the TBI children and from their matched controls (Table 44). There was no significant difference in the mean number of streptococcal species between the 2 groups or in the frequency isolation of individual species from the oral rinses at baseline except for *S. oralis* (Table 45). The isolation frequency of *S. oralis* was significantly greater in the TBI group ($\chi^2 = 4.06$, df 1, $p < 0.04$). There were no differences in the mean streptococcal counts except for *S. salivarius,* which was greater in the control group ($p < 0.02$). The most numerous and frequently isolated streptococcal species were *S. mitis*, *S. oralis* and *S. salivarius.* *S. anginosus* was isolated from 1 child in the TBI group and *S. constellatus* from 1 child in the matched control group.

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the oral streptococcal count as a percentage of the total anaerobic count between the TBI and control groups (Table 46).

Table 44: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline: TBI Group and Matched Control Groups

Groups	TBI Group (n = 23)						Matched Control Group (n = 23)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	17.4	0.67	1.52	nd	nd	4.78	30.4	0.95	1.53	nd	nd	4.26
S. mitis	60.9	3.58	3.05	4.85	nd	7.85	60.9	3.43	2.90	4.45	nd	7.08
S. oralis	87	5.25	2.29	5.90	nd	7.73	60.9	3.74	3.11	5.90	nd	7.90
S. sanguis	26.1	1.54	2.70	nd	nd	7.52	34.8	1.91	2.68	nd	nd	6.15
S. parasanguis	39.1	2.15	2.80	nd	nd	7.00	62.2	2.75	2.70	4.70	nd	6.15
S. gordonii	17.4	0.98	2.20	nd	nd	6.28	4.4	0.22	1.04	nd	nd	5.00
S. salivarius	78.3	4.47[a]	2.53	5.54	nd	7.38	95.7	5.80[a]	1.37	6.04	nd	6.95
S. vestibularis	17.4	0.96	2.17	nd	nd	6.700	13.0	0.79	2.09	nd	nd	6.42
S. anginosus	4.3	0.24	1.15	nd	nd	5.52	nd				nd	
S. intermedius	nd				nd		4.4	0.25	1.19	nd	nd	5.700
Total Aerobic Count	100	7.39[b]	0.73	7.76	5.76	8.32	100	7.15[b]	0.47	7.20	5.97	7.73
Total Anaerobic Count	100	7.56	0.59	7.76	6.38	8.48	100	7.45	0.51	7.28	6.61	8.36

nd = not detected

% = percentage of children

a = significantly greater in matched control group, $p < 0.02$

b = significantly greater in TBI group, $p < 0.03$

Table 45: Mean Number of Species of Viridans Streptococci Isolated from the Oral Rinses at Baseline: TBI and Matched Control Groups

TBI Group (n = 23)			Matched Control Group (n = 23)			
Mean	**sd**	**Median**	**Mean**	**sd**	**Median**	**Sig**
3.26	1.10	3.00	3.67	0.92	4.00	ns

Sig = statistical significance
ns = not statistically significant

Table 46: Mean Total Streptococcal Count as a Percentage of the Total Anaerobic Count: TBI and Matched Control Groups

	Mean	**sd**	**Median**	**Min**	**Max**	**Sig**
Control Group	19.36	18.43	12.80	0.46	60.91	
TBI Group	19.69	20.63	15	0.28	73.86	ns

Sig = statistical significance
ns = not statistically significant

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

The proportion of *S. salivarius* as a percentage of the total streptococcal count was significantly greater in the controls when compared with the TBI group ($p < 0.02$). There were no other significant differences in the proportion of any other individual streptococcal species at baseline (Table 47).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The only difference between the 2 groups was a significantly larger proportion of *S. salivarius* as a percentage of the total anaerobic count in the control group ($p < 0.02$) (Table 48).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the '*oralis* group' as a percentage of the total anaerobic count between the 2 groups at baseline. Members of the '*oralis* group' were not isolated from 2 control children and 1 TBI subject.

Candida Species

Candida species were isolated from 7 children in the TBI group and from 3 of the matched controls and this difference in the isolation frequency between the 2 groups was not significant. Although *C. albicans* was the predominant species in the TBI group, *C. glabrata* and *C. tropicalis* were also isolated from 2 children. *C. albicans* alone was isolated from the control group (Table 49).

Enterobacteriaceae and Enterococci

Enterobacteriaceae and enterococci were not isolated from the oral rinses of either the control or TBI group at baseline.

Summary of Results at Baseline: TBI and Matched Control Groups

There were a few differences in the oral flora of the TBI children and their matched controls:

(1) There was a significantly greater total aerobic bacterial count in the TBI group.

(2) The isolation frequency of *S. oralis* was significantly greater in the TBI group.

(3) The proportion of *S. salivarius* as a percentage of the total streptococcal count was significantly greater in the controls when compared with the TBI group.

(4) There was a significantly larger proportion of *S. salivarius* as a percentage of the total anaerobic count in the control group.

(5) The isolation frequency of *Candida* species was greater in the TBI children, but not significantly.

There were no differences in the proportions of any other species of viridans streptococci as a percentage of either the total streptococcal count or the total anaerobic count.

Table 47: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline: TBI and Matched Control Groups

Groups	TBI Group (n =23)					Matched Controls (n =23)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.03	0.09	< 0.001	nd	0.34	0.08	0.25	< 0.001	nd	1.14
S. mitis	18.29	23.83	8.33	nd	75.22	15.58	23.75	3.09	nd	72.97
S. oralis	34.79	28.34	30.01	nd	100	21.94	25.22	15.46	nd	79.21
S. sanguis	6.66	13.61	< 0.01	nd	44.89	6.02	10.92	< 0.01	nd	34.15
S. parasanguis	7.89	15.59	< 0.01	nd	60.02	8.29	14.14	1.10	nd	57.14
S. gordonii	1.95	5.57	< 0.01	nd	25.00	0.48	0.23	< 0.01	nd	1.10
S. salivarius	25.33[a]	29.27	9.98	nd	86.54	43.13[a]	26.64	45.83	nd	97.75
S. vestibularis	0.96	2.49	< 0.01	nd	9.40	4.39	14.19	0.00	nd	64.67
S. anginosus	4.10	19.66	< 0.01	nd	94.29	nd			nd	
S. intermedius	nd			nd		0.53	2.54	< 0.01		12.2

nd = not detected

a = significantly greater in control group, $p < 0.02$

Table 48: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline: TBI Group and Matched Control Groups

Groups	TBI Group(n = 23)					Matched Control Group (n = 23)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.003	0.01	< 0.001	nd	0.05	0.008	0.30	< 0.001	nd	0.15
S. mitis	4.37	11.96	0.27	nd	55.56	3.14	5.62	0.42	nd	22.88
S. oralis	6.87	9.70	4.23	nd	41.54	5.95	10.92	0.84	nd	40.50
S. sanguis	1.90	5.50	< 0.01	nd	25.39	0.62	1.93	< 0.01	nd	9.09
S. parasanguis	1.37	3.21	< 0.01	nd	10.53	0.78	1.25	0.10	nd	4.63
S. gordonii	0.22	0.58	< 0.01	nd	2.14	0.004	0.21	< 0.01	nd	0.13
S. salivarius	4.63[a]	8.15	0.86	nd	31.25	6.88[a]	7.51	3.91	nd	27.03
S. vestibularis	0.18	0.56	< 0.01	nd	2.53	1.94	8.22	< 0.01	nd	39.39
S. anginosus	0.31	1.50	< 0.01	nd	7.17				nd	
S. constellatus	nd			nd	nd				nd	
S. intermedius	nd			nd	nd	0.04	0.20	< 0.01	nd	0.96

nd = not detected

a = significantly greater in control group, $p < 0.02$

Table 49: Mean Number of *Candida* Isolated at Baseline: TBI and Matched Control Groups

	Mean	**sd**	**Median**	**Min**	**Max**	**%**
TBI Group	101	317.04	< 0.01	nd	1500	30.4
Control Group	2.17	6	< 0.01	nd	20	13.0

nd = not detected
% = percentage of children

The Oral Flora Seven Days Post - transplantation

Total Bacterial Counts

There were significant decreases in both the mean total aerobic ($p < 0.0003$) and mean anaerobic bacterial counts ($p < 0.0002$) from baseline to 7 days transplantation (Table 50 and Figs 9 and 10). The mean decreases were considerable, from $\log_{10}$ 7.39 to 6.21 for the aerobic count and from $\log_{10}$ 7.56 to 6.45 for the anaerobic count.

Viridans Streptococci

Isolation Frequency

There was a significant decrease in the mean number of streptococcal species isolated from the oral rinses ($p < 0.0003$) (Table 51) and in the isolation frequencies of *S. sanguis* ($p < 0.03$), *S. parasanguis* ($p < 0.008$) and *S salivarius* ($p < 0.00001$). There were no changes in the isolation frequency of *S. mitis, S. oralis* and *S. parasanguis, S. gordonii* and *S. anginosus* and were each isolated from one child only, *S. salivarius* from 2 children and *S. gordonii* from 3 others. *S. mutans, S. sanguis* and *S. vestibularis* were not isolated 7 days post - transplantation. There was a significant decrease from baseline in the numbers of *S. sanguis* ($p < 0.03$), *S. parasanguis* ($p < 0.03$) and *S. salivarius* ($p < 0.0003$) per ml of oral rinse. There was also a decrease, although not statistically significant, in the numbers of *S. mutans* ($p < 0.07$) and *S. vestibularis* ($p < 0.07$). The numbers of *S. mitis, S. oralis* and *S. gordonii* were unchanged. (Table 50). The predominant species isolated were *S. mitis* and *S. oralis.* Viridans streptococci were not isolated from 2 subjects 7 days post- transplantation.

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was a significant increase from baseline in the proportion of the total streptococcal count as a percentage of the total anaerobic count ($p < 0.006$) (Table 52).

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There was a significant increase from baseline in the proportion of *S. oralis* as a percentage of the total streptococcal count ($p < 0.0009$). There were decreases in the proportion of several other species as a percentage of the total streptococcal count; *S. sanguis* ($p < 0.03$), *S. parasanguis* ($p < 0.01$) and *S. salivarius* ($p < 0.0003$). There was also a decrease, but not statistically significant, in the proportion of *S. mutans* ($p < 0.07$) and *S. vestibularis* ($p < 0.07$). The mean proportion of *S. mitis, S. gordonii and S. anginosus* were not significantly different 7 days post - transplantation (Table 53). This was probably due to their low frequency isolation.

Fig 9: Total Aerobic Bacterial Counts: TBI Group

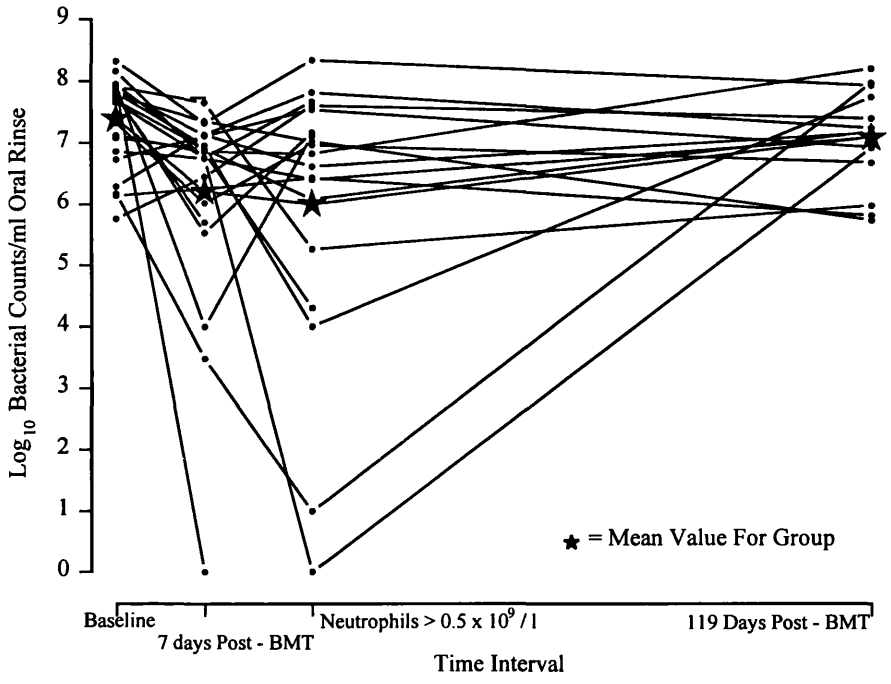


Fig 10: Total Anaerobic Bacterial Counts: TBI Group

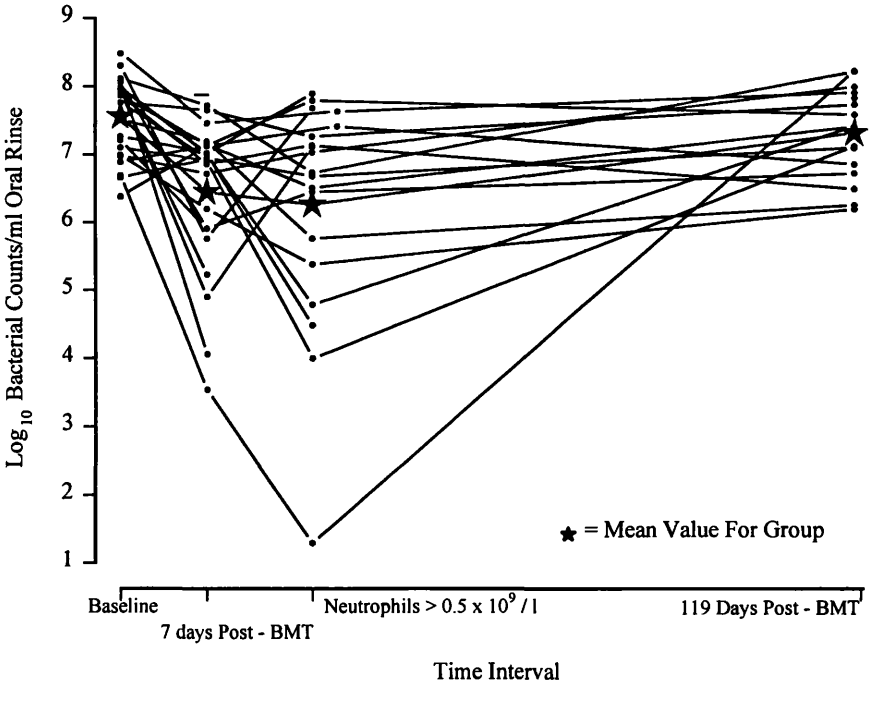


Table 50: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): TBI Group

	Baseline (n = 23)						Day 7 Post - Transplantation (n = 23)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	17.4	0.67	1.52	nd	nd	4.78	nd				nd	
S. mitis	60.9	3.58	3.05	4.85	nd	7.85	43.5	2.55	3.01	nd	nd	6.74
S. oralis	87	5.25	2.29	5.90	nd	7.73	87	5.28	2.31	6.24	nd	7.67
S. sanguis	26.1[a]	1.54	2.70	nd	nd	7.52	nd[a]				nd	
S. parasanguis	39.1[b]	2.15	2.80	nd	nd	7.00	4.3[b]	0.24	1.14	nd	nd	5.48
S. gordonii	17.4	0.98	2.20	nd	nd	6.28	13.0	0.74	1.96	nd	nd	6.08
S. salivarius	78.3[c]	4.47	2.53	5.54	nd	7.38	8.7[c]	0.49	1.65	nd	nd	6.26
S. vestibularis	17.4	0.96	2.17	nd	nd	6.700	nd				nd	
S. anginosus	4.3	0.24	1.15	nd	nd	5.52	4.3	0.28	1.36	nd	nd	6.53
S. intermedius	nd				nd		nd				nd	
Total Aerobic Count	100	7.39[d]	0.73	7.76	5.76	8.32	100	6.21[d]	1.69	6.85	0.00	7.65
Total Anaerobic Count	100	7.56[e]	0.59	7.76	6.38	8.48	100	6.45[e]	1.11	6.93	3.54	7.72

nd = not detected

% = percentage of children

a = significant decrease 7 days post - BMT, $p < 0.03$

b = significant decrease 7 days post - BMT, $p < 0.03$

c = significant decrease 7 days post - BMT, $p < 0.0003$

d = significant decrease 7 days post - BMT, $p < 0.0003$

e = significant decrease 7 days post - BMT, $p < 0.0002$

Table 51: Mean Number of Species of Viridans Streptococci Isolated at Baseline and 7 days Post - Transplantation: TBI Group (n = 23)

	Mean	sd	Median	Min	Max	Sig
Baseline	3.26	1.10	3.00	1.00	6.00	
7 Days Post-BMT	1.70	0.70	2.00	0	3.00	**$p < 0.0003$**

Sig = statistical significance

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There was a significant increase from baseline in the proportion of *S. oralis* as a percentage of the total anaerobic count ($p < 0.0005$). There were significant decreases in percentage of *S. sanguis* ($p < 0.03$), *S. parasanguis* ($p < 0.05$) and *S. salivarius* ($p < 0.006$) but the proportion of *S. mitis, S. gordonii* and *S. anginosus* were not significantly different from baseline (Table 54).

Table 52: Change in Mean Total Streptococcal Count from Baseline to 7 Days Post - Transplantation: TBI Group (n = 23)

	Mean	sd	Median	Min	Max	Sig
Baseline	19.68	20.63	15.00	0.28	73.86	
7 Days Post-BMT	46.21	34.01	46.15	nd*	100.00	**$p < 0.006$**

nd* = not detected
Sig = statistical significance

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was a significant increase in the proportion of the *'oralis* group' as a percentage of the total anaerobic count from 11.24% at baseline to 44.49% 7 days post - transplantation ($p < 0.001$) (Table 55 and Fig 11). Members of the *'oralis* group' were not isolated from 2 subjects 7 days post - transplantation.

Blood Cultures

Four children had positive blood or Hickman line cultures for the ' *oralis* group' between baseline and 7 days post - transplantation. One child had positive blood cultures on days 6 and 7 post - transplantation, a second child on day 7 and a third child had a positive Hickman line culture on day 6 post - transplantation.. A fourth child had a positive Hickman line culture for *S. sanguis* 2 days before transplantation.

Candida Species

Candida species were isolated from 3 children, one of whom also had oral Candida carriage at baseline. The isolates from 2 of the children were *C. albicans* and from the third child, *C. tropicalis.* There was no difference in the isolation frequency of Candida between baseline and 7 days post - transplantation. (Table 56).

Enterobacteriaceae and Enterococci

Enterococcus faecalis was isolated from 1 child, 7 days post - transplantation. The number of colonies was 7,000 colonies per ml of oral rinse. There were no *Enterobacteriaceae* isolated.

