Influence of Periodontitis on the Experience of Oral Mucositis in Cancer Patients Undergoing Head and Neck Radiotherapy

A report submitted to the University of Adelaide
in partial fulfilment of the requirements of the
Degree of Doctor of Clinical Dentistry
in the speciality of Periodontics

Arlene Bee Hong KHAW
BDS (Malaya)

School of Dentistry

January 2014

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Background and aim: Virtually all patients who receive head and neck radiotherapy develop some degree of oral mucositis. Severe oral mucositis may necessitate an interruption of the course of radiotherapy and thus can serve as a dose-limiting factor. Periodontitis is a host-driven inflammatory response to a pathogenic bacterial biofilm in the subgingival environment, resulting in the progressive destruction of the tissues that support the teeth, specifically the gingiva, periodontal ligament, and alveolar bone. This disease affects more than 50% of the population. Considering that radiation-induced oral mucositis and periodontitis are both characterised by the continuing presence of systemic inflammation, they may be associated through a primed inflammatory response as proposed by the "two-hit" model. Alternatively, both conditions may be correlated as they represent a dysregulation of the inflammatory response. To date, no studies have looked into the association between these conditions. The aim of this study is to determine whether the severity of oral mucositis is associated with the severity of periodontitis in cancer patients undergoing head and neck radiotherapy.

Materials and methods: Eighty-five consecutive patients seeking dental clearance prior to head and neck radiotherapy were assessed for their eligibility for participation in the study. Forty-one patients met the inclusion criteria. The severity of oral mucositis was measured according to the WHO system. The severity of periodontitis was assessed clinically and radiographically. Gingival crevicular fluid was sampled and levels of eight cytokines were determined using a multiplexed bead immunoassay. The association between radiation-induced oral mucositis and periodontitis was analysed using logistic and linear regression, and two-way contingency tables.

Results: The mean age of the whole study population was 63.3 ± 11.0 years (range 44.8 to 82.9 years). The majority of patients were male (73%). The primary tumour site was most commonly the oral cavity and salivary gland (45%), followed by the pharynx (33%) and larynx and others (21%). The duration of radiotherapy was significantly associated with the severity of oral mucositis (p-value=0.038). A trend towards increased pocket depth and clinical attachment levels was noted in patients with oral mucositis grades 1-4, but this was not statistically significant.

Conclusion: Patients seeking dental clearance prior to head and neck radiotherapy at the Special Needs Unit, Adelaide Dental Hospital, were a good representation of the general head and neck cancer population. The resultant lack of association between radiation-induced oral mucositis and periodontitis was attributed to the extraction of teeth prior to periodontal examination, lack of uniformity of cancer treatment regimens and lack of statistical power. Hence, larger studies with a tighter inclusion criteria (e.g. similar radiotherapy protocol, without chemotherapy or surgery) are now required to follow-up on these preliminary findings.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

_____

Dr Arlene KHAW Bee Hong

January 2014

Acknowledgements

This thesis has benefited greatly from the support of many people, some of whom I would like to sincerely thank here.

Foremost, I wish to express my gratitude to my course supervisors, Professor Mark Bartold and Dr. Bryon Kardachi, for accepting me into the programme, and for their invaluable mentorship over the past three years.

My research supervisors, Professor Mark Bartold, Professor Dorothy Keefe, Professor Richard Logan and Dr. Sharon Liberali, for their continuing guidance and support.

Dr. Sharon Liberali, specialist in Special Needs Dentistry, for helping me with data collection despite her numerous commitments. This study would not have been possible without her.

Dr. Elizabeth Coates, Dr. Bronwyn Scopacasa and staff members at the John Goodhart Clinic, Adelaide Dental Hospital, for accommodating and assisting me while I recruited patients at the clinic.

Ms. Ceilidh Marchant, from the School of Dentistry, University of Adelaide, for her laboratory guidance, assistance and friendship.