Summary of Results 7 Days Post - Transplantation: TBI Group

There were several important changes in the oral flora in the TBI children at 7 days post - transplantation:

(1) There were significant decreases in both the total aerobic and anaerobic bacterial counts

(2) Although there was a significant decrease in the mean number of streptococcal species isolated from the oral rinses, there was a significant increase in the proportion of the streptococcal count as a percentage of the total anaerobic count. The streptococcal species contributing to the increased proportion were predominantly members of the '*oralis* group', particularly *S. oralis.* There were significant decreases in several other species and *S. mutans, S. sanguis* and *S. vestibularis* were not isolated.

(3) The proportion of the '*oralis* group' as a percentage of the total anaerobic count increased significantly from baseline to 7 days post - transplantation.

(4) Four children had positive blood or Hickman line cultures between baseline and 7 days post - transplantation. Except for one child, these all occurred within 24 hours of the oral rinse.

(4) *E. faecalis* was isolated from the oral rinse of 1 child.

Table 53: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline and 7 Days Post - transplantation (Neutrophils < 10^8/l): TBI Group

Groups	Baseline (n = 23)					7 Days Post - Transplantation (n = 23)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.03	0.09	< 0.001	nd	0.34	nd			nd	
S. mitis	18.29	23.83	8.33	nd	75.22	13.78	24.97	< 0.01	nd	86.96
S. oralis	34.79[a]	28.34	30.01	nd	100	72.29[a]	37.47	89.36	nd	100
S. sanguis	6.66[b]	13.61	< 0.01	nd	44.89	nd[b]			nd	
S. parasanguis	7.89[c]	15.59	< 0.01	nd	60.02	0.25[c]	1.18	< 0.01	nd	5.66
S. gordonii	1.95	5.57	< 0.01	nd	25.00	1.93	6.18	< 0.01	nd	24.00
S. salivarius	25.33[d]	29.27	9.98	nd	86.54	0.76[d]	3.00	< 0.01	nd	14.13
S. vestibularis	0.96	2.49	< 0.01	nd	9.40				nd	
S. anginosus	4.10	19.66	< 0.01	nd	94.29	2.27	10.91	< 0.01	nd	52.31
S. intermedius	nd			nd					nd	

nd = not detected

a = significant increase from baseline, $p < 0.0009$

b = significant decrease from baseline, $p < 0.03$

c = significant decrease from baseline, $p < 0.01$

d = significant decrease from baseline, $p < 0.0003$

Table 54: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): TBI Group

Groups	**Baseline (n = 23)**					**7 Days Post - transplantation (n = 23)**				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.003	0.01	< 0.001	nd	0.05	nd			nd	
S. mitis	4.37	11.96	0.27	nd	55.56	7.09	14.49	< 0.01	nd	53.40
S. oralis	6.87[a]	9.70	4.23	nd	41.54	37.40[a]	32.62	25.71	nd	100
S. sanguis	1.90[b]	5.50	< 0.01	nd	25.39	nd[b]			nd	
S. parasanguis	1.37[c]	3.21	< 0.01	nd	10.53	0.18[c]	0.87	< 0.01	nd	4.17
S. gordonii	0.22	0.58	< 0.01	nd	2.14	0.76	2.33	< 0.01	nd	8.45
S. salivarius	4.63[d]	8.15	0.86	nd	31.25	1.87[d]	7.35	< 0.01	nd	34.62
S. vestibularis	0.18	0.56	< 0.01	nd	2.53	nd			nd	
S. anginosus	0.31	1.50	< 0.01	nd	7.17	0.33	1.58	< 0.01	nd	7.56
S. constellatus	nd			nd	nd	nd			nd	
S. intermedius	nd			nd	nd	nd			nd	

nd = not detected

a = significant increase from baseline, $p < 0.0005$

b = significant decrease from baseline, $p < 0.03$

c = significant decrease from baseline, $p < 0.05$

d = significant decrease from baseline, $p < 0.006$

Table 55: Change in '*oralis* group' between Baseline to 7 Days Post -Transplantation: TBI Group (n = 23)

	Mean	sd	Median	Min	Max	Sig
Baseline	11.24	16.50	6.65	nd	73.81	
7 Days Post-BMT	44.49	34.17	46.15	nd	100	**p < 0.001**

Sig = statistical significance
nd = not detected

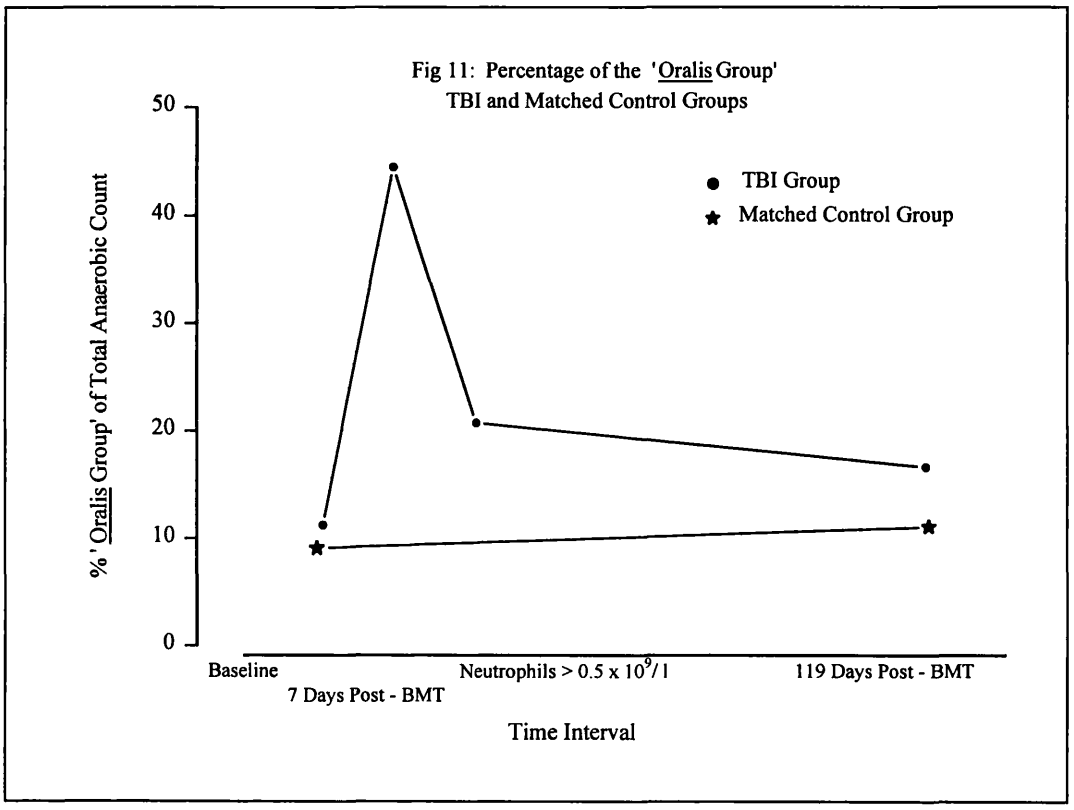


Table 56: Mean Number of *Candida* at Baseline and 7 Days Post - Transplantation: TBI Group (n = 23)

	Mean	sd	Median	Min	Max	%
Baseline	101	317.04	0	nd*	1500	30.4
7 Days Post-BMT	53.48	179.41	0	nd	830	13.0

nd* = not detected
% = percentage of children

The Oral Flora After Recovery of the Peripheral Neutrophil Count (> 0.5 x 10^9 / l)

Total Bacterial Counts

The mean total bacterial counts remained significantly lower than at baseline; the aerobic count $p < 0.02$ and anaerobic count $p < 0.003$ (Table 57 and Figs 9 and 10).

Viridans Streptococci

Isolation Frequency

The mean number of streptococcal species isolated was significantly less than at baseline ($p < 0.0004$) (Table 58). There was a significant decrease in the isolation frequency of *S. mitis* ($p < 0.04$) and *S salivarius* ($p < 0.003$) from baseline, but no differences in the isolation frequency of *S. oralis, S. parasanguis, S. sanguis* or *S. gordonii.* There were no isolates of *S. gordonii, S. mutans, S. vestibularis* or *S. anginosus* from the TBI group at this sampling time (Table 57). The numbers of *S. salivarius* recovered was significantly lower than at baseline ($p < 0.002$) and also the numbers of *S. sanguis* ($p < 0.07$) and *S. gordonii* ($p < 0.07$), but not significantly. The counts of *S. mitis, S. oralis* and *S. parasanguis* were not significantly different from those at baseline. Viridans streptococci were not isolated from 3 children.

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no significant difference in the total streptococcal count as a percentage of the total anaerobic count from baseline.

Each Streptococcal Species as a Percentage of the Streptococcal Count

The proportion of *S. oralis* as a percentage of the total streptococcal count was significantly increased from baseline ($p < 0.02$) and that of *S. salivarius* was significantly decreased ($p < 0.005$). There were no significant differences from baseline for *S. mitis, S. sanguis S. parasanguis or S. gordonii* (Table 59).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There was a significant decrease in the proportion of *S. salivarius* ($p < 0.01$) and the change in the proportion of *S. gordonii* approached significance ($p < 0.07$). There were no significant differences in the proportion of *S. mitis, S. oralis, S. sanguis* and *S. parasanguis* (Table 60).

Table 57: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline and and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9 /l: TBI Group

	Baseline (n = 19)						Neutrophils > 0.5 x 10^9/l (n = 19)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	15.7	0.67	1.52	< 0.001	nd	4.78	nd				nd	
S. mitis	63.2	3.59	2.92	4.85	nd	7.04	26.3	1.62	2.81	< 0.01	nd	7.04
S. oralis	89.5	5.24	2.07	5.63	nd	7.73	68.4	3.92	2.93	5.18	nd	7.26
S. sanguis	31.6	1.86	2.87	< 0.01	nd	7.52	10.5	0.50	1.51	< 0.01	nd	4.85
S. parasanguis	47.1	2.60	2.89	< 0.01	nd	7.00	15.8	0.93	2.22	< 0.01	nd	6.58
S. gordonii	21.1	1.19	2.37	< 0.01	nd	6.28	nd				nd	
S. salivarius	89.5	5.15[a]	2.0	5.70	nd	7.38	31.6	1.53[a]	2.35	< 0.01	nd	6.08
S. vestibularis	15.7	0.93	2.23	< 0.01	nd	6.700	nd				nd	
S. anginosus	nd				nd		nd				nd	
S. intermedius	nd				nd							
Total Aerobic Count	100	7.33[b]	0.78	7.69	5.76	8.32	100	6.00[b]	2.25	6.82	0.00	8.33
Total Anaerobic Count	100	7.53[c]	0.58	7.76	6.38	8.48	100	6.26[c]	1.66	6.73	1.30	7.89

nd = not detected

% = percentage of children

a = significant decrease from baseline, $p < 0.002$

b = significant decrease from baseline, $p < 0.02$

c = significant decrease from baseline, $p < 0.003$

Table 58: Mean Number of Species of Viridans Streptococci Isolated at Baseline and After Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9 / l: TBI Group (n = 19)

	Mean	sd	Median	Min	Max	Sig
Baseline	3.26	1.10	3.00	1.00	6.00	
N* >0.5x10^9/l	1.79	0.79	2.00	1.00	4.00	**p < 0.0004**

N* = neutrophil count
Sig = statistical significance

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no significant difference from baseline in the proportion of the *'oralis* group' as a percentage of the total anaerobic count. This group of streptococci was not isolated from 5 subjects.

Candida Species

Candida species were isolated from 2 children one of whom also had oral *Candida* carriage at 7 days post - transplantation but not at baseline. The isolates were *C. albicans.* There was no difference in the isolation frequency of *Candida* from the baseline.

Enterobacteriaceae and Enterococci

Eneterobacter cloaca was isolated from 1 child, after the peripheral neutrophil count had recovered > 0.5 x 10^9/l. The number of colonies was 5,500 colonies per ml of oral rinse. There were no enterococci.

Summary of Results after Recovery of the Peripheral Neutrophil Count: TBI Group

As the peripheral neutrophil count recovered, the oral flora of the TBI children remained significantly different from baseline:

(1) The total bacterial counts were significantly lower than at baseline.

(2) Although the mean number of streptococcal species isolated was significantly lower than at baseline, the proportion of the total streptococcal count as a percentage of the total anaerobic counts was not significantly different from baseline.

(3) The isolation frequencies of *S. mitis* and *S. salivarius* were significantly lower than baseline and the numbers of *S. salivarius* were lower. The proportion of *S. salivarius* only, as a percentage of both the total streptococcal and total anaerobic counts, was significantly decreased from baseline.

(4) There was no significant difference in the proportion of the '*oralis* group' as a percentage of the total anaerobic count although the proportion *of S. oralis* as a percentage of the total streptococcal count was significantly increased from baseline.

(5) *E. cloaca* was isolated from one child.

Table 59: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9: TBI Group

Groups	Baseline (n = 19)					Neutrophils < 0.5 x 10^9/l (n = 19)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.03	0.09	< 0.001	nd	0.34	nd			nd	
S. mitis	16.30	21.34	8.33	nd	60.53	16.38	33.73	< 0.01	< 0.01	98.91
S. oralis	32.27[a]	24.98	30.01	nd	80	46.72[a]	44.68	35.71	< 0.01	100
S. sanguis	8.06	14.65	< 0.01	nd	44.89	10.53	31.53	< 0.01	< 0.01	100
S. parasanguis	9.55	16.74	< 0.01	nd	60.02	7.14	21.24	< 0.01	< 0.01	70.37
S. gordonii	2.36	6.07	< 0.01	nd	25.00	nd			nd	
S. salivarius	30.56[b]	29.68	28.33	nd	86.54	3.45[b]	7.51	< 0.01	< 0.01	25.00
S. vestibularis	0.86	2.49	< 0.01	nd	9.40	nd			nd	
S. anginosus	nd			nd	94.29	nd			nd	
S. intermedius	nd			nd		nd			nd	

nd = not detected

a = significantly increased from baseline, $p < 0.02$

b = significantly decreased from baseline, $p < 0.005$

Table 60: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: TBI Group

Groups	Baseline (n = 19)					Neutrophils > 0.5 x 10^9/l (n = 19)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.0008	0.003	< 0.001	nd	0.05	nd			nd	
S. mitis	2.24	4.71	0.27	nd	18.97	2.00	4.68	< 0.01	nd	17.42
S. oralis	6.0	9.57	2.08	nd	41.54	18.73	27.03	1.88	nd	87.31
S. sanguis	1.90	5.50	< 0.01	nd	25.39	0.15	0.58	< 0.01	nd	2.50
S. parasanguis	1.66	3.48	< 0.01	nd	10.53	1.82	3.61	< 0.01	nd	14.06
S. gordonii	0.27	0.63	< 0.01	nd	2.13	nd			nd	
S. salivarius	5.60[a]	8.69	1.60	nd	31.25	1.33[a]	4.77	< 0.01	nd	20.83
S. vestibularis	0.20	0.61	< 0.01	nd	2.53	nd			nd	
S. anginosus	nd			nd		nd			nd	
S. constellatus	nd			nd	nd	nd			nd	
S. intermedius	nd			nd	nd	nd			nd	

nd = not detected

a = significantly decreased from baseline, $p < 0.01$

The Oral Flora 119 Days Post - transplantation

Total Bacterial Counts

The were no significant differences in either the mean total aerobic or anaerobic counts between baseline and 119 days post - transplantation (Tables 44 and 61).

Viridans Streptococci

Isolation Frequency

There was no difference in the mean number of species or in the isolation frequency of the viridans streptococci between baseline and 119 days post - transplantation. *S. anginosus, S. constellatus* and *S. intermedius* were isolated although these species were not found at baseline. There was no difference in the streptococcal counts between baseline and the end of the study (Tables 44 and 61).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between baseline and 119 days post - transplantation.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There were no differences between baseline and 119 days post - transplantation (Tables 47 and 62).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The proportion of *S. mitis* as a percentage of the total anaerobic count was greater than at baseline, but not significantly ($p < 0.06$). There was no difference in the percentages of the other streptococcal species (Tables 48 and 63).

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between baseline and 119 days post - transplantation. The *'S. oralis* group' was not isolated from 1 subject

Candida Species

Candida species were isolated from 7 children. Four of these children had oral *Candida* carriage at baseline only. In a fifth child *Candida* only appeared as the neutrophils recovered and in a further child *Candida* was found at 7 days post - transplantation and when the neutrophils had recovered but not at baseline. In the seventh child *Candida* was isolated only at 118 days post - transplantation. Six isolates were *C. albicans* and the seventh isolate was

C. glabrata. The species had changed in 1 child from *C. glabrata* at baseline to *C. albicans* and in a second child from *C. tropicalis* to *C. glabrata* 119 days post - transplantation. There was no difference in the isolation frequency.

Enterobacteriaceae and Enterococci

There were no *Enterobacteriaceae* or enterococci in the oral rinses 119 days post - transplantation.

Summary of Results 119 Days Post - Transplantation: TBI Group

The oral flora of the TBI children 119 days post - transplantation was similar to baseline. There were no significant differences in the mean total bacterial counts or the streptococcal populations. *S. anginosus* and *S. intermedius* were isolated from the oral rinse of I child each and *S. constellatus* from 2 others. These species were not isolated at baseline. There was an increase in the proportion of *S. mitis* as a percentage of the total anaerobic count, but this was not significant.

Table 61: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at End of Study: TBI Group and Matched Control Groups

Groups	TBI Group (n =16)						Matched Control Group (n = 16)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	nd [a]				nd		31.3	1.01[a]	1.67	< 0.001	nd	4.81
S. mitis	62.5	3.80	3.11	5.36	nd	7.61	81.3	4.82	2.43	5.76	nd	6.60
S. oralis	75	4.39	2.74	5.45	nd	7.18	81.3	4.86	2.54	5.99	nd	7.61
S. sanguis	18.8	1.07	2.32	< 0.01	nd	6.56	31.3	1.80	2.76	< 0.01	nd	6.23
S. parasanguis	25	1.25[b]	2.29	< 0.01	nd	6.20	62.5	3.42[b]	2.79	4.89	nd	6.43
S. gordonii	12.5	0.76	2.06	< 0.01	nd	6.08	6.3	0.36	1.42	< 0.01	nd	5.70
S. salivarius	87.5	5.26	2.21	6.09	nd	7.40	93.8	5.61	1.60	5.88	nd	6.99
S. vestibularis	6.3	0.45	1.79	< 0.01	nd	7.18	12.5	0.76	2.09	< 0.01	nd	6.71
S. anginosus	6.3	0.40	1.60	< 0.01	nd	6.38	nd				nd	
S. constellatus	12.5	0.77	2.12	< 0.01	nd	6.86	nd				nd	
S. intermedius	6.3	0.33	1.33	< 0.01	nd	5.30	nd				nd	
Total Aerobic Count	100	7.07	0.74	7.15	5.72	8.18	100	7.21	0.39	7.08	6.76	7.86
Total Anaerobic Count	100	7.31	0.67	7.41	6.19	8.22	100	7.48	0.47	7.62	6.51	8.20

nd = not detected

% = percentage of children

a = significantly greater in control group, $p < 0.02$

b = significantly greater in control group, $p < 0.03$

Table 62: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at End of Study: TBI and Matched Control Groups

Groups	TBI Group (n = 16)					Matched Control Group (n = 16)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	nd [a]			nd		0.06[a]	0.21	< 0.001	nd	0.86
S. mitis	30.82	32.15	17.98	nd	95.08	20.50	19.56	15.07	nd	67.68
S. oralis	21.82	22.46	13.22	nd	60.00	31.45	32.47	19.14	nd	90.41
S. sanguis	4.10	12.12	< 0.01	nd	48.00	5.91	12.07	< 0.01	nd	42.71
S. parasanguis	7.76	20.07	< 0.01	nd	67.42	5.00	6.64	2.43	nd	21.59
S. gordonii	3.19	11.13	< 0.01	nd	44.44	0.45	1.81	< 0.01	nd	7.25
S. salivarius	27.70	27.22	24.85	nd	100	30.58	20.41	27.20	nd	78.38
S. vestibularis	1.16	4.63	< 0.01	nd	18.52	6.05	16.85	< 0.01	nd	57.30
S. anginosus	0.55	2.21	< 0.01	nd	8.86	nd			nd	
S. constellatus	2.61	8.23	< 0.01	nd	32.14	nd			nd	
S. intermedius	0.30	1.19	< 0.01	nd	4.76	nd			nd	

nd = not detected

a = significantly greater in control group, $p < 0.02$

Table 63: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at End of Study: TBI and Matched Control Groups

Groups	TBI Group (n = 16)					Matched Control Group (n = 16)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	nd [a]			nd		0.008[a]	0.02	< 0.001	nd	0.09
S. mitis	10.69	17.39	2.72	nd	64.10	2.61	2.51	1.58	nd	8.16
S. oralis	5.95	7.90	2.12	nd	25.64	9.38	20.43	3.69	nd	83.67
S. sanguis	0.43	1.37	< 0.01	nd	5.46	1.10	3.13	< 0.01	nd	12.59
S. parasanguis	0.58	1.66	< 0.01	nd	6.40	1.73	4.97	0.14	nd	20.15
S. gordonii	1.14	4.28	< 0.01	nd	17.14	0.07	0.3	< 0.01	nd	1.19
S. salivarius	5.85	5.78	3.85	nd	16.29	8.28	17.67	3.35	nd	73.13
S. vestibularis	0.58	2.33	< 0.01	nd	9.32	0.77	2.38	< 0.01	nd	9.20
S. anginosus	0.90	0.36	< 0.01	nd	1.45	nd			nd	
S. constellatus	0.64	2.25	< 0.01	nd	9.00	nd			nd	
S. intermedius	0.10	0.41	< 0.01	nd	1.64	nd			nd	

nd = not detected

a = significantly greater in control group, $p < 0.02$

Comparison of the Oral Flora at the End of the Study: TBI Matched Control Groups

Total Bacterial Counts

There was no difference in either the mean total aerobic or total anaerobic counts between the TBI and matched controls groups (Table 61).

Viridans Streptococci

Isolation Frequency

There was no difference in the mean number of streptococcal species isolated from the oral rinses of either group of children. There was a greater frequency isolation of *S. mutans* ($\chi^2 = 5.93$, df 1, $p < 0.01$) and *S. parasanguis* ($\chi^2 = 4.57$, df 1, $p < 0.03$) in the control group. The colony counts for *S. mutans* ($p < 0.02$) and *S. parasanguis* ($p < 0.03$) were significantly greater in the matched control group (Table 61).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between the TBI and the control children.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

The proportion of *S. mutans* as a percentage of the total streptococcal count was signficantly greater in the matched control group ($p < 0.02$), and the proportion of *S. parasanguis* was also greater, but not significantly ($p < 0.07$) (Table 62).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

Only the proportion of *S. mutans* formed a greater percentage of the total anaerobic count ($p < 0.02$) in the matched control group (Table 63).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the 2 groups at the end of the study. The '*oralis* group' was not isolated from 1 control and 1 TBI child.

<u>Candida</u> Species

Candida species were isolated from 2 control and 7 TBI children. Both the isolation frequency

($\chi^2 = 6.0$, df 1, $p < 0.01$) and the colony counts ($p < 0.02$) were significantly greater in the TBI group.

Enterobacteriaceae and Enterococci

There were no *Enterobacteriaceae* or enterococci in the oral rinses of either group of children at the end of the study.

Summary of Results at the End of the Study: TBI and Matched Control Groups

(1) There were no significant differences in the mean total bacterial counts between the TBI and matched control groups.

(2) There were no significant differences between the mean number of streptococcal species or the proportion of the total streptococcal count as a percentage of the total anaerobic count. There was a significant decrease in isolation frequencies and numbers of *S. mutans* and *S. parasanguis.*

(3) The proportion of *S. mutans* as a percentage of both the total streptococcal and total anaerobic counts was significantly lower in the TBI group.

(4) The isolation frequency and numbers of *Candida* species were significantly greater in the TBI group.

CHANGES IN THE ORAL FLORA OF THE CTO GROUP

Comparison of the Oral Flora at Baseline: CTO and Matched Control Groups

The baseline oral flora of the CTO children (n = 11) and that of their matched controls (n = 11) were compared at baseline. The results are as follows:-

Total Bacterial Counts

There were no differences in either the mean total aerobic or total anaerobic counts between the 2 groups at baseline (Table 64).

Viridans Streptococci

Isolation Frequency

There were significantly fewer species isolated from the oral rinses of the CTO group ($p < 0.01$) (Table 65). There were no isolates of *S. vestibularis*, , *S. constellatus* or *S. intermedius* from the oral rinses of either group. There were no isolates of *S. gordonii* from the CTO children and no isolates of *S. anginosus* from the matched controls (Table 64). There were no differences in the isolation frequency of the streptococci but the colony count for *S. salivarius* was greater in the CTO children ($p < 0.07$) although not significantly. The main species isolated from both groups at baseline were *S. mitis, S. oralis* and *S. salivarius*.

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between the CTO and matched control children (Table 66).

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There were no differences between the CTO and control groups at baseline (Table 67).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There were no differences between the chemotherapy and control groups at baseline (Tables 68).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the 2 groups at baseline. Members of the '*oralis* group' were not isolated from 1 child in each group.

Table 64: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline: CTO and Matched Control Groups

Groups	CTO Group (n = 11)						Matched Control Group (n = 11)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	9.1	0.44	1.47	nd	nd	4.88	36.4	1.04	1.57	nd	nd	3.95
S. mitis	72.7	4.40	2.94	5.85	nd	7.23	54.5	3.10	3.00	4.70	nd	6.20
S. oralis	72.7	4.42	2.89	5.60	nd	6.67	81.8	4.88	2.48	5.90	nd	6.69
S. sanguis	36.4	2.18	3.07	nd	nd	7.00	54.5	3.31	3.22	5.04	nd	6.95
S. parasanguis	45.5	2.58	3.02	nd	nd	6.78	45.5	2.61	3.03	nd	nd	6.15
S. gordonii	nd				nd		9.1	0.57	1.90	nd	nd	6.30
S. salivarius	90.9	5.94	1.99	6.45	nd	7.02	90.9	5.39	1.91	6.04	nd	6.81
S. anginosus	9.1	0.54	1.78	nd	nd	5.90	nd				nd	
S. constellatus	nd				nd		nd				nd	
S. intermedius	nd				nd		18.2	1.09	2.43	nd	nd	6.30
Total Aerobic Count	100	7.44	0.83	7.61	5.40	8.68	100	7.35	0.46	7.26	6.74	8.22
Total Anaerobic Count	100	7.58	0.77	7.79	5.42	8.18	100	7.53	0.47	7.65	6.91	8.37

nd = not detected

Table 67: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline: CTO Matched Control Groups

Groups	CTO Group (n =11)					Matched Control Group (n =11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.08	0.26	< 0.001	nd	0.85	0.03	0.08	< 0.001	nd	0.26
S. mitis	18.15	18.92	16.78	nd	55.32	9.87	12.01	0.83	nd	33.33
S. oralis	27.09	30.65	13.42	nd	88.46	25.65	22.23	25.97	nd	69.01
S. sanguis	6.24	11.93	< 0.01	nd	32.31	21.23	27.50	2.90	nd	81.82
S. parasanguis	4.26	5.86	< 0.01	nd	16.67	11.97	19.28	< 0.01	nd	56.91
S. gordonii	nd			nd		2.36	7.83	< 0.01	nd	25.97
S. salivarius	43.6	29.16	40.43	nd	88.24	25.64	20.33	18.18	nd	57.93
S. anginosus	0.58	1.91	< 0.01	nd	6.35	nd			nd	
S. intermedius	nd			nd		3.25	7.24	< 0.01	nd	18.18

nd = not detected

Table 68: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline: CTO and Matched Control Groups

Groups	Chemotherapy Only (n =11)					Matched Controls (n =11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.01	0.05	< 0.001	nd	0.16	0.001	0.02	< 0.001	nd	0.01
S. mitis	6.49	12.04	2.17	nd	40.00	2.59	4.25	0.22	nd	12.22
S. oralis	10.73	25.96	3.09	nd	88.46	6.22	11.72	1.78	nd	40.50
S. sanguis	1.46	3.23	< 0.01	nd	9.52	2.63	4.12	0.31	nd	11.84
S. parasanguis	1.41	2.68	< 0.01	nd	7.69	2.95	5.62	< 0.01	nd	14.44
S. gordonii	nd			nd		0.47	1.55	< 0.01	nd	5.13
S. salivarius	7.79	8.79	3.88	nd	29.23	4.46	4.47	4.93	nd	14.22
S. anginosus	0.05	0.16	< 0.01	nd	0.53	nd			nd	
S. intermedius	nd			nd		0.33	0.82	< 0.01	nd	2.63

nd = not detected

Candida Species

The only yeast species isolated from the CTO group was *C. albicans.* This was isolated from 1 child in the CTO group and from 3 of the matched controls. There was no difference in the isolation frequency between the 2 groups (Table 68).

Table 69: Mean Number of *Candida albicans* Isolated at Baseline: CTO and Matched Control Groups

	Mean	sd	Median	Min	Max	%
Controls	23	59	0.00	0.00	200	27.3
CTO	410	123	0.00	0.00	410	9.1

% = percentage of children

Enterobacteriaceae and Enterococci

There were no *Enterobacteriaceae* or enterococci isolated from the oral rinses of either the control or CTO group at baseline.

Summary of Result at Baseline: CTO and Matched Control Groups

There were no significant differences in the mean total bacterial counts between the 2 groups at baseline but there were some differences in the components of the oral streptococcal flora. Significantly fewer streptococcal species were isolated from the CTO group. There were no differences in the isolation frequency of the streptococci but the numbers of *S. salivarius* were greater in the CTO group, although not significantly.

The Oral Flora Seven Days Post - transplantation

Total Bacterial Counts

There was a significant decrease in both the mean total aerobic ($p < 0.03$) and anaerobic counts ($p < 0.009$) from baseline to 7 days transplantation (Table 70 and Figs 12 and 13). The mean decreases were considerable, the aerobic count decreased from $\log_{10}$ 7.44 to 6.68 and the mean anaerobic count from $\log_{10}$ 7.58 to 6.81

Viridans Streptococci

Isolation Frequency

There was a significant decrease in the mean number of species isolated ($p < 0.01$) (Table 71) and in the isolation frequency of *S salivarius* only, ($p < 0.008$). There was a decrease in the colony counts for *S. salivarius* ($p < 0.05$), and also *S. parasanguis* ($p < 0.07$) but not significantly. There were no changes in the isolation frequency or counts of *S. mitis, S. oralis* or *S. anginosus*. The main species isolated were *S. mitis* and *S. oralis*. *S. mutans* and S. *sanguis* were not isolated (Table 70).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

The proportion of the total streptococcal count as a percentage of the total anaerobic count increased from 27.94% at baseline to 34.33% at 7 days post - transplantation, but not significantly.

Each Streptococcal Species as a Percentage of the Streptococcal Count

There was a significant decrease from baseline in the proportion of *S. salivarius* as a percentage of the total streptococcal count ($p < 0.005$). There were significant decreases in the percentages of *S. sanguis* ($p < 0.07$) and *S. parasanguis* ($p < 0.07$) but not significantly. The predominant species in 2 children was *S. anginosus* which comprised 17.65% of the total streptococcal count 7 days post - transplantation (Table 72).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There was a decrease from baseline in the proportion of *S. sanguis* ($p < 0.07$) and *S. salivarius* ($p < 0.07$) as a percentage of the total anaerobic count, although not significantly. There were no significant changes in the proportion of *S. mitis, S. oralis* or *S. anginosus* (Table 73).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was an increase in the proportion of the '*oralis* group' as a percentage of the total anaerobic count from baseline, but this was not significant (Fig 14). The '*oralis* group' was not isolated from 1 subject and in another the count was 0.004% of the total anaerobic count.

Fig 12: Total Aerobic Bacterial Counts: CTO Group

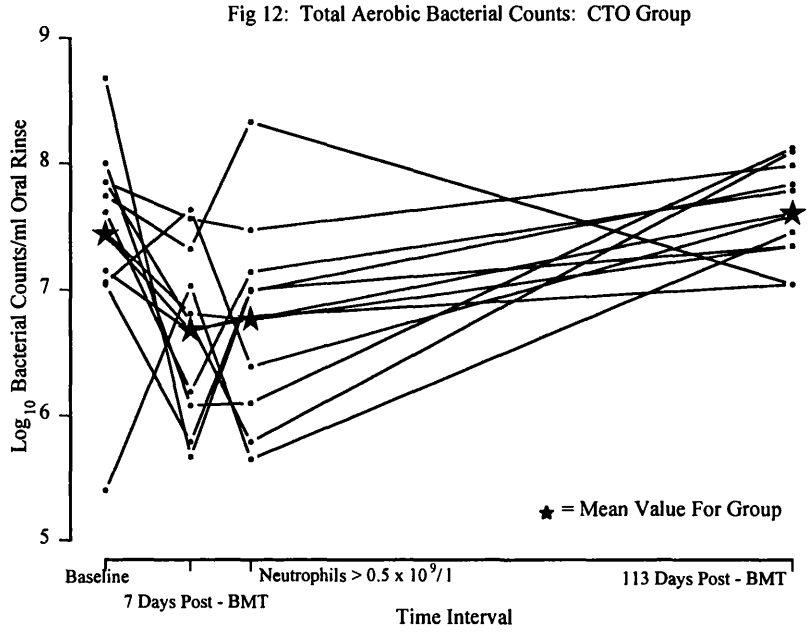


Fig 13: Total Anaerobic Bacterial Counts: CTO Group

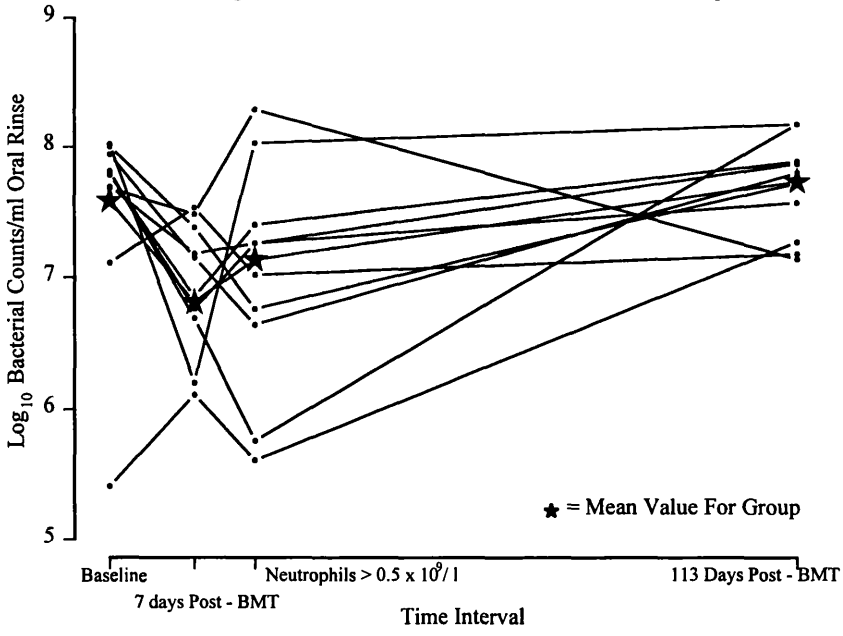


Table 70: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline and Day 7 Post - Transplantation (Neutrophils < 10^8/l): CTO Group

	Baseline (n = 11)						7 Days Post -Transplantation (n = 11)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	9.1	0.44	1.47	nd	nd	4.88	-	-	-	-	-	-
S. mitis	72.7	4.40	2.94	5.85	nd	7.23	63.6	3.39	2.96	5.18	nd	6.60
S. oralis	72.7	4.42	2.89	5.60	nd	6.67	72.7	4.35	2.87	5.48	nd	7.48
S. sanguis	36.4	2.18	3.07	nd	nd	7.00	-	-	-	-	-	-
S. parasanguis	45.5	2.58	3.02	nd	nd	6.78	9.1	0.43	1.42	nd	nd	4.70
S. salivarius	90.9	5.94[a]	1.99	6.45	nd	7.02	18.2	1.09[a]	2.46	nd	nd	6.93
S. anginosus	9.1	0.54	1.78	nd	nd	5.90	18.2	0.71	1.63	nd	nd	4.78
Total Aerobic Count	100	7.44[b]	0.83	7.61	5.40	8.68	100	6.68[b]	0.68	6.74	5.67	7.63
Total Anaerobic Count	100	7.58[c]	0.77	7.79	5.42	8.18	100	6.81[c]	0.62	6.85	5.62	7.53

nd = not detected

% = percentage of children

a = significant decrease from baseline, $p < 0.05$

b = significant decrease from baseline, $p < 0.03$

c = significant decrease from baseline, $p < 0.009$

Table 71: Mean Number of Species of Viridans Streptococci Isolated at Baseline and 7 days Post - Transplantation: CTO Group (n = 11)

	Mean	sd	Median	Min	Max	Sig
Baseline	3.00	0.63	3.00	2.00	4.00	
7 Days Post-BMT	2.00	0.63	2.00	1.00	3.00	**0.01**

Sig = statistical significance

Blood Cultures

One child had a positive Hickman line culture 4 days post - transplantation and a positive blood culture 7 days post - transplantation for the '*oralis* group.