Dr. David Sharkey, from the Robinson Institute & Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, University of Adelaide, for his assistance with the Luminex 200 bead analyser and the Xponent version 3.1 software program.

Ms Suzanne Edwards, Mr. Thomas Sullivan and Ms. Nancy Briggs, from the Data Management & Analysis Centre, Discipline of Public Health, University of Adelaide, for their statistical support and friendship.

All forty-one patients who consented to participate in this study with the intention of helping others, despite knowing that there would be no immediate benefit to them.

The Australian Periodontology Research Foundation Student Grants for their support.

The Ministry of Health Malaysia for their generous sponsorship.

My immediate family in Malaysia, for their love, support and encouragement; and my extended family in Australia, for giving me a home away from home.

Finally, I would like to thank my husband and best friend, Dr. Tan Wee Han. His determination to pursue his speciality of interest has greatly influenced me in persuing mine. This journey has been more memorable being shared with him.

Chapter 1: Literature review

Arlene Khaw[1,2], Richard Logan[1], Dorothy Keefe[3], P. Mark Bartold[1,2]

[1] School of Dentistry, Faculty of Health Sciences, University of Adelaide

[2] Colgate Australian Clinical Dental Research Centre, University of Adelaide

[3] School of Medicine, Faculty of Health Sciences, University of Adelaide

1.1 Introduction

In 2013, approximately 124,910 Australians are expected to be diagnosed with cancer. An estimated 149,990 are expected to be diagnosed in 2020 (Australian Institute of Health and Welfare, 2012). Head and neck cancers present with a 5-year relative survival of 82.4% when diagnosed at an early stage. This 5-year relative survival drops to 33.2% when the cancer has metastasised to distant sites (Howlader et al., 2011). As extensive surgical treatment may be disfiguring, there has been substantial research into non-surgical treatment options such as radiotherapy (Furness et al., 2011). Radiotherapy can be used either as a monotherapy, an adjuvant to surgery, or in combination with chemotherapy to treat cancers (Glenny et al., 2010, Fanucchi et al., 2006, Calvo et al., 2006, Connell and Hellman, 2009).

Virtually all patients who receive head and neck radiotherapy develop some degree of oral mucositis (Sonis, 2011). Most patients consistently report oral mucositis as the most debilitating acute side effect of radiotherapy that they experience. Severe oral mucositis may necessitate an interruption in the course of radiotherapy, and can thus serve as a dose-limiting factor (Denham et al., 1999, Sonis et al., 1999, Vera-Llonch et al., 2006, Rosenthal, 2007). The pathogenesis of radiation-induced oral mucositis involves the production of a range of inflammatory molecules and proteins such as interleukin (IL)-1β, IL-6 and tumour-necrosis factor, that lead to apoptosis and tissue injury (Sonis, 2002). These factors are not only damaging, but also provide a positive feedback loop that drives the destructive process forward.

Periodontitis is a host-driven inflammatory response to a pathogenic bacterial biofilm in the subgingival environment, resulting in the progressive destruction of the tissues that support the teeth, specifically the gingiva, periodontal ligament, and alveolar bone (Kornman, 2008). It affects more than 50% of the population (Roberts-Thomson, 2007, Hugoson et al., 2008, Eke et al., 2012). Given its high prevalence, periodontitis can be considered an

important global health problem in terms of quality of life. Despite the localised nature of periodontitis, a plethora of systemic inflammatory markers associated with this disease, such as C-reactive protein (CRP), IL-6, IL-1β and TNF, have been reported and it is speculated that these contribute to systemic diseases such as cardiovascular disease, diabetes mellitus and rheumatoid arthritis (Loos, 2005, Madianos et al., 2010).

Considering that radiation-induced oral mucositis and periodontitis are both characterised by the continuing presence of systemic inflammation, they may be associated through a primed inflammatory response as proposed by the "two-hit" model (Golub et al., 2006). Alternatively, both conditions may be correlated as they represent a dysregulation of the inflammatory response (Sonis et al., 2004b, Bartold et al., 2010).