Candida Species

C. albicans (1.57 x 10^4 colonies per ml oral rinse) was isolated from 1 child who had not had oral *Candida* carriage at baseline.

Enterobacteriaceae and Enterococci

Neither *Enterobacteriaceae* nor enterococci were isolated from the oral rinses 7 days post - transplanation.

Table 72: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline and Day 7 Post - Transplantation (Neutrophils < 10^8/l): CTO Group

Groups	Baseline (n = 11)					Day 7 Post- Transplantation (n = 11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.08	0.26	< 0.001	nd	0.85	nd			nd	
S. mitis	18.15	18.92	16.78	nd	55.32	29.29	38.17	11.77	nd	100
S. oralis	27.09	30.65	13.42	nd	88.46	45.62	40.72	39.27	nd	100
S. sanguis	6.24	11.93	< 0.01	nd	32.31	nd			nd	
S. parasanguis	4.26	5.86	< 0.01	nd	16.67	0.58	1.91	< 0.01	nd	6.33
S. salivarius	43.6[a]	29.16	40.43	nd	88.24	6.87[a]	18.41	< 0.01	nd	60.71
S. anginosus	0.58	1.91	< 0.01	nd	6.35	17.65	39.28	< 0.01	nd	100

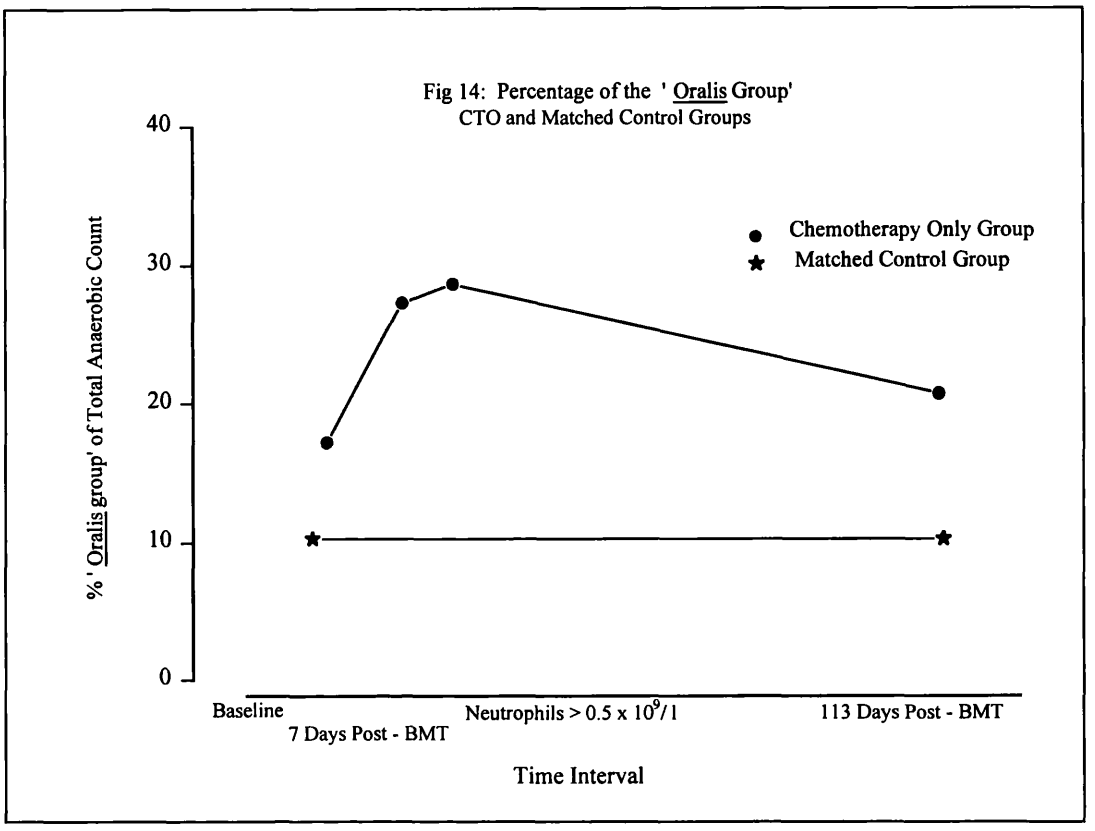
nd = not detected

a = significant decrease from baseline, $p < 0.005$

Table 73: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): CTO Group

Groups	Baseline (n = 11)					Day 7 Post- Transplantation (n = 11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.01	0.05	< 0.001	nd	0.16	nd			nd	
S. mitis	6.49	12.04	2.17	nd	40.00	5.58	5.97	3.33	nd	13.33
S. oralis	10.73	25.96	3.09	nd	88.46	21.68	33.94	2.45	nd	88.24
S. sanguis	1.46	3.23	< 0.01	nd	9.52	nd			nd	
S. parasanguis	1.41	2.68	< 0.01	nd	7.69	0.93	0.31	< 0.01	nd	1.02
S. salivarius	7.79	8.79	3.88	nd	29.23	5.66	18.27	< 0.01	nd	60.71
S. anginosus	0.05	0.16	< 0.01	nd	0.53	1.31	4.31	< 0.01	nd	14.29

nd = not detected



Summary of Results 7 Days Post Transplantation: CTO Group

There were several changes in the oral flora 7 days post - transplantation,

(1) There were significant decreases in the mean total aerobic and anaerobic counts from baseline.

(2) There was a significant decrease in the mean number of species isolated and a significant decrease in the isolation frequency of *S. salivarius*. There was a decrease in the numbers of *S. salivarius* and also of *S. parasanguis,* but not significantly.

(3) There was no significant difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count from baseline.

(3) There was a significant decrease from baseline in the proportion of *S. salivarius* as a percentage of the total streptococcal count. There were decreases in the proportions of *S. parasanguis* and *S. sanguis,* but not statistically significantly .

(4) There was a decrease from baseline in the proportion of *S. salivarius* and *S. sanguis* as a percentage of the total anaerobic count although not statistically significant.

(5) There was no difference in the proportion of the '*oralis* group' from baseline.

(6) One child had both a positive Hickman line and blood culture during the neutropenic period.

The Oral Flora After Recovery of the Peripheral Neutrophil Count (> 0.5 x 10^9 / l)

Total Bacterial counts

The mean total aerobic count remained significantly lower than the baseline figure ($p < 0.05$). The mean total anaerobic count had recovered and was no different from the baseline figure (Table 74 and Figs 12 and 13).

Viridans Streptococci

Isolation Frequency

The mean number of species of streptococci was significantly decreased from baseline ($p < 0.02$) (Table 75). There was a significant decrease in the isolation frequency of *S salivarius* ($p < 0.004$) from baseline, but no differences in the isolation frequency of *S. mitis, S. oralis, S. sanguis, S. parasanguis* and *S. anginosus. S. mutans* was not isolated (Table 74). The predominant species isolated were *S. mitis* and *S. oralis.* The numbers of *S. parasanguis* ($p < 0.04$) and *S. salivarius* ($p < 0.007$) were decreased and also of *S. mitis* ($p < 0.07$) but not significantly. The counts of *S. oralis, S. sanguis* and *S. anginosus* were not significantly different from those at baseline (Table 74).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count from baseline.

Each Streptococcal Species as a Percentage of the Streptococcal Count

The proportion of *S. oralis* as a percentage of the total streptococcal count was significantly increased from baseline ($p < 0.05$), but decreased for *S. parasanguis* ($p < 0.04$) and *S. salivarius* ($p < 0.008$). There were no significant differences from baseline in the proportions of *S. mitis, S. sanguis* and *S. anginosus* (Table76).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There were significant decreases in the proportion of *S. parasanguis* ($p < 0.04$) and *S. salivarius* ($p < 0.03$) as a percentage of the total anaerobic count. There were no significant differences from baseline in the proportions of *S. mitis, S. oralis, S. sanguis* and *S. anginosus* (Table 77).

Table 74: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group

	Baseline (n = 11)						Neutrophils > 0.5 x 10^9/l (n = 11)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	9.1	0.44	1.47	nd	nd	4.88	-	-	-	-	-	-
S. mitis	72.7	4.40	2.94	5.85	nd	7.23	45.5	2.32	2.77	nd	nd	6.45
S. oralis	72.7	4.42	2.89	5.60	nd	6.67	72.7	4.28	2.81	5.30	nd	6.80
S. sanguis	36.4	2.18	3.07	nd	nd	7.00	9.1	0.53	1.76	nd	nd	5.85
S. parasanguis	45.5	2.58[a]	3.02	< 0.01	nd	6.78	18.2	0.64[a]	1.44	nd	nd	4.00
S. salivarius	90.9	5.94[b]	1.99	6.45	nd	7.02	9.1	0.59[b]	1.96	nd	nd	6.51
S. anginosus	9.1	0.54	1.78	nd	nd	5.90	18.2	0.79	1.78	nd	nd	5.0
Total Aerobic Count	100	7.44[c]	0.83	7.61	5.40	8.68	100	6.77[c]	0.77	6.79	5.65	8.33
Total Anaerobic Count	100	7.58	0.77	7.79	5.42	8.18	100	7.14	0.93	7.26	5.61	8.45

nd = not detected

% = percentage of children

a = significant decrease from baseline, $p < 0.04$

b = significant decrease from baseline, $p < 0.007$

c = significant decrease from baseline, $p < 0.05$

Table 75: Mean Number of Species of Viridans Streptococci Isolated at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group (n = 11)

	Mean	sd	Median	Min	Max	Sig
Baseline	3	0.63	3	2	4	
Neutrophils >0.5x10^9/l	1.91	0.94	2	0	3	**p < 0.02**

Sig = statistical significance

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count from baseline. The '*oralis* group' was not isolated from 1 subject and the count was 0.004 per ml of oral rinse in 2 others.

Candida Species

C. albicans (110 colonies per ml of oral rinse) was isolated from the same subject who also had oral carriage at 7 days post - transplantation.

Enterobacteriaceae and Enterococci

Klebsiella pneumoniae was isolated from 1 child, after the peripheral neutrophil count had recovered. The number of colonies was 200 colonies per ml of oral rinse. No enterococci were isolated.

Summary of Results after Recovery of the Peripheral Neutrophil Count: CTO Group

There were several changes in the oral flora from baseline, as the peripheral neutrophil count recovered. These were as follows:-

(1) Although the mean total anaerobic bacterial counts had recovered to the baseline level, the mean total aerobic bacterial counts remained significantly decreased.

(2) The mean number of species of streptococci was significantly decreased from baseline There was a significant decrease in the isolation frequency of *S salivarius.* The numbers of *S. salivarius* and *S. parasanguis* were significantly decreased.

(3) The proportion of *S. oralis* as a percentage of the total streptococcal count was significantly increased from baseline, but the proportion decreased for *S. parasanguis* and *S. salivarius.*

(4) There were significant decreases in the proportion of *S. parasanguis* and *S. salivarius* as a percentage of the total anaerobic count.

(5) *Klebsiella pneumoniae* was isolated from 1 child.

Table 76: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group

Groups	Baseline (n = 11)					Neutrophils > 0.5 x 10^9/l				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.08	0.26	< 0.001	nd	0.85	nd			nd	
S. mitis	18.15	18.92	16.78	nd	55.32	20.87	32.69	< 0.01	nd	100
S. oralis	27.09[a]	30.65	13.42	nd	88.46	53.92[a]	39.67	66.32	nd	100
S. sanguis	6.24	11.93	< 0.01	nd	32.31	1.22	4.06	< 0.01	nd	13.46
S. parasanguis	4.26[b]	5.86	< 0.01	nd	16.67	1.59[b]	4.03	< 0.01	nd	13.10
S. salivarius	43.6[c]	29.16	40.43	nd	88.24	3.06[c]	10.16	< 0.01	nd	33.68
S. anginosus	0.58	1.91	< 0.01	nd	6.35	10.25	22.80	< 0.01	nd	57.14

nd = not detected

a = significant increase from baseline, $p < 0.05$

b = significant decrease from baseline, $p < 0.04$

c = significant decrease from baseline, $p < 0.008$

Table 77: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group

Groups	Baseline (n = 11)					Neutrophils > 0.5 x 10^9/l				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.01	0.05	< 0.001	nd	0.16	nd		< 0.01	nd	
S. mitis	6.49	12.04	2.17	nd	40.00	9.76	18.79	< 0.01	nd	49.12
S. oralis	10.73	25.96	3.09	nd	88.46	18.88	21.24	16.35	nd	59.55
S. sanguis	1.46	3.23	< 0.01	nd	9.52	0.35	1.17	< 0.01	nd	3.89
S. parasanguis	1.41[a]	2.68	< 0.01	nd	7.69	0.22[a]	0.74	< 0.01	nd	2.44
S. salivarius	7.79[b]	8.79	3.88	nd	29.23	1.15[b]	3.80	< 0.01	nd	12.60
S. anginosus	0.05	0.16	< 0.01	nd	0.53	0.004	0.01	< 0.01	nd	0.04

nd = not detected

a = significant decrease from baseline, $p < 0.04$

b = significant decrease from baseline, $p < 0.03$

Oral Flora 113 Days Post - Transplantation

Total Bacterial counts

There were no significant differences in either the mean total aerobic or anaerobic counts between baseline and 113 days post - transplantation (Tables 64 and 78).

Viridans Streptococci

Isolation Frequency

There was an increase in the mean number of streptococcal species from baseline, but this was not significant (Table 79). There were no differences in the isolation frequency of the viridans streptococci or the streptococcal counts between baseline and 113 days post - transplantation.

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between baseline and 113 days post - transplantation.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There were no differences between baseline and 113 days post - transplantation (Tables 67 and 80).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There were no differences between baseline and 113 days post - transplantation (Tables 68 and 81).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no difference in the '*oralis* group' as a percentage of the total anaerobic count between baseline and 113 days post - transplantation.

<u>Candida</u> Species

C. albicans was isolated from 3 children. *Candida* species had not been isolated from 2 of these subjects on any other occasion. The third child also had oral *Candida* carriage at 7 days post - transplantation and when the neutrophil count had recovered > 0.5 x 10^9/l, but not at baseline. The numbers of colonies per ml of oral rinse were 150, 410 and 1500. There was no difference in the isolation frequency.

Table 78: Total Bacterial Counts as Colony - Forming Units / ml Oral Rinse ($\log_{10}$) at End of Study: CTO and Matched Control Groups

Groups	CTO Group Only (n = 11)						Matched Control Group (n = 11)					
Species	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**	**%**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	36.4	1.13	1.91	nd	nd	5.03	36.4	0.88	1.32	nd	nd	3.60
S. mitis	54.5	3.56	3.34	5.70	nd	7.60	63.6	3.94	3.17	5.70	nd	7.78
S. oralis	100	6.30	0.86	6.30	4.85	7.64	81.8	5.00	2.57	6.20	nd	7.28
S. sanguis	54.5	1.19	2.65	nd	nd	6.79	45.5	2.76	3.20	nd	nd	7.08
S. parasanguis	54.5	3.31	3.20	5.30	nd	6.90	54.5	3.12	3.02	4.48	nd	6.32
S. gordonii	nd				nd		9.1	0.57	1.88	nd	nd	6.23
S. salivarius	100	6.40[a]	0.59	6.34	5.70	7.51	90.9	5.28[a]	1.83	5.85	nd	6.36
S. anginosus	9.1	0.50	1.65	nd	nd	5.46	nd				nd	
S. intermedius	nd				nd		9.1	0.63	2.08	nd	nd	6.90
Total Aerobic Count	100	7.60[b]	0.39	7.58	7.04	8.12	100	7.19[b]	0.45	7.08	6.53	8.00
Total Anaerobic Count	100	7.73	0.40	7.80	7.14	8.27	100	7.60	0.52	7.69	6.94	8.38

nd = not detected

% = percentage of children

a = significantly greater in chemotherapy only group, $p < 0.03$

b = significantly greater in chemotherapy only group, $p < 0.04$

Table 79: Mean Number of Species of Viridans Streptococci Isolated 113 Days Post - Transplantation CTO Group (n = 11)

	Mean	sd	Median	Min	Max	Sig
Baseline	3.00	0.63	3	2	4	
113 Days Post-BMT	3.64	0.81	3	3	5	**ns**

Sig = statistical significance
ns = not significant

Enterobacteriaceae and Enterococci

There were no *Enterobacteriaceae* or enterococci isolated from any of the oral rinses 113 days post - transplantation.

Summary of Results 113 Days Post - Transplantation: CTO Group

The oral flora of the CTO group 113 days post - transplantation was similar to baseline. There were no significant differences in the total aerobic and anaerobic bacterial counts. The was a non-significant increase in the mean number of streptococcal species and no other significant differences in the streptococcal populations.

Table 80: Proportion of Each Streptococcal Species as a Percentage of the Total Streptococcal Count at End of Study: CTO Matched Controls

Groups	CTO Group (n = 11)					Matched Control Group (n = 11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.13	0.42	< 0.001	nd	1.39	0.008	0.02	< 0.001	nd	0.07
S. mitis	15.44	18.38	4.81	nd	44.69	19.34	21.63	13.64	nd	59.41
S. oralis	29.63	27.03	19.72	4.76	90.39	26.74	22.25	25.58	nd	69.56
S. sanguis	8.22	23.22	< 0.01	nd	77.22	12.08	18.30	< 0.01	nd	57.69
S. parasanguis	12.30	16.72	4.64	nd	48.65	12.74	16.43	7.40	nd	47.72
S. gordonii	nd			nd		3.59	11.92	< 0.01	nd	39.53
S. salivarius	31.71	18.74	32.87	4.81	57.75	24.77	28.10	16.28	nd	99.99
S. anginosus	1.79	5.95	< 0.01	nd	19.72	nd			nd	
S. intermedius	nd			nd		0.72	2.39	< 0.01	nd	7.92

nd = not detected

Table 81: Proportion of Each Streptococcal Species as a Percentage of the Total Anaerobic Count at End of Study: CTO Matched Control Groups

Groups	CTO Group (n = 11)					Matched Control Group (n = 11)				
Species	**Mean**	**sd**	**Median**	**Min**	**Max**	**Mean**	**sd**	**Median**	**Min**	**Max**
S. mutans	0.02	0.06	< 0.001	nd	0.20	0.0005	0.001	< 0.001	nd	0.005
S. mitis	7.93	13.40	0.48	nd	38.41	4.63	7.52	0.71	nd	24.90
S. oralis	12.73	16.68	2.70	nd	50.00	4.92	5.52	3.89	nd	17.78
S. sanguis	2.70	6.06	< 0.01	nd	16.49	1.48	2.26	< 0.01	nd	6.34
S. parasanguis	1.80	2.56	0.46	nd	6.98	1.71	2.64	0.31	nd	7.32
S. gordonii	nd			nd		0.55	1.81	< 0.01	nd	6.01
S. salivarius	8.07[a]	8.59	4.72	0.96	24.32	1.74[a]	1.05	1.95	nd	3.07
S. anginosus	0.03	0.11	< 0.01	nd	0.37	nd			nd	
S. intermedius	nd			nd		0.30	1.00	< 0.01	nd	3.32

nd = not detected

a = significantly greater in CTO group, $p < 0.02$

Comparison of The Oral Flora at the End of the Study: CTO and Matched Control Groups

Baseline

Total Bacterial Counts

The mean total aerobic count in the CTO group was significantly greater at the end of the study ($p < 0.04$) but there was no difference in the mean total anaerobic counts (Table 78).