1.2 Head and neck cancers

Head and neck cancers are a related group of cancers that involve the oral cavity, pharynx and larynx (IARC (WHO), 2011). For the purpose of standardisation, the International Classification of Diseases and Related Health Problems 10th Revision (ICD-10) has been introduced by the World Health Organization (WHO) to code various diseases including head and neck cancers (Table 1) (WHO, 2012).

Code	Subtype of head and neck cancers
C00	Malignant neoplasm of lip
C01	Malignant neoplasm of base of tongue
C02	Malignant neoplasm of other and unspecified parts of tongue
C03	Malignant neoplasm of gum
C04	Malignant neoplasm of floor of mouth
C05	Malignant neoplasm of palate
C06	Malignant neoplasm of other and unspecified parts of mouth
C07	Malignant neoplasm of parotid gland
C08	Malignant neoplasm of other and unspecified major salivary glands
C09	Malignant neoplasm of tonsil
C10	Malignant neoplasm of oropharynx
C11	Malignant neoplasm of nasopharynx
C12	Malignant neoplasm of piriform sinus
C13	Malignant neoplasm of hypopharynx
C14	Malignant neoplasm of other and ill-defined sites in the lip, oral cavity and pharynx
C32	Malignant neoplasm of larynx

Table 1: Coding of various head and neck cancers according to the International Classification of Diseases and Related Health Problems 10th Revision (ICD-10)

Apart from their anatomical proximity, these cancers have been grouped together for several other reasons. Firstly, they frequently share the same histologic manifestation – more than 90% are squamous cell carcinoma (Sugerman and Savage, 2002, Ramos et al., 2010). Other less common histological variations include malignant melanoma, adenocarcinoma and Kaposi's sarcoma (Sugerman and Savage, 2002). Secondly, head and neck cancers share similar aetiologic factors – at least 75% of head and neck cancers are associated with tobacco and alcohol use (IARC (WHO), 2011). The human papillomavirus (HPV), especially type 16, has also been implicated, particularly in young male patients who are neither smokers nor alcohol users (La Vecchia et al., 1997, Lindel et al., 2001, Mork et al., 2001, Hansson et al., 2005, Hammarstedt et al., 2006, Sturgis and Cinciripini, 2007, D'Souza et al., 2007, Ryerson et al., 2008, Adelstein et al., 2009, Attner et al., 2010, Ramos et al., 2010, Psyrri et al., 2011). Thirdly, head and neck cancers are managed in a fairly similar manner – surgery, radiotherapy, chemotherapy, or their combination (Cancer Council Australia, 2011).

Nevertheless, the grouping of patients with various head and neck cancers into one single disease entity has been criticised (Adelstein et al., 2009) because they are generally diagnosed at different stages and therefore managed in different ways (Bessell et al., 2011). Generally, oral cancers present at an early stage and the primary treatment is surgery, radiotherapy, or both, while oropharyngeal cancers are likely to present at an advanced stage and these patients are more likely to require radiotherapy with or without chemotherapy.

1.2.1 Incidence and mortality

1.2.1.1 Worldwide

Head and neck cancer ranked seventh worldwide, both in terms of estimated new cases (633,000), and deaths (355,000), in 2008 (Table 2) (Ferlay et al., 2010). These estimates have risen by approximately 15% since 2000 (Parkin et al., 2001).

Cancer sites	Incidence	Mortality
Oral (including lip)	263,000	127,000
Pharyngeal	219,000	146,000
Laryngeal	151,000	82,000

Table 2: Estimates of worldwide burden of oral (including lip), pharyngeal and laryngeal cancer in 2008 (Ferlay et al., 2010)

1.2.1.2 Australia

The incidence and mortality of lip, tongue and laryngeal cancer in Australia are reported in the Australian Cancer Incidence and Mortality (ACIM) books (Table 3). Among these sites, lip cancer was associated with the highest incidence but lowest mortality, while laryngeal cancer was associated with the lowest incidence but highest mortality. These cancers also occurred more frequently in males than in females (Australian Institute of Health and Welfare, 2011).