Viridans Streptococci

Isolation Frequency

There was no difference in the mean number of streptococcal species or in the frequency isolation of any species of viridans streptococci from the oral rinses. The numbers of *S. salivarius* were significantly higher in the CTO group ($p < 0.03$) (Table78).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between the chemotherapy only and matched control groups.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There were no differences between the CTO and control children. (Table 80).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The proportion of *S. salivarius* as a percentage of the total anaerobic count was significantly greater in the CTO group ($p < 0.02$) (Table 81).

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the CTO and matched control groups.

Candida Species

C. albicans was isolated from the same control child as at baseline and from 3 CTO children. There was no difference in the isolation frequency between the CTO and control groups.

Enterobacteriaceae and Enterococci

There were no *Entrobacteriaceae* or enterococci in the oral rinses at the end of the study.

Summary of Results at the End of the Study: CTO and Matched Control Groups

There were a few differences in the oral flora of the CTO children and their matched controls at the end of the study:

(1) The total aerobic bacterial counts were significantly greater in the CTO group although there were no differences in the total anaerobic counts.

(2) The streptococcal flora was similar except for a significant increase in both the numbers of *S. salivarius* and the proportion of *S. salivarius* as a percentage of the total anaerobic count in the CTO group.

Comparison of the Oral Flora: TBI and CTO Groups

Baseline

Total Bacterial counts

There was no significant difference in the total aerobic and total anaerobic counts between the TBI and CTO groups (Tables 44 and 64).

Viridans Streptococci

Isolation Frequency

There was no significant difference in the number of streptococcal species or the frequency isolation of any species of viridans streptococci. *S. gordonii* and *S. vestibularis* were not isolated from the oral rinses of the children in the CTO group. (Tables 44 and 64). The numbers of *S. salivarius* were significantly greater in the CTO group ($p < 0.005$).

Total Streptococcal Count as a Percentage of the Total Streptococcal Count

There was no significant difference in the proportion total streptococcal count as a percentage of the total anaerobic count between the TBI and CTO children.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

There was no significant difference between the TBI and CTO groups (Tables 47 and 67).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The proportion of *S. salivarius* as a percentage of the total anaerobic count was significantly greater in the CTO group, but not significantly ($p < 0.06$) (Tables 48 and 68).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the TBI and CTO children.

<u>Candida</u> Species

There was no difference in the isolation frequency or colony counts of *Candida.*

<u>Enterobacteriaceae</u> and Enterococci

There were no *Enterobacteriaceae* or enterococci isolated from the oral rinses of either group.

Summary of Results at Baseline: CTO and TBI Groups

The only differences in the oral flora of the TBI and the CTO children were in the streptococcal population. *S. gordonii* and *S. vestibularis* were not isolated from the oral rinses of the children in the CTO group. The numbers of *S. salivarius* only were significantly greater in the CTO group and the proportion of *S. salivarius* as a percentage of the total anaerobic count was also greater in the CTO group, but not significantly. The baseline oral floras were clearly very similar.

The Oral Flora Seven Days Post - Transplantation

Total Bacterial counts

There was no significant difference in the mean total aerobic or anaerobic counts between the TBI and CTO groups (Tables 50 and 70).

Viridans Streptococci

Isolation Frequency

There was no significant difference in the number of streptococcal species or the frequency isolation of any species of viridans streptococci, or the colony counts (Tables 50 and 70).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the total streptococcal count as a percentage of the total anaerobic count between the TBI and CTO groups.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

The proportion of *S. oralis* only, as a percentage of the total streptococcal count was significantly greater in the TBI group ($p < 0.03$) (Tables 53 and 72).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

There was no significant difference between the TBI and CTO children (Tables54 and 73).

The '<u>oralis</u> group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the TBI and CTO groups.

<u>Candida</u> Species

There was no significant difference in the isolation frequency or colony counts of *Candida.*

Enterobacteriaceae and Enterococci

Enterococcus faecalis was isolated from the oral rinse of 1 child in the TBI group.

Summary of Results 7 Days Post - Transplantation: TBI and CTO Groups

The only significant difference between the TBI and CTO groups was a significantly greater proportion of *S. oralis* as a percentage of the total streptococcal count in the TBI children.

The Oral Flora After Recovery of the Peripheral Neutrophil Count (> 0.5 x 10^9 /l)

Total Bacterial Counts

There was no significant difference in the total aerobic or anaerobic counts between the TBI and CTO groups (Table 57 and 74).

Viridans Streptococci

Isolation Frequency

There was no difference in the number of species isolated between the 2 groups. The frequency isolation of *S. anginosus* was significantly greater in the CTO group ($\chi^2 = 3.70$, df 1, $p < 0.05$). There was no difference between the number of colonies for each species (Tables 57 and 74).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between the TBI and CTO groups.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

The proportion of *S. anginosus* as a percentage of the total streptococcal counts was greater in the CTO group but not significantly ($p < 0.07$) (Tables 59 and 76).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The proportion of *S. anginosus* as a percentage of the total anaerobic count was greater in the CTO group but not significantly ($p < 0.06$) (Tables 60 and 77).

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the *'oralis* group' as a percentage of the total anaerobic count between the TBI and CTO groups.

Candida Species

There was no significant difference in the isolation frequency or colony counts of *Candida*

Enterobacteriaceae and Enterococci

Enterobacter cloaca was isolated form the oral rinse of only 1 child in the TBI group; Klebsiella pneumoniae was isolated from the oral rinse of 1 child in the CTO group.

Summary of Results after Recovery of the Peripheral Neutrophil Counts: TBI and CTO Groups

The only differences in the oral flora between the TBI and the CTO group were in the streptococcal population. The frequency isolation of *S. anginosus* only, was significantly greater in the chemotherapy group. The proportion of *S. anginosus* as a percentage of the total streptococcal count and the total anaerobic count was greater in the CTO, but not significantly. *Enterobacteriacea* were isolated from 1 child in each group.

The Oral Flora at the End of the Study

Total Bacterial counts

The total aerobic count was significantly higher in the CTO group ($p < 0.05$) but there was no difference in the total anaerobic count between the TBI and CTO groups (Tables 61 and78).

Viridans Streptococci

Isolation Frequency

There was no significant difference in the number of species isolated between the 2 groups. The frequency isolation of *S. mutans* was greater in the CTO, group ($\chi^2 = 6.83$, df 1, $p < 0.009$) and also of *S. oralis*, although not significantly ($p < 0.07$). The numbers of *S. mutans* ($p < 0.01$) and *S. oralis* ($p < 0.05$) were significantly greater in the CTO group and of *S. parasanguis* ($p < 0.06$), although not significantly (Tables 61 and 78).

Total Streptococcal Count as a Percentage of the Total Anaerobic Count

There was no difference in the proportion of the total streptococcal count as a percentage of the total anaerobic count between the TBI and CTO groups.

Each Streptococcal Species as a Percentage of the Total Streptococcal Count

The proportion of *S. mutans* as a percentage of the total streptococcal count was significantly greater in the CTO group ($p < 0.01$) (Tables 62 and 80).

Each Streptococcal Species as a Percentage of the Total Anaerobic Count

The proportion of *S. mutans* as a percentage of the total anaerobic count was significantly greater in the CTO group ($p < 0.01$) (Tables 63 and 81).

The 'oralis group' as a Percentage of the Total Anaerobic Count

There was no significant difference in the proportion of the '*oralis* group' as a percentage of the total anaerobic count between the 2 groups. Members of the '*oralis* group' were not isolated from 1 child in the TBI group.

Candida Species

There was no significant difference in the isolation frequency or colony counts of Candida

Enterobacteriaceae and Enterococci

There were no *Enterobacteriaceaeceae* bacteria or enterococci isolated form the oral rinses of either group.

Summary of Results at the End of the Study: TBI and CTO Groups

There were several differences in the oral flora between the TBI and the CTO children:

(1) The total aerobic bacterial count only was significantly higher in the CTO group.

(2) The isolation frequency of *S. mutans* was significantly greater in the CTO, group and also of *S. oralis*, although not significantly. The numbers of *S. mutans* and *S. oralis* per ml of oral rinse were significantly greater in the CTO groups and also of *S. parasanguis*, although not significantly.

(3) The proportion of *S. mutans* as a percentage of the total streptococcal count and the total anaerobic count was significantly greater in the CTO group.

IMMUNOLOGY RESULTS

Optimisation of Assays

Coating Layer

Total IgA, Secretory IgA and IgG

Three dilutions of rabbit anti-human IgA were prepared and each one used to coat a separate microtitre tray. These were 1/500, 1/1000 and 1/2000. Four oral rinses were added to each tray in four doubling dilutions and each assay completed in the same manner. The results were compared and the 1/1000 concentration was selected, since this showed the best linear relationship between O.D. and concentration. The procedure was repeated using rabbit anti - human IgG to establish the optimum coating layer concentration for the IgG assays, which was also 1/1000.

Detection Layer

Total IgA, and Secretory IgA and IgG

The detection layer for total IgA was mouse anti - human IgA monoclonal antibody, for secretory IgA (S - IgA), monoclonal anti- secretory component and for IgG mouse anti - human IgG. For each of these, 3 dilutions 1/500, 1/1000 and 1/2000, were prepared and tested. The best results were obtained from 1/1000 dilution. The linear relationship between O.D. and concentration was similar for all 3 dilutions and the 1/1000 was accepted.

Conjugate

Total IgA, Secretory IgA and IgG

The conjugate concentration was established by comparing concentrations of 1/300 and 1/500 for the IgA and S - Iga and concentrations of 1/500 and 1/1000 for IgG. Concentration of 1/500 for IgA and S - IgA and 1/1000 for IgG were accepted.

Oral Rinse Concentration

Many assays were run using a wide range of concentrations of the oral rinses; the lowest starting dilution which covered four points on the standard curve was accepted. The optimum starting concentration was found to be 1/50 for total IgA and S - IgA, 1/10 for IgG and 1/2.5 for salivary antibodies to *S. mitis* and *S. oralis.*

Reproducibility of Methods

Inter - Assay Variation

The same 4 samples were repeated in each assay and the inter-assay variation was less than 20%.

Intra - Assay Variation

The intra-assay variation for the oral rinses was below 12% and most of those with a variation greater than this were repeated. However, in a few cases, there was insufficient oral rinse to do this.

SALIVARY IMMUNOGLOBULINS OF THE WHOLE CONTROL GROUP

The concentrations of total salivary IgA, S - IgA, IgA antibodies to *S. mitis* and *S. oralis* and total salivary IgG were recorded at baseline and 118 days later for those matched with the TBI children and 117 days later for those matched with the chemotherapy only children. The immunological data have been analysed for the entire control group (n = 33), excluding results which were greater than 2 standard deviations from the mean.

There were no significant differences in the concentration of salivary IgA, S - IgA and salivary IgA antibodies to *S. mitis* and *S. oralis* between baseline and 117/118 days later (Table 82).

Total Salivary IgG

The mean concentration of total salivary IgG was significantly lower at the end of the study ($p < 0.02$) (Table 82). The sample size was small and this difference was most probably due to a type I statistical error (Altman 1992).

Correlations

The correlations between the concentration of total salivary IgA and S - IgA ($p < 0.0001$) and between the concentration of total salivary IgA and salivary IgA antibodies to *S. mitis* ($p < 0.02$) were significant. There was no significant correlation between the concentration of total salivary IgA and salivary IgA antibodies to *S. oralis* (Table 83).

Table 82: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline and 117/118 Days Later: Whole Control Group (n = 33)

	Baseline						117/118 Days					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	32	7.33	5.56	7.76	0.60	23.40	31	9.71	6.47	8.23	0.94	22.54
Secretory IgA (μg/ml)	32	18.30	9.05	17.25	4.03	37.03	31	18.38	10.39	14.20	4.72	42.05
Antibodies to *S. mitis* (E.U./ml)	31	72.57	48.09	61.51	15.89	183.05	32	66.48	56.30	43.76	1	223.65
Antibodies to *S. oralis* (E.U./ml)	30	35.70	26.03	32.13	1	83.33	31	35.14	25.73	29.30	2.31	102.70
Total Salivary IgG (ng/ml)	31	120.65[a]	71	111.10	11.28	289.30	30	72.64[a]	55.06	54.35	15.99	254.60

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 33.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs: IgA: n = 30, S - IgA: n = 31, *S. mitis:* n = 31, *S. oralis:* n = 29, IgG: n = 28
Sig = statistical significance
ns = no significant difference
a = IgG significantly greater at end of study, $p < 0.02$

Table 83: Correlation Coefficients of Total Salivary IgA at Baseline: Whole Control Group (n = 33)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.72	**p < 0.0001**
IgA Antibodies to *S. mitis*	0.42	**p < 0.02**
IgA Antibodies to *S. oralis*	0.33	p <0.08

* Spearman Rank Correlation Coefficient
Sig = statistical significance

CHANGES IN THE SALIVARY IMMUNOGLOBULINS OF THE TBI GROUP

Salivary Immunoglobulins at Baseline

The baseline salivary immunoglobulins of the TBI children (n = 23) and their matched controls were compared at baseline. The results are as follows:

There were no significant differences in the mean concentrations of either total salivary IgA, S - IgA, salivary IgA antibodies to *S. mitis* and *S. oralis* and total salivary IgG between the TBI and matched control groups at baseline (Table 84).

Correlations

The correlations between the concentration of total salivary IgA and the concentrations of S - IgA ($p < 0.0001$) and salivary IgA antibodies to *S. mitis* ($p < 0.003$) and *S. oralis* ($p < 0.05$) were statistically significant (Table 85).

Table 84: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline: TBI Group and Matched Controls

	TBI Group (n = 23)						**Matched Control Group (n = 23)**					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	22	11.84	10.11	8.64	0.97	33.63	22	6.28	4.82	4.75	1.04	15.14
Secretory IgA (μg/ml)	22	19.26	14.78	17.49	2.16	45.58	22	15.06	7.53	13.50	4.03	29.56
Antibodies to *S. mitis* (E.U./ml)	21	60.21	65.32	31.23	11.95	266.60	22	71.58	52.01	59.67	15.89	183.05
Antibodies to *S. oralis* (E.U./ml)	21	27.26	28.78	17.81	nd	88.41	21	29.18	24.27	20.48	1	75.54
Total Salivary IgG (ng/ml)	21	123.83	123.52	76.95	25.71	450	21	104.42	68.94	99.17	11.28	248.65

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 23.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
TBI and matched controls at baseline: IgA: n = 21, S - IgA: n = 21, *S. mitis:* n = 20, *S. oralis*: n = 19, IgG: n = 19
Sig = statistical significance
nd = not detected

Table 85: Correlation Coefficients of Total Salivary IgA at Baseline: TBI Group (n = 23)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.82	**P < 0.0001**
IgA Antibodies to *S. mitis*	0.61	**p < 0.003**
IgA Antibodies to *S. oralis*	0.4	**p < 0.05**

* Spearman Rank Ccorrelation Coefficient
Sig = statistical significance

Salivary Immunoglobulins Seven Days Post - Transplantation

Total Salivary IgA and Secretory IgA

There were no significant differences in the mean concentrations of either total salivary IgA or S - IgA between baseline and 7 days post - transplantation (Table 86).

Salivary IgA Antibodies to S. mitis and S.oralis

There was no significant difference in the mean concentration of salivary IgA antibodies to *S. mitis* (Table 85). There was an increase in the mean concentrations of salivary IgA antibodies to *S. oralis* from 27.26 to 48.05 E.U. / ml, although this was not significant.

Total Salivary IgG

There was a significant increase in the mean concentration of total salivary IgG 7 days post - transplantation ($p < 0.01$) compared with baseline (Table 86).

Correlations

The correlations between the concentration of total salivary IgA and S - IgA ($p < 0.0001$) and the concentrations of salivary IgA antibodies to *S. mitis* ($p < 0.006$) and *S. oralis* ($p < 0.03$) were statistically significant (Table 87).

Table 86: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline and 7 Days Post - transplantation (Neutrophils < 10^8/l): TBI Group

	Baseline (n = 23)						Day 7 Post - transplantation (n = 23)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	22	11.84	10.11	8.64	0.97	33.63	21	15.58	23.33	6.95	0.36	85.35
Secretory IgA (μg/ml)	22	19.26	14.78	17.49	2.16	45.58	22	18.02	25.63	7.63	0.70	97.65
Antibodies to *S. mitis* (E.U./ml)	21	60.21	65.32	31.23	11.95	266.60	21	65.56	78.82	36.32	3.29	289.55
Antibodies to *S. oralis* (E.U./ml)	21	27.26	28.78	17.81	nd	88.41	20	48.05	46.28	35.41	1	205.40
Total Salivary IgG (ng/ml)	21	123.83[a]	123.52	76.95	25.71	450	21	657.20 [a]	822.21	238.40	52.08	2571.0

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 23 and 19
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs between baseline and 7 days post - transplantation: IgA: n = 21, S - IgA: n = 21, *S. mitis:* n = 20, *S. oralis:* n = 19, IgG: n = 19
Sig = statistical significance
ns = no significant difference
nd = not detected
a = significantly increased day 7 post - transplantation, $p < 0.01$

Table 87: Correlation Coefficients of Total Salivary IgA 7 Days Post - transplantation: TBI Group (n = 23)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.73	**$p < 0.0001$**
IgA Antibodies to *S. mitis*	0.60	**$p < 0.006$**
IgA Antibodies to *S. oralis*	0.5	**$p < 0.03$**

* Spearman Rank Correlation Coefficient
Sig = statistical significance

Salivary Immunoglobulins After Recovery of the Peripheral Neutrophil Count ($< 0.5 \times 10^9$ l)

Total Salivary IgA and Secretory IgA

There were significant decreases in the mean concentrations of total salivary IgA ($p < 0.01$) and S - IgA ($p < 0.001$) between baseline and time of recovery of the peripheral neutrophil count (Table 88).

Salivary IgA Antibodies to S. mitis and S. oralis

There was a significant decrease in the mean concentration of salivary IgA antibodies to *S. mitis* ($p < 0.002$) from baseline but no significant difference in the mean concentration of salivary IgA antibodies to *S. oralis* (Table 88).

Total Salivary IgG

There was a significant decrease in the mean concentration of total salivary IgG compared with baseline ($p < 0.03$) (Table 88).

Table 88: Concentrations Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: TBI Group

	Baseline (n = 19)						Neutrophil Count > 0.5 x 10^9/l (n = 19)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (µg/ml)	19	12.71[a]	10.64	10.39	0.97	33.63	18	6.16[a]	5.97	3.63	0.19	19.66
Secretory IgA (µg/ml)	19	21.21[b]	14.99	19.45	2.16	45.58	18	6.60[b]	6.93	5.14	0.48	24.42
Antibodies to *S. mitis* (E.U./ml)	18	64.10[c]	69.95	33.90	11.95	266.60	17	19.32[c]	18.80	19.85	1	58.78
Antibodies to *S. oralis* (E.U./ml)	17	20.63	26.20	9.14	nd	84.03	18	23.54	21.48	20.24	1	69.86
Total Salivary IgG (ng/ml)	18	120.79[d]	126.53	75.98	25.71	450	17	84.10[d]	103.89	34.64	6.58	412.35

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 23 and 19.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs between baseline and neutrophils > 0.5 x 10^9/l: IgA: n = 18, S - IgA: n = 18, *S. mitis:* n = 16, *S. oralis:* n = 16, IgG: n = 16
Sig = statistical significance
ns = no significant difference
nd = not detected
a = significantly decreased when neutrophils < 0.05 x 10^9/l, $p < 0.01$
b = significantly decreased when neutrophils < 0.05 x 10^9/l, $p < 0.001$
c = significantly decreased when neutrophils < 0.05 x 10^9/l, $p < 0.002$
d = significantly decreased when neutrophils < 0.05 x 10^9/l, $p < 0.03$

Correlations

The correlations between the concentration of total salivary IgA and secretory IgA ($p < 0.0001$)and the concentration of salivary IgA antibodies to *S. mitis* ($p < 0.03$) were statistically significant. There was no significant correlation between the concentrations of total salivary IgA and salivary IgA antibodies to *S. oralis* (Table 89).

Table 89: Correlation Coefficients of Total Salivary IgA after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: TBI Group (n = 19)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.77	**p < 0.0001**
IgA Antibodies to *S. mitis*	0.53	**p < 0.03**
IgA Antibodies to *S. oralis*	-0.3	p < 0.23

* Spearman Rank Correlation Coefficient
Sig = statistical significance

Salivary Immunoglobulins 119 Days Post - transplantation

Total Salivary IgA and Secretory IgA

The total salivary IgA concentration was significantly greater 119 days post - transplantation than at baseline ($p < 0.005$). There was no significant difference in the concentrations of S - IgA between baseline and the end of the study (Tables 84 and 90).

Salivary IgA Antibodies to S. mitis and S.oralis

There was an increase in the concentration of salivary IgA antibodies to *S. mitis*, from baseline but this was not significant (Tables 84 and 90). There was a significant increase in the mean concentration of salivary IgA antibodies to *S. oralis* ($p < 0.002$).

Table 90: Concentrations of Total Salivary Immunoglobulins and Antibodies to *S. mitis* and *S. oralis* at End of Study: TBI Group and Matched Controls

	TBI Group (n =16)						Matched Control Group (n = 16)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (µg/ml)	15	31.61[a]	21.75	25.73	2.39	77.55	16	10.03[a]	6.47	8.96	2.45	22.54
Secretory IgA (µg/ml)	15	29.54	24.52	21.22	0.11	84.21	16	17.74	10.56	12.90	5.57	36.60
Antibodies to *S. mitis* (E.U./ml)	14	109.14	63.18	122.00	22	212.35	16	77.64	60.68	47.23	7.77	196.25
Antibodies to *S. oralis* (E.U./ml)	16	136.68[b]	92.51	105.72	10.68	270.05	16	32.73[b]	22.42	28.85	4.98	77.65
Total Salivary IgG (ng/ml)	15	36.35	33.73	25.73	2.39	135.00	15	68.80	53.89	47.99	15.99	169.45

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 16.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
TBI Matched pairs between baseline and 119 Days post - transplantation: IgA: n = 15, S - IgA: n = 15, *S. mitis*: n = 14, *S. oralis*: n = 16, IgG: n = 14
TBI and matched control pairs at end of study: IgA: n = 15, S - IgA: n = 15, *S. mitis:* n = 14, *S. oralis:* n = 16, IgG: n = 14
Sig = statistical significance
ns = no significant difference
a = significantly greater in TBI group, $p < 0.001$
b = significantly greater in TBI group, $p < 0.0001$

Total Salivary IgG

There was no significant difference in the mean concentrations of total salivary IgG between baseline and 119 days post - transplantation (Tables 84 and 90).

Correlations

The correlation between the mean concentrations total salivary IgA and S - IgA ($p < 0.0001$) and salivary IgA antibodies to *S. mitis* ($p < 0.04$) were statistically significant. There was no significant correlation between total salivary IgA and salivary IgA antibodies to *S. oralis* (Table 91).

Table 91: Correlation Coefficients of Total Salivary IgA 119 Days Post - Transplantation: TBI Group (n =16)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.91	**p < 0.0001**
IgA ntibodies to *S. mitis*	0.55	**p < 0.04**
IgA Antibodies to *S. oralis*	0.14	p < 0.63

* Spearman correlation coefficient
Sig = statistical significance

Comparison of Salivary Immunoglobulins at the End of the Study: TBI Group and Matched Control Groups

Total Salivary IgA and Secretory IgA

The mean concentration of total salivary IgA was significantly greater in the TBI group ($p < 0.001$) at the end of the study (Table 90). Although the concentration S - IgA was greater in the TBI children, the difference was not significant.

Salivary IgA Antibodies to S. mitis and S. oralis

The mean concentration of salivary IgA antibodies to *S. mitis* was greater in the TBI group although not significantly. Salivary IgA antibodies to *S. oralis* were significantly greater in the TBI group ($p < 0.0001$) (Table 90).

Total Salivary IgG

There was no significant difference in the mean concentration of total salivary IgG between the TBI and matched control group at the end of the study, although the concentration was greater

in the control group (Table 90).

Summary of Results for Salivary Immunoglobulins: TBI and Matched Control Groups

There were no significant differences in the concentrations of salivary immunoglobulins or antibodies to *S. oralis* and *S. mitis* between the TBI and matched control group at baseline. There were some changes in the salivary immunoglobulins in the TBI group during the transplantation period:

(1) There was a significant increase in the concentration of total salivary IgG 7 days post - transplantation.
(2) There were significant decreases in the mean concentrations of total salivary IgA, S - IgA, salivary IgA antibodies to *S. mitis* and total salivary IgG between baseline and recovery of the peripheral neutrophil count.
(3) There was a significant increase in the mean concentration of total salivary IgA and salivary IgA antibodies to *S. oralis* between baseline and 119 days post - transplantation.
(4) The mean concentrations of total salivary IgA, and salivary IgA antibodies to *S. oralis* were significantly greater in the TBI group than the matched control group at the end of the study.

CHANGES IN THE SALIVARY IMMUNOGLOBULINS OF THE CTO GROUP

The conditioning regimen for the children in this group comprised high dose chemotherapy followed by autologous bone marrow rescue (n = 7) or matched sibling allograft (n = 4). The salivary immunoglobulins for the autografts and the allografts recipients were compared and there was no statistical differences between these 2 small subgroups. The results are for the whole group (n = 11)

Salivary Immunoglobulins at baseline

The baseline concentrations salivary immunoglobulins of the CTO children (n = 11) and their matched controls were compared at baseline. The results are as follows:-

Total Salivary IgA and Secretory IgA

There was no significant difference in the mean concentrations of total salivary IgA (Table 92) but the mean concentration of S - IgA was significantly greater in the control children ($p < 0.02$) at baseline.

There were no significant differences in the mean concentrations of salivary IgA antibodies to *S. mitis* and *S. oralis* and total IgG between the CTO and matched controls groups at baseline (Table 92).

Correlations

The correlations between the concentration of total salivary IgA and the concentrations of S - IgA ($p < 0.004$) and salivary IgA antibodies to *S. mitis* ($p < 0.005$) and *S. oralis* ($p < 0.006$) were statistically significant (Table 93).

Table 92: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline: CTO Group and Matched Controls

	CTO Group (n = 11)						Matched Control Group (n = 11)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	10	11.00	6.67	11.03	3.40	23.38	11	7.46	4.27	8.56	0.60	15.34
Secretory IgA (μg/ml)	10	13.37[a]	11.47	9.75	3.13	38.87	11	25.33[a]	7.80	24.35	9.18	37.03
Antibodies to *S. mitis* (E.U./ml)	11	167.52	132.89	100.15	28.46	432	10	78.57	36.99	80.57	34.51	156.30
Antibodies to *S. oralis* (E.U./ml)	11	45.92	42.55	30.93	0.79	104.40	10	47.17	25.57	45.87	17.06	83.33
Total Salivary IgG (ng/ml)	10	96.36	97.08	56.53	23.31	309.20	10	126.42	52.40	119.35	39.83	248.10

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 11.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs at baseline: IgA: n = 10, S - IgA: n = 10, *S. mitis:* n = 10, *S. oralis:* n = 10, IgG: n = 10
Sig = statistical significance
ns = no significant difference
a = significantly greater in control children, $p < 0.02$

Table 93: Correlation Coefficients of Total Salivary IgA at Baseline: CTO Group (n = 11)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.82	**p < 0.004**
IgA Antibodies to *S. mitis*	0.81	**p < 0.005**
IgA Antibodies to *S. oralis*	0.79	**p < 0.006**

* Spearman Rank Correlation Coefficient
Sig = statistical significance

Salivary Immunoglobulins Seven Days Post - transplantation

Total Salivary IgA and Secretory IgA

There were no significant differences in the mean concentration of total salivary IgA between baseline and 7 days post - transplantation (Table 94) but the concentration S - IgA was significantly reduced ($p < 0.008$) (Table 94).

Salivary IgA Antibodies to S. mitis and S. oralis

There was a significant decrease in the mean concentration of salivary IgA antibodies to *S. mitis* ($p < 0.03$). The mean concentration of salivary IgA antibodies to *S. oralis* was greater than at baseline but not significantly (Table 94).

Total Salivary IgG

There was a significant increase in the mean concentration of total salivary IgG 7 days post - transplantation ($p < 0.02$) (Table 94).

Correlations

There were no significant correlations between the concentration of total salivary IgA and the concentrations of S - IgA and salivary IgA antibodies to *S. mitis*. The correlation between the concentrations of total salivary IgA and salivary IgA antibodies to *S. oralis* was statistically significant ($p < 0.02$) (Table 95).

Table 94: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline and 7 Days Post - Transplantation (Neutrophils < 10^8/l): CTO Group

	Baseline (n = 11)						Day 7 Post - transplantation (n =11)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	10	11.00	6.67	11.03	3.40	23.38	11	7.52	9.39	2.67	0.97	25.83
Secretory IgA (μg/ml)	10	13..37 [a]	11.47	9.75	3.13	38.87	10	5.04[a]	5.17	2.49	1	17.21
Antibodies to *S. mitis* (E.U./ml)	11	167.52[b]	132.89	100.15	28.46	432	10	41.90[b]	46.04	30.01	4.41	160.65
Antibodies to *S. oralis* (E.U./ml)	11	45.92	42.55	30.93	0.79	104.40	11	82.54	61.13	74.23	1	201.60
Total Salivary IgG (ng/ml)	10	80.83[c]	88.84	51.17	23.31	309.20	10	249.27[c]	292.23	133.9	40.68	819.10

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 11.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs between baseline and 7 days post - transplantation: IgA: n =10, S - IgA = 9, *S. mitis:* n =10, *S. oralis:* n =10, IgG: n =9
Sig = statistical significance
ns = no significant difference
a = significantly decreased 7 days post - BMT, $p < 0.008$
b = significantly decreased 7 days post - BMT, $p < 0.03$
c = significantly increased 7 days post - BMT, $p < 0.02$

Table 95: Correlation Coefficients of Total Salivary IgA 7 Days Post - transplantation: CTO Group (n = 11)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	-0.33	$p < 0.35$
IgA ntibodies to *S. mitis*	0.35	$p < 0.33$
IgA Antibodies to *S. oralis*	0.67	**$p < 0.02$**

* Spearman Rank Correlation Coefficient
Sig = statistical significance

Salivary Immunoglobulins After Recovery of the Peripheral Neutrophil Count ($< 0.5 \times 10^9$ / l)

Total Salivary IgA and Secretory IgA

There was a significant decrease in the mean concentration of total salivary IgA ($p < 0.02$) and in the concentration of S - IgA although not significantly, between baseline and the recovery of the peripheral neutrophil count (Table 96).

Salivary IgA Antibodies to S. mitis and S. oralis

The mean concentration of salivary IgA antibodies to *S. mitis* was decreased from baseline, although not significantly. There was no significant difference in the mean concentration of salivary IgA antibodies to *S. oralis* (Table 96).

Salivary IgG

The mean concentration of salivary IgG was greater than at baseline but not significantly (Table 96).

Correlations

There were no significant correlations between the concentration of total salivary IgA and the concentrations of secretory IgA and salivary IgA antibodies to *S. mitis*. The correlation between the concentration of total salivary IgA and salivary IgA antibodies to *S. oralis* was statistically significant ($p < 0.03$) (Table 97).

Table 96: Concentrations Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at Baseline and after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group

	Baseline (n = 11)						**Neutrophil Count > 0.5 x 10^9/l (n = 11)**					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (µg/ml)	10	11.00[a]	6.67	11.03	3.40	23.38	10	3.84[a]	7.18	0.95	0.15	22.65
Secretory IgA (µg/ml)	10	13..37	11.47	9.75	3.13	38.87	11	8.14	8.24	4.18	1.23	22.12
Antibodies to *S. mitis* (E.U./ml)	11	167.52	132.89	100.15	28.46	432	11	76.08	65.98	54.87	3.64	191.20
Antibodies to *S. oralis* (E.U./ml)	11	45..92	42.55	30.93	0.79	104.40	10	41.14	36.44	36.98	1	110.90
Total Salivary IgG (ng/ml)	10	96.36	97.08	56.53	23.31	309.20	9	159.85	189.03	127.20	1	598.05

NB: Results greater than 2 standard deviations from the mean have been excluded so that **n** is less than 11.
As a consequence the matched pairs derived in this way have been excluded, which accounts for the following:
Matched pairs between baseline and neutrophils > 0.5 x 10^9/l: IgA: n = 9, S - IgA: n = 10, *S. mitis*: n = 11, *S. oralis*: n = 10, IgG: n = 9
Sig = statistical significance
ns = no significant difference
a = significant decrease when neutrophils > 0.5 x 10^9/l, $p < 0.02$

Table 97: Correlation Coefficients of Total Salivary IgA after Recovery of the Peripheral Neutrophil Count > 0.5 x 10^9/l: CTO Group (n = 11)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.49	$p < 0.15$
IgAAntibodies to *S. mitis*	-0.29	$p < 0.43$
IgA Antibodies to *S. oralis*	0.72	**$p < 0.03$**

* Spearman Rank Correlation Coefficient
Sig = statistical significance

Salivary Immunoglobulins 113 Days Post - transplantation

Total Salivary IgA and Secretory IgA

There was a significant decrease in the mean concentration of total salivary IgA between baseline and 113 days post - transplantation ($p < 0.04$). There was no significant difference in the mean concentration of S - IgA between baseline and the end of the study (Tables 92 and 98).

Salivary IgA Antibodies to S. mitis and S. oralis

Although the concentration of salivary IgA antibodies to *S. mitis* was greater than at baseline, the difference was not significant. There was no significant difference in the mean concentration of salivary IgA antibodies to *S. oralis* between baseline and 113 days post - transplantation (Tables 92 and 98).

Total Salivary IgG

There was no significant difference in the mean concentration of total salivary IgG between baseline and 113 days post - transplantation.

Correlation

There were no significant correlations between the mean concentration of total salivary IgA and the concentrations of S - IgA and salivary IgA antibodies to *S. mitis* and *S. oralis* (Table 99).

Table 98: Concentrations of Total Salivary Immunoglobulins and IgA Antibodies to *S. mitis* and *S. oralis* at End of Study: CTO and Matched Controls

	CTO Group (n =11)						Matched Control Group (n = 11)					
(per ml oral rinse)	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**	**n**	**Mean**	**sd**	**Median**	**Min**	**Max**
Total Salivary IgA (μg/ml)	10	4.65[a]	4.01	3.02	1.02	12.42	10	12.16[a]	6.62	11.48	2.81	22.51
Secretory IgA (μg/ml)	11	16.59	10.88	13.75	4.97	32.71	11	28.48	15.03	23.54	11.37	56.45
Antibodies to *S. mitis* (E.U./ml)	11	75.06	65.28	78.13	1	173	10	77.41	57.49	59.67	31.32	223.65
Antibodies to *S. oralis* (E.U./ml)	10	36.85	37.10	23.07	1	110.60	10	50.14	34.54	40.76	11.47	120.60
Total Salivary IgG (ng/ml)	10	98.88	87.71	103.20	1	272.10	11	151.56	106.63	89.65	40.54	313.40

NB: Results greater than 2 standard deviations from the mean have been excluded which accounts so that n is less than 11.
In addition the matched pairs have been excluded, which account for the reduction in the number of pairs for each analysis as follows:
CTO matched pairs between baseline and 113 days post - transplantation: IgA: 9, S - IgA: 10, *S. mitis*: 11, *S. oralis:*10, IgG: 9
CTO and matched control pairs at end of study: IgA: 9, S - IgA:11, *S. oralis*, 9, *S. mitis*: 10, IgG: 9
a = significantly greater in control group, $p < 0.008$

Table 99: Correlation Coefficients of Total Salivary IgA 113 Days Post - Transplantation: CTO Group (n = 11)

Correlation with Total Salivary IgA		
	rho*	**Sig**
Secretory IgA	0.32	$p < 0.37$
IgA Antibodies to *S. oralis*	-0.07	$p < 0.87$
IgA Antibodies to *S. mitis*	-0.14	$p < 0.70$

* Spearman Rank Correlation Ccoefficient
Sig = statistical significance

Comparison of Salivary Immunoglobulins at the End of the Study: CTO and Matched Control Groups

Total Salivary IgA and Secretory IgA

The mean concentration of total salivary IgA was significantly greater in the control group ($p< 0.008$) as was also the concentration of S - IgA but not significantly, at the end of the study (Table 98).