Cancer sites	Incidence			Mortality		
	Male	Female	All	Male	Female	All
Lip	659	244	903	12	3	15
Tongue	401	199	600	134	51	185
Laryngeal	526	57	583	192	22	214

Table 3: Incidence and mortality of lip, tongue and laryngeal cancer in Australia in 2007 (Australian Institute of Health and Welfare, 2011)

The incidence of oropharyngeal cancer (including tonsillar and the base of tongue), which is potentially related to the human papilloma virus (HPV), has been increasing year to year and is more striking within recent birth cohorts (Hocking et al., 2011). Between 2000 and 2005, 1315 cases were reported compared to 757 cases between 1982 and 1987. Male pharyngeal cancer mortality in Australia has increased sharply from the early 1960s, and peaked in the late 1980s. Over this period, the increase was as high as 1.1% per year. Female pharyngeal cancer mortality on the other hand remained steady and well below that of the rate of males (Adair et al., 2011).

1.2.1.3 South Australia

Cancer incidence, mortality and case fatality (survival) in South Australia may be obtained from the South Australian Cancer Registry (SACR) (South Australian Cancer Registry, 2010).

Table 4 shows gender-specific rates and percentage of head and neck cancers for incidence and mortality, and the lifetime risk for incidence. The male to female ratio for both incidence and mortality were 2:1 and 3.5:1 respectively. The trend for total new cases and deaths has not changed greatly over the last thirty years (Figure 1). The data also shows that head and neck cancer more commonly affects an older population, with a steep rise in incidence after the age of 40 (Figure 2).

Gender	Incidence				Mortality		
	No.	Rate	%	Risk	No.	Rate	%
Males	106	12.3	2.1	1 in 115	53	6.1	2.8
Females	53	5.3	1.4	1 in 263	15	1.4	1.0

Table 4: Incidence and mortality rates per 100,000. Rates age-standardised to the Australian 2001 population (South Australian Cancer Registry, 2010)

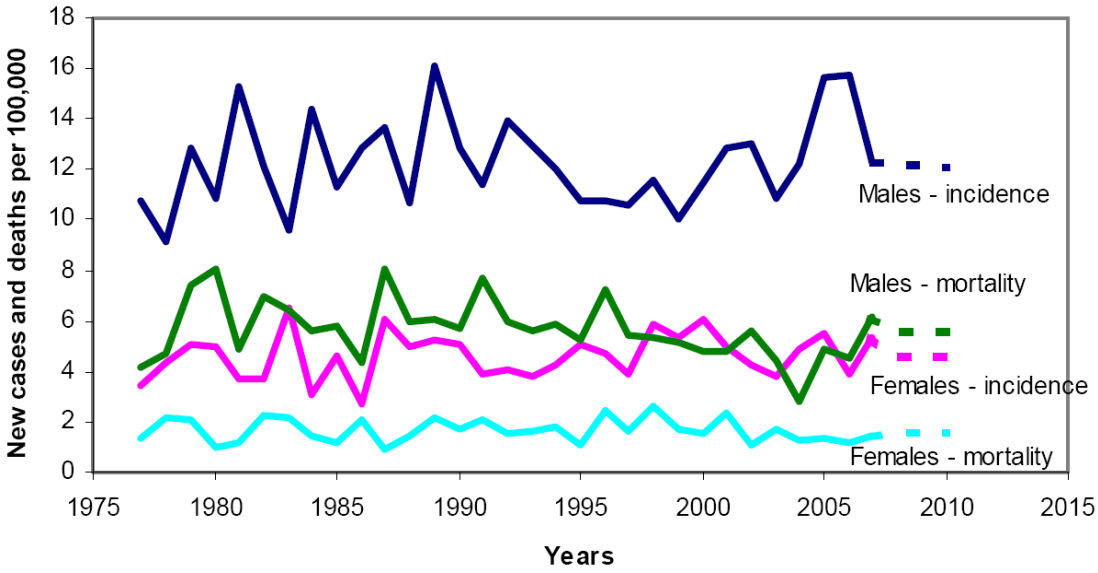


Figure 1: Total new cases and deaths for head and neck cancer for the period 1977-2007 (South Australian Cancer Registry, 2010)