Salivary IgA Antibodies to S. mitis and S. oralis

There were no significant differences in the mean concentrations of salivary IgA antibodies to *S. mitis* and *S. oralis* between the CTO and the matched control groups (Table 98).

Total Salivary IgG

Although the mean concentration of total salivary IgG was greater in the matched control children at the end of the study, this difference was not significant (Table 98).

Summary of Results for Salivary Immunoglobulins: CTO and Matched Control Groups

The mean concentration of S - IgA was significantly greater in the matched control children at baseline. The mean concentration of total salivary IgG was also greater, but not significantly. There were some changes in the salivary immunoglobulins in the CTO group during the transplantation period:

(1) There were significant decreases in the concentrations of S - IgA and salivary IgA antibodies to *S. mitis* 7 days post - transplantation and a significant increase in the concentration total salivary IgG.

(2) There was a significant decrease in the concentrations of total IgA as the peripheral neutrophil count recovered to > 0.5 x 10^9/ l.

(3) There was a significant decrease in the mean concentration of total salivary IgA between baseline and 113 days post - transplantation .

(4) The concentration of total salivary IgA in the control group was significantly greater in the matched controls children than in the CTO children at the end of the study.

Comparison of Salivary Immunoglobulins TBI and CTO Groups

Salivary Immunoglobulins at baseline

Total Salivary IgA and Secretory IgA

There were no significant differences in the mean concentrations of total salivary IgA and S - IgA between the TBI and CTO groups at baseline (Tables 84 and 92).

Salivary IgA Antibodies to S. mitis and S. oralis

The concentration of salivary IgA antibodies to *S. mitis* were significantly greater in the TBI group ($p < 0.004$). There was no significant difference in the mean concentration of salivary IgA antibodies to *S. oralis* between the TBI and CTO groups (Tables 84 and 92).

Total Salivary IgG

The was no significant difference in the mean concentration of total salivary IgG between the 2 transplant groups (Tables 84 and 92).

Salivary Immunoglobulins Seven Days Post - transplantation

There were no significant differences in the mean concentrations of total salivary IgA, S - IgA, salivary IgA antibodies to *S. mitis* and *S. oralis* and total Salivary IgG between the TBI and CTO children (Tables 86 and 94).

Salivary Immunoglobulins After Recovery of the Peripheral Neutrophil Count ($< 0.05 \times 10^9$ / l)

Total Salivary IgA and Secretory IgA

There were no significant differences in the mean concentrations of total salivary IgA and S - IgA between the TBI and CTO children (Tables 88 and 96).

Salivary IgA Antibodies to S. mitis and S. oralis

The mean concentration of salivary IgA antibodies to *S. mitis* was significantly greater in the CTO group ($p < 0.004$). There was no significant difference in the mean value of salivary IgA antibodies to *S. oralis* between the 2 groups (Tables 88 and 96).

Total Salivary IgG

There was no significant difference in the mean value of total salivary IgG between the TBI and CTO groups (Tables 88 and 96).

Salivary Immunoglobulins at the End of the Study

Total Salivary IgA and Secretory IgA

The mean concentration of total salivary IgA was significantly greater in the TBI group ($p < 0.0001$), but there was no significant difference in the mean concentration of S - IgA between the 2 groups (Tables 90 and 98).

IgA Antibodies to S. mitis and S. oralis

There was no significant difference in the mean concentration of salivary IgA antibodies to *S. mitis* but the mean concentration of salivary IgA antibodies to *S. oralis* was significantly greater in the TBI group ($p < 0.002$) (Tables 90 and 98).

Total Salivary IgG

There was no significant difference in the mean concentration of total salivary IgG between the TBI and CTO groups (Tables 90 and 98).

Summary of Results for Salivary Immunoglobulins: TBI and CTO Groups

There were a few differences between the TBI and CTO groups during the transplantation period:

(1) The mean concentration of IgA antibodies to *S. mitis* was significantly greater in the CTO group at baseline.

(2) The mean concentrations of IgA antibodies to *S. mitis* was significantly greater in the CTO group when the peripheral neutrophil count had recovered to $> 0.5 \times 10^9/l$.

(3) The mean concentrations of total salivary IgA and IgA antibodies to *S. oralis* were greater in the TBI group at the end of the study.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

The relationship between the composition and abundance of the oral flora and oral bacterial loading and systemic infection in sick children is clearly an important issue. One particular aspect is bacteraemia from oral micro - organisms in profoundly immunosuppressed and neutropenic children. The background for this is the interaction between the treatment procedures and sequelae and their effect on the oral bacterial flora. These factors include a period of intense immunosuppression, the administration of systemic antibiotics, mucosal inflammation including widespread gingivitis, the change from oral to parenteral nutrition during the period of severe oral discomfort and the resultant difficulty in maintaining adequate oral hygiene. There is some evidence to support this complex of interrelationships from the results of a study carried out at The Great Ormond Street Hospital For Children. Twelve percent of septicaemic episodes in children treated in the Haematology / Oncology unit were associated with oral streptococci. This provides indirect support for the findings reported here, that oral streptococci enter the blood stream during periods of mucositis / gingivitis related to intensive chemotherapy (Holzel and de Saxe 1992).

Oral Lesions

Dental Caries

The dmft for the control children in the present study was within the range reported in the Great Britain dental health surveys co-ordinated by the British Association of Community Dentistry (Pitts and Palmer, 1994). The range of dmft in the Great Britain survey was 1.16 to 2.82 and the mean value for the controls in the present study was 1.42. This mean value also falls between those from 2 local dental surveys. The dmft for 5 years old in an affluent area of Surrey was 1.16 (Harris 1994) and for Greenwich, an inner city area of London, 2.4 (Ob 1994). Schools for the control children in the present study were of mixed socio-economic status. The DMFT of 0.14 for the controls was lower than the national survey range of 1.12 to 2.1. The dmft for the TBI children, 2.53, was just within the range of the national survey, but the dmfs was significantly greater than the that of the matched controls. The dmft for the CTO children, 4.14, was greater than the matched controls, but not significantly, and was also greater than the national survey. The mean percentage of the decayed component of the dmfs in the TBI group was 16.67, sd 38.92 and for the CTO group was 27.20 sd 26.65. These figures are an indication of the amount of bacterial activity from caries present in the mouth before immunosuppression. It is important to ensure that children do not start the conditioning

regimen with active caries because of the risk of opportunistic infection arising from an abscessed tooth which could form a focus of infection.

Bacterial Dental Plaque and Gingivitis

An important part of the mouthcare regimen is rinsing with 0.2% chlorhexidine, which if used effectively, reduces plaque accumulation (and bacterial loading) (Schiott et al. 1970; Joyston - Bechal and Hernaman 1993). This has been reported by several groups of investigators (McGaw and Belch, 1985; Ferretti et al. 1987; Ferretti et al. 1990), as has a reduction in the frequency isolation of the viridans streptococci (Ferretti et al. 1987; Ferretti et al. 1990). Other workers have reported a temporary increase in viridans streptococci on oral epithelial surfaces and this increase should be considered as a possible risk for infection in medically compromised patients (Vaahtoniemi et al. 1995). Most of the children in the present study were unable to use the chlorhexidine effectively for 2 main reasons. Chlorhexidine has an unpleasant taste and the 7% alcohol base causes a burning sensation of the already inflamed mucosa.

A significant deterioration in gingival health occurred during the period of intense immunosuppression, associated with a large increase in plaque accummulation, around the gingival margin. This was probably due to the concurrent problems of oral pain and it was understandable that compliance with oral hygiene procedures was often poor. Approximately 4 months after transplantation, the oral health indices had returned to baseline levels. However, it is during this period of immunosuppression and profound neutropenia that infections are most likely to arise from the oral cavity.

Mucositis

This occurred from approximately day 5 post transplantation and persisted for several days, the clinical manifestations being more pronounced and long-lasting in the TBI group than the CTO group. Mucositis is reported to be strongly associated with oral bacteraemias (Valteau et al. 1991; Bochud et al. 1994b). The sites affected may be discrete, but often most if not all of the oral mucosa is involved. Widespread erythema with ulceration and bleeding of the mucosal surfaces and the areas around the salivary gland ducts provide a large area for systemic ingress of bacteria. It is highly likely that an important site of systemic entry is through the inflamed gingival tissues.

Changes in the Oral Flora

There were 2 main changes in the oral flora in both the TBI and the CTO groups at 7 days post - transplantation. The streptococcal flora was simplified now consisting predominately of

2 species, *S. mitis* and *S. oralis*, collectively the '*oralis* group'. There was a simultaneous decrease in the mean total aerobic and anaerobic counts of both transplant groups. The proportion of the '*oralis* group' of the total anaerobic count was increased in both groups, but only significantly in the TBI group.

When the neutrophil count had recovered above 0.5×10^9 / l, the proportion of the '*oralis* group' and the mean total anaerobic count were not significantly different from baseline in the CTO group. Although the proportion of the '*oralis* group' was not significantly different from baseline in the TBI group, the mean total anaerobic count remained significantly lower. At the end of the study, the proportion of the '*oralis* group' was no different from baseline and the mean total anaerobic counts were no different from baseline in either transplant group. The total aerobic count was significantly greater in the chemotherapy only group compared with the matched controls.

The survival and increase in proportion of the '*oralis* group' during the period of intense immunosuppression predisposes the children to the risk of opportunistic infection from the oral cavity. Other investigators have identified a number of features which help to explain the ability of this particular group of streptococci to survive the adverse conditions created by the conditioning regimen, antibiotic therapy and the change from oral nutrition to parenteral feeding. Of all the oral streptococci, the '*oralis* group' have the greatest ability to proliferate on host - derived nutrients which may in part be due to their greater proteolytic activity (Homer et al. 1990).

Members of the '*oralis* group' are important early colonisers of enamel surfaces(Nyvad and Kilian, 1990) with the ability to bind mucins in the salivary pellicle (Hsu et al. 1994). The '*oralis* group' have the greatest affinity for binding transferrin This is an important component of serum and enters the oral cavity in the gingival crevicular fluid or through bleeding gingival tissues (Curtis et al. 1988). It is probable that transferrin binding by the '*oralis* group' may be involved in the initial attachment of these organisms to tooth or mucosal surfaces (Beighton et al. 1990). Although the transplant children did not have periodontal disease, the mean gingival score was significantly increased on day 7 post - transplantation, a few had spontaneous gingival bleeding and all had some degree of mucositis (Figs 7 and 8). Gingival inflammation results in an increase in the production of crevicular fluid. This would facilitate the passage of transferrin across the gingival margin, thus providing a substrate for the initial attachment of these streptococci.

The survival of the ' *oralis* group' in the oral cavity in the absence of oral nutrition is dependent upon finding an alternative source of carbon. It was found that in monkeys (*Macaca Fascicularis*) following 18 hours of fasting significantly greater numbers of *S. mitior* (which has since been designated *S. oralis*) were recovered from the plaque on the molar teeth

Beighton et al. 1981). Further work indicated that *S. oralis* is able to hydrolyse the oligosaccharide side chain of host - derived glycoproteins in saliva and plaque and immediately utilise the released carbohydrates (Homer et al. 1996). It is highly probable that *S. oralis* survives well in the mouths of the neutropenic children because of the readily available transferrin for primary binding and the carbon source available from saliva and plaque glycoproteins. Furthermore, *S. oralis, S. mitis* and the other members of the Mitis group of viridans streptococci are able to utilise N - acetylneuraminic acid (sialidase) as a sole carbon source (Byers et al. 1996) which is likely to be an important factor in their persistence in the mouth of these children. By the time the neutrophil count had recovered above 0.5 x 10^9/l the children were eating again and were no longer supported by parenteral nutrition. The numbers of the '*oralis* group' isolated from the saliva were not significantly different from baseline. A further factor is that the '*oralis* group' seem to be less sensitive to the antibiotic regimens than the other species of oral streptococci.

There was no evidence of oral candidiasis at the examination times during the transplantation period. This was mainly because of the anti fungal drugs. Oral acquisition of *Enterobacteriaceae* and enterococci was negligible which reflects the high standard of care that the children receive. While the children are in isolation the care protocols are strictly observed.

Opportunistic Infection

Four children in the TBI group, 17.4%, had either positive blood or Hickman line cultures for the '*oralis* group' between 2 days pre - and 7 days post - transplantation. One of these children had positive blood cultures on 2 consecutive days. From the chemotherapy only group 1 child had a positive Hickman line culture and blood culture on days 4 and 7 post - transplantation, respectively. The DNA was extracted from *S. oralis* blood culture isolate and an *S. oralis* oral isolate which were both obtained on day 7 post - transplantation from the same child. The DNA pattern was identical for both isolates (Fig 15). The child from whom the *S. oralis* blood culture was isolated was suffering from mild mucositis, with no evidence of ulceration to the naked eye but there was extensive gingivitis. Although the widely held view is that entry into the blood stream occurs through mucosal ulcerations it could just as likely to enter through the gingival margin.

Investigators in France found that oral streptococci with the same ribotype as blood isolates were recovered from 7 patients with bacteraemia (Richard et al. 1995). They also stated that although oral ulcerations are believed to be the usual site of entry, many studies fail to demonstrate a significant association between mucositis and viridans streptococcal infections.

One of the conclusions of the present study is that opportunistic infection may gain early ingress into the systemic circulation via the gingival margin, especially when there is inflammation.

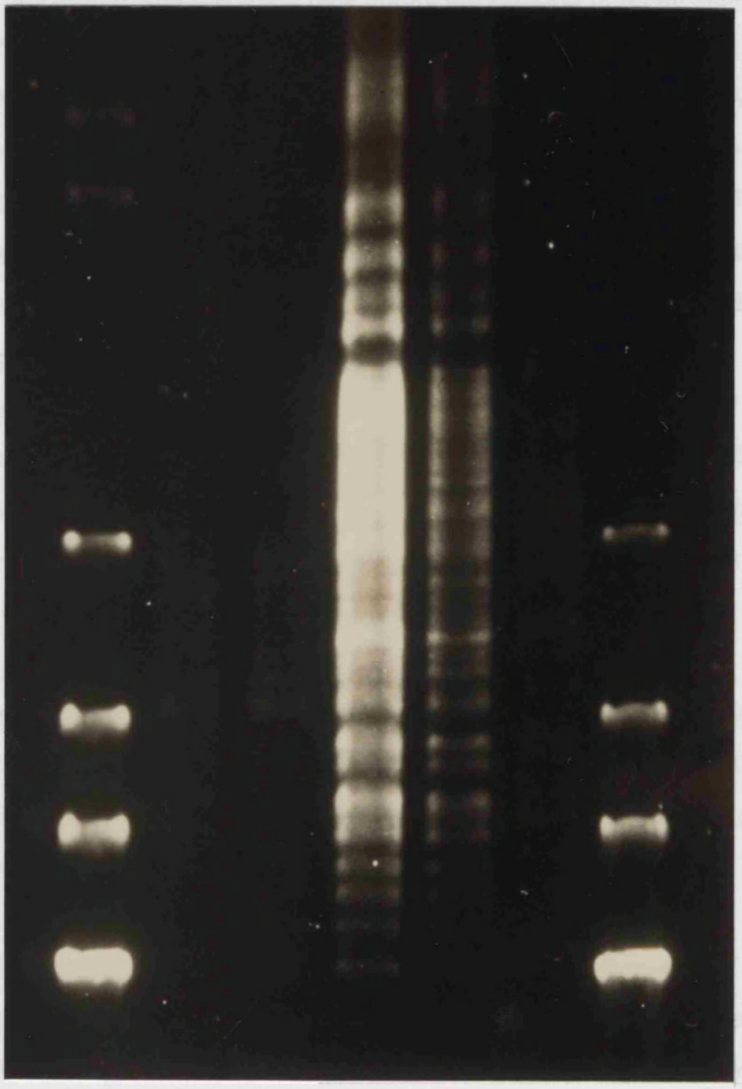


Fig 15: *S. oralis* isolates from a blood culture (left) and saliva (right) from the same child 7 days post - transplantation (DNA extracted by Christina Batzavali)

Mucosal Immunity

The changes in the mucosal immunity were difficult to interpret. They are similar in part, to the changes found in another group of bone marrow transplant patients (Chaushu et al. 1994a). In this group the investigators found that following a decrease in immunoglobulin concentrations after the conditioning regimen, the early post - transplant phase is associated with a rapid increase in salivary immunoglobulin levels. An increase in the concentrations of salivary immunoglobulins was detected as early as day 4 post - transplantation which steadily increased and levelled out 2 to 3 weeks later. The concentrations then decreased and remained

low for approximately 3 months. It was suggested that the origin of the salivary immunoglobulins was the donor, the donor B cells migrating to the recipient's salivary glands. The original donor cell lines die after about 3 weeks which explains the decrease in immunoglobulin production and it is some time before the humoral immune system becomes re - established. This assumption was tested with tetanus toxoid with comparable results (Chaushu et al. 1994b). Although it is not known how much the concentration of salivary immunoglobulins changed immediately after the conditioning regimen, the mean concentration of IgG was significantly increased on day 7 post - transplantation in this study. This was coincident with a significant increase in the gingival index which together with the mucositis would enable increased transudation of serum IgG across the gingival margin and the inflamed mucosal surfaces. However, the children were receiving weekly infusions of immunoglobulins, predominately IgG, and the increased concentrations observed were probably mostly from the infusion and only partly donor derived. The mean total salivary IgA was not significantly different from baseline in either transplant group and since the intravenous immunoglobulin contains only a trace of IgA, the salivary concentrations were almost certainly donor derived.

The mean concentrations of S - IgA and IgA antibodies to *S. mitis* were unchanged from baseline in the TBI group but were significantly lower than at baseline in the CTO group. The mean concentrations of antibodies to *S. oralis* were increased in both transplant groups although not significantly.

As the neutrophil count recovered above 0.5 x 10^9/l, which occurred between 15 - 35 days in the TBI group and 13 - 26 days in the chemotherapy only group, the mean concentrations of total salivary IgA were significantly decreased from the baseline values in both transplant groups. The mean concentrations of S - IgA and IgA antibodies to *S. mitis* were decreased in both groups compared with baseline, although not significantly in the CTO group. However, the concentration of antibodies to *S. mitis* was significantly greater in the chemotherapy only children compared with the TBI children.

At the end of the study, approximately 16 - 17 weeks after transplantation, the concentrations of total IgA and IgA antibodies to *S. oralis* were significantly greater than at baseline in the TBI group and also greater than those in the CTO group. The concentration of S - IgA in the chemotherapy group was significantly less than at baseline. These findings were in agreement with results from a study of 42 bone marrow transplant recipients in whom the S - IgA and IgG were significantly decreased on day 39 post - transplantation (Dens et al. 1996). In a different group of 85 bone marrow transplant patients all classes of

immunoglobulins were significantly decreased before and up to 5 years post - transplantation (Norhagen et al. 1994). The immunoglobulins in the present study have not been estimated after 4 months post - transplantation and no useful comparison can be made. The changes in antibody concentrations are more erratic and difficult to explain. There is probably cross - reactivity between *S. mitis* and *S. oralis* which is why the concentration of antibodies to only 1 or both species may change. There is no correlation between the numbers of *S. mitis* and *S. oralis* with the antibody concentrations possibly because the responses have to be re - established by the new immune system as it grows and develops in the recipient.

Microbiological Implications

The most important clinical and oral microbiological changes in children undergoing bone marrow transplantation occur during the period of intense immunosuppression and neutropenia. There is a significant increase in bacterial plaque and gingivitis which combined with mucositis and the survival and persistence of the '*oralis* group' of the viridans streptococci increase the risk of oro - dental bacteraemias and systemic infection from these particular species of the oral streptococci. There is sufficient evidence that infections are caused by the oral streptococci although there is no clinical proof of the actual site of entry into the bloodstream. Is it through the inflamed oral mucosa, the gingival crevice or both?

Clinical Implications

It is clear that children undergoing bone marrow transplantation need to be dentally fit before the conditioning regimen is started. This means not only the absence of caries but the children and parents must also have a good understanding of the importance of reducing oral bacterial loading. Although 2% chlorhexidine mouthrinse is known to reduce bacterial plaque levels and gingivitis, the taste prohibits it effective use in children.

One of the late complications in bone marrow transplant patients is the phenomenom of 'rebound caries' particularly in children who have been conditioned with TBI (Fig 16). This is due to a combination of reduced salivary flow, re - colonisation of the oral cavity with *S. mutans* and refined carbohydrate and occurs after the first year following transplantation. This is more difficult to deal with since children who are effectively discharged from the department should be seen regularly by their general dental practitioners and there is some evidence that this does not always occur. It is important to ensure that effective dental prevention is continued at home as soon as the children are well after transplant. Other long - term dental effects which occur as a result of TBI include incomplete root development (Dahllof et al. 1988c) and varying degrees of craniomandibular dysfunction following irradiation (Dahllof et al. 1994).

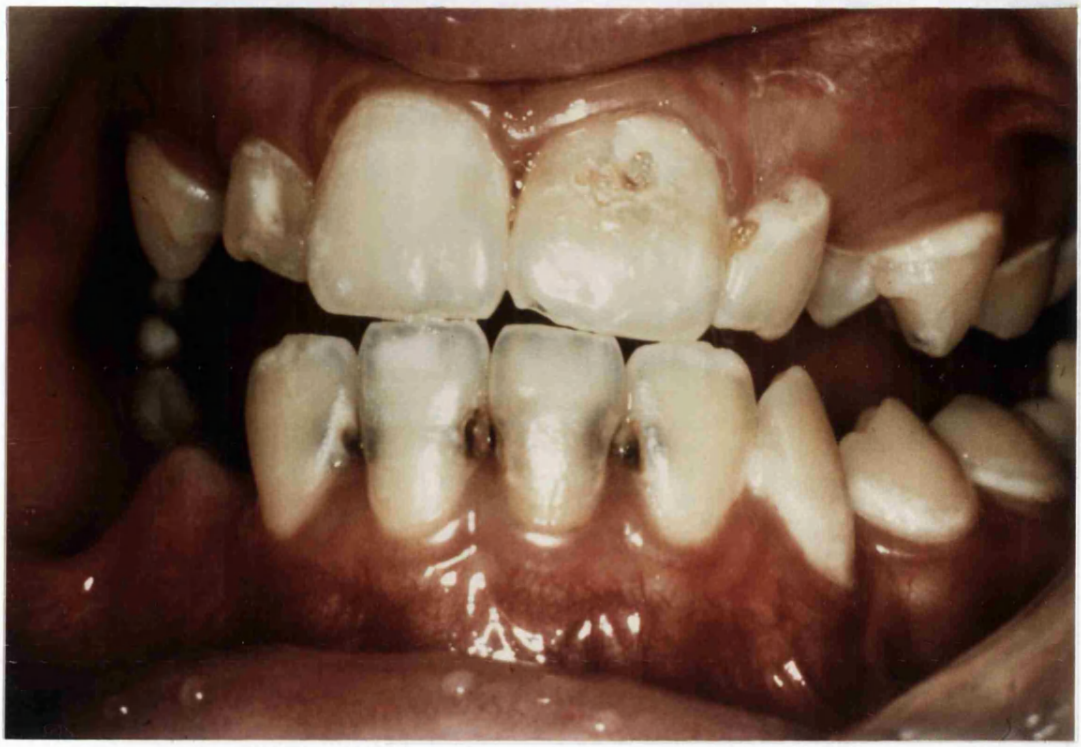


Fig 16: Extensive dental caries 18 months post - transplantation

Future Work

The main conclusion from this study is that it is necessary to improve the standard of oral health throughout the peri - transplantation period to prevent infections arising from the mouth. This would involve changing the composition of the oral flora to prevent selection of the '*oralis* group' of the viridans streptococci which are associated with febrile neutropenic episodes. One way would be to remove the viridans streptococci either by sterilisation of the oral cavity, which is a theoretical possibility only, certainly at present. The use of the antibiotic vancomycin, to which the viridans streptococci are sensitive unfortunately leads to the emergence of resistant enterococci. Alternatively, the selection of the '*oralis* group' could be prevented by providing another source of carbon, for example sucrose or glucose so that these particular streptococci do not select out. However, this would not reduce the amount of bacterial plaque and gingival inflammation. It is therefore, appropriate to reduce plaque accumulation with good oral hygiene before the conditioning regimen and one way would be to prescribe daily chlorhexidine mouthrinse in addition to toothbrushing for I week before the conditioning regimen. Compliance is essential and the way to ensure this is to use the chlorhexidine without the alcohol base with a different flavour, for example, bubble gum flavour is popular. This would help to reduce the risk of opportunistic infection from the oral streptococci. For such recommendations to be widely accepted it would be necessary to demonstrate their efficacy using the methodology of this study.

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APPENDIX 1

Explanation Sheet and Consent Form for the Bone Marrow Transplant Children

Maxillofacial and Dental Department
Hospital for Sick Children
Great Ormond Street
London WC1N 3JH

Changes in the Oral Microflora as a Result of Chemotherapy and Total Body Irradiation

Dear Parent,

During the time that your child is in hospital, he/she will be given many medicines. These medicines make the mouth feel very dry and this can also make it feel very sore. As a result of this, your child may not feel like brushing his/her teeth as well as normally. If the teeth are not cleaned so well, germs collect around the teeth and gums. Because of the complicated treatment there is a risk that your child may get an infection from the germs in the mouth. We want to study this so that we can find ways of preventing such infections. The samples we need to take from the mouth will not cause any harm or discomfort. In addition, it is important to record if your child has any decayed teeth and inflammation of the gums, so that we can arrange any treatment required.

When your child is admitted to hospital, we would like to take a sample of saliva and on three other separate occasions. The saliva will be collected by giving your child a mouthwash for 30 seconds.

We should be most grateful if you and your child would help us with this important work.

Victoria S. Lucas (Clinical Research Fellow)
Graham J. Roberts (Consultant and Reader in Paediatric Dentistry, Dental Department)

.....

CONSENT

I agree to my child.....taking part in the above study

Signed.....Parent/Guardian

Date.....

Explanation Sheet and Consent Form for the Matched Control Children

Dental Department
Hospital For Sick Children
Great Ormond Street
London WC1N 3JH

Changes in the Oral Microflora as a Result of Chemotherapy and Total Body Irradiation

Dear Parent,

We should be very grateful if you and your child would help us with our research. This is to help other children who need to have bone marrow transplants. While these children are in hospital for their transplant they are given many medicines which make their mouths very dry and sore. As a result of this, the children do not clean their teeth as well as normally and germs collect around the teeth and gums. These germs can cause very serious infections which do not occur in healthy children like yours.

We want to study the germs so that we can find ways of preventing such infections. To do this, we need to take small samples from the mouth and compare the germs that we find with similar samples from healthy children.

We would like to take a sample of your child's saliva, on four separate occasions. We would also like to check the teeth for decay and the gums for redness on the first occasion only. This information together with your child's identity, is strictly confidential and will remain so.

The normal high standards for cross-infection prevention will apply.

This research is not related to the School Dental Inspections carried out by the Community Dental Service and will not affect your child's usual dental treatment in any way.

Victoria S. Lucas (Clinical Research Fellow)

Graham J. Roberts (Consultant, Maxillo Facial & Dental Department)

.....

CONSENT

I agree to my child.....taking part in the above study

Signed.....Parent/Guardian

Date.....

APPENDIX 2

Examples of Conditioning Regimens

CONDITIONING:	CYCLOPHOSPHAMIDE / TBI
BMT DONOR:	MATCHED SIBLING DONOR
GVHD PROPHYLAXIS:	CYCLOSPORIN AND METHOTREXATE

DAY	**D = DAY**	**TREATMENT**
Tuesday	D - 10	Admit to hospital
Wednesday	D - 9	Insertion of double lumen Hickman line
Thursday	D - 8	
Friday	D - 7	Cyclophosphamide 60 mg / kg + MESNA iv
Saturday	D - 6	Cyclophosphamide 60 mg / kg + MESNA iv
Sunday	D - 5	
Monday	D - 4	TBI day 1 (180 cGy x 1#)
Tuesday	D - 3	TBI day 2 (180 cGy x 2#)
Wednesday	D - 2	TBI day 3 (180 cGy x 2#)
Thursday	D - 1	Cyclosporin 1.5 mg / kg iv bd
		TBI day 4 (180 cGy x 2#)
Friday	D0	TBI day 5 (180 cGy x 1#)
		Reinfuse bone marrow
		? volume reduction / ? red cell depletion
Monday	D + 3	Methotrexate 10 mg / m^2 iv
Thursday	D + 6	Methotrexate 10 mg / m^2 iv
Tuesday	D + 11	Methotrexate 10 mg / m^2 iv
Tuesday	D + 18	Methotrexate 10 mg / m^2 iv
		(only if neutrophils > 0.2 x 10^9 /l)

D -: Days before transplant

D0: Day of transplant

D +: Days after transplant

CONDITIONING: CAMPATH / CYCLOPHOSPHAMIDE / TBI

BMT DONOR: MATCHED UNRELATED DONOR

GVHD PROPHYLAXIS: CYCLOSPORIN AND METHOTREXATE

DAY	D = DAY	TREATMENT
Thursday	D - 15	Admit to hospital
Friday	D - 14	Insertion of double lumen Hickman line
		Back - up bone marrow harvest
Monday	D - 11	Allopurinol 4 mg / kg po tds for 5 days
Tuesday	D - 10	Campath IG 0.2 mg / kg iv + premed
Wednesday	D - 9	Campath IG 0.2 mg / kg iv + premed
Thursday	D - 8	Campath IG 0.2 mg / kg iv + / - premed
Friday	D - 7	Campath IG 0.2 mg / kg iv + / - premed
Saturday	D - 6	Campath IG 0.2 mg / kg iv + / - premed
Friday	D - 7	Campath IG 0.2 mg / kg iv + / - premed
		Cyclophosphamide 60 mg / kg + MESNA iv
Saturday	D - 6	Campath IG 0.2 mg / kg iv + / - premed
		Cyclophosphamide 60 mg / kg + MESNA iv
Sunday	D - 5	
Monday	D - 4	TBI Day 1 (180 cGy x 1#)
Tuesday	D - 3	TBI Day 2 (180 cGy x 2#)
Wednesday	D - 2	TBI Day 3 (180 cGy x 2#)
Thursday	D - 1	Cyclosporin 1.5 mg / kg iv bd
		TBI Day 4 (180 cGy x 2#)
Friday	D0	TBI Day 5 (180 cGy x 1#)
		Reinfuse bone marrow
		? T cell depletion
		? volume reduction / ? red cell depletion
Monday	D + 3	Methotrexate 10 mg / m^2 iv
Thursday	D + 6	Methotrexate 10 mg / m^2 iv
Tuesday	D + 11	Methotrexate 10 mg / m^2 iv
Tuesday	D + 18	Methotrexate 10 mg / m^2 iv
		(only if neutrophils > 0.2 x 10^9 /l)

CONDITIONING: BUSULPHAN / CYCLOPHOSPHAMIDE

BMT DONOR: MATCHED SIBLING DONOR

GVHD PROPHYLAXIS: CYCLOSPORIN AND METHOTREXATE

DAY	**D = DAY**	**TREATMENT**
Thursday	D - 15	Admit to hospital
Friday	D - 14	Insertion of double lumen Hickman line
Monday	D - 11	
Tuesday	D - 10	Busulphan 4 or 5 mg / kg via nasogastric tube in 2 divided doses (as protocol)
Wednesday	D - 9	Busulphan 4 or 5 mg / kg in 2 divided doses
Thursday	D - 8	Busulphan 4 or 5 mg / kg in 2 divided doses
Friday	D - 7	Busulphan 4 or 5 mg / kg in 2 divided doses
Saturday	D - 6	Cyclophosphamide 50 mg / kg + MESNA iv
Sunday	D - 5	Cyclophosphamide 50 mg / kg + MESNA iv
Monday	D - 4	Cyclophosphamide 50 mg / kg + MESNA iv
Tuesday	D - 3	Cyclophosphamide 50 mg / kg + MESNA iv
Wednesday	D - 2	Rest day
Thursday	D - 1	Cyclosporin 1.5 mg / kg iv bd
Friday	D0	Reinfuse bone marrow ? volume reduction / ? red cell depletion
Monday	D + 3	Methotrexate 10 mg / m^2 iv
Thursday	D + 6	Methotrexate 10 mg / m^2 iv
Tuesday	D + 11	Methotrexate 10 mg / m^2 iv
Tuesday	D + 18	Methotrexate 10 mg / m^2 iv (only if neutrophils > 0.2 x 10^9 /l)

CONDITIONING:	CYCLOPHOSPHAMIDE PRIMING
	HIGH DOSE MELPHALAN CONDITIONING
BMT DONOR:	AUTOLOGOUS BONE MARROW TRANSPLANT
GVHD PROPHYLAXIS:	NONE

DAY	**D = DAY**		**TREATMENT**
Friday	D - 7		Cyclophosphamide prime 300 mg / m^2 iv bolus
Tuesday	D - 3		Admit to hospital
Wednesday	D - 2	0 hour	Pre-melphalan hydration 4% dextrose / 0.18% saline + 20mmol / L Kcl at 200 ml / m^2 / hour for 3 hours
		3 hours	Melphalan 200 mg / m^2 as iv bolus followed by 0.45% saline / 2.5% dextrose at 3 litres / m^2 for 24 hours + 40 mmol / m^2 / 24 hours Kcl
		27 hours	Complete post - melphalan hydration
Friday	D0		Reinfuse bone marrow, with premedication, 48 hours after melphalan

APPENDIX 3

ELISA BUFFERS

Phosphate buffer saline (PBS) pH 7.2	**gram / litre**
Sodium chloride	8
Potassium chloride	0.2
Disodium hydrogen phosphate	1.15
Sodium dihydrogen phosphate	0.2
Distilled water to make up to 1 litre	

PBS + Azide

Prepare PBS

Sodium azide 0.2

PBS + bovine serum (BSA) + tween 20 (T20)

Prepare PBS

BSA - concentration of 0.5%

T20 - concentration of 0.05%

Diethanolamine pH 9.8

Magnesium chloride MgCl. $6H_20$ dissolved in 800 ml distilled water 101 mg

Diethanolamine 97 ml

Adjust to pH 9.8 with concentrated HCL and make up to 1 litre with distilled water

Store in dark at 4°C

Methyl glyoxal

95 ml distilled water

5 ml of 40% methyl glyoxal

pH adjusted to 8.0 with 10% sodium bicarbonate

The concentration of the methyl gloxal used for the ELISA assays was 0.3%

Buffered formalin	**per litre**
Formaldehyde	100 ml
Distilled water	900 ml
Disodium phosphate (anhydrous) Na_2HP0_4	6.5g
Sodium dihydrogen phosphate $NaH_2P0_4.H_20$	4.0g

Sodium Hydroxide

200 ml distilled water

24g sodium hydroxide

Preparation of bacterial cell suspension for coating the solid phase for the estimation of antibodies to *S. mitis* and *S. oralis* by ELISA after (Czerkinsky et al. 1983)

Each streptococcal species was inoculated onto Columbia blood agar and incubates anaerobically at 37° for 3 days. The growth was harvested and suspended in 20 ml of buffered formalin overnight at room temperature. The suspension was centrifuged at 2500g for 5 minutes, the supernatant discarded and the pellet of bacterial cells washed 3 times with PBS + azide. The pellet was suspended in 20 ml of PBS + azide and the concentration of cells adjusted to an O.D. of 1.85 at 540 nm using a spectrophotometer. An O.D. of 1.85 was approximately equivalent to 8 x 10^9 cells / ml (Challacombe et al. 1984). The coating layer was prepared from the stock methyl glyoxal and bacterial suspension.

PUBLICATIONS

Abstracts

Oral streptococcal changes following total body irradiation and chemotherapy for bone marrow transplantation(BMT). Presidents invitation, Section of Odontology, Royal Society of Medicine jointly with D. Beighton and G.J. Roberts 1995.

Oral streptococcal changes in bone marrow transplantation children following chemotherapy/irradiation, jointly with D. Beighton and G.J. Roberts. BSDR Manchester, 1995 Abstract No: 517.

Oral streptococcal changes in bone marrow transplantation children following chemotherapy/irradiation, jointly with D. Beighton and G.J. Roberts. IAPD Gothenborg, 1995.

Changes in the Oral Streptococcal Flora following Irradiation/Chemotherapy for Bone Marrow Transplantation, Jointly with D. Beighton and G.J. Roberts. X111th Lancefield Symposium on Streptococci and Streptococcal Diseases, Paris 1996.

Papers

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