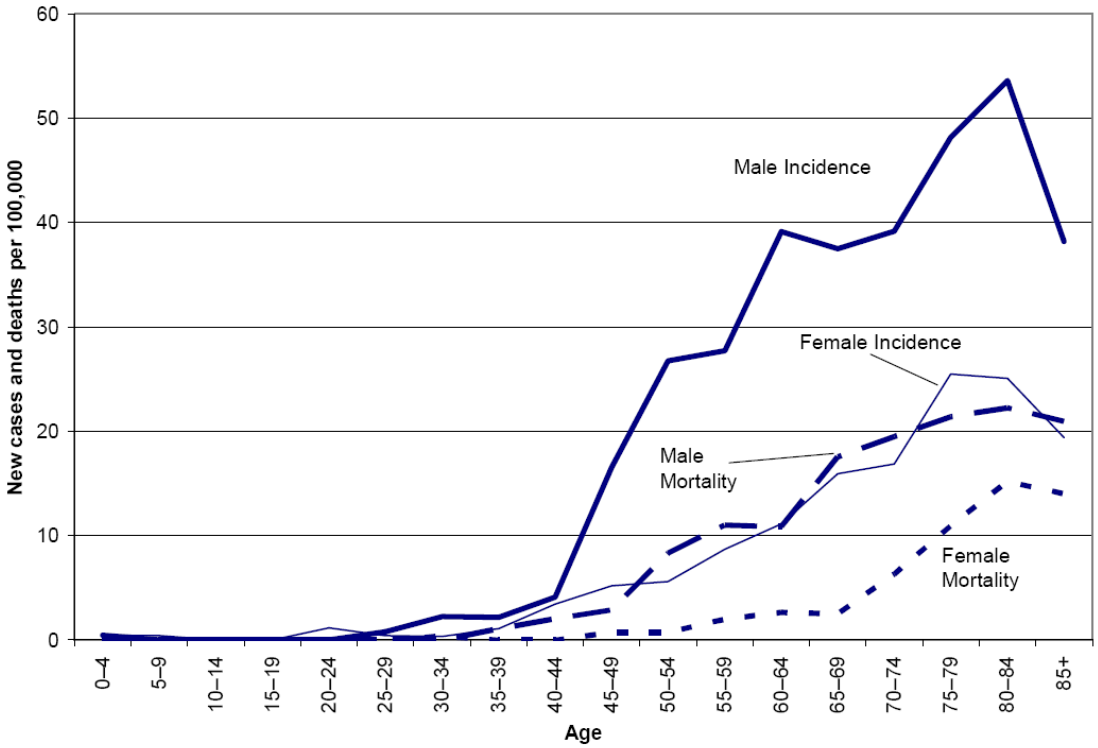


Figure 2: Head and neck cancer incidence and mortality by age group for the period 2001-2007 (South Australian Cancer Registry, 2010)

1.2.2 Risk factors

The incidence of lip cancer is high in Australia due to solar irradiation, particularly among Australian-born individuals (Parkin et al., 2005). It has been shown that some migrant groups, particularly from cultures where a lighter complexion is seen as desirable, or where concealing clothing is worn for religious reasons, continue to protect themselves by staying out of direct sunlight (Benson and Skull, 2007).

For intraoral cancer, the most commonly cited risk factors are tobacco smoking, excess alcohol consumption, use of smokeless tobacco, betel quid chewing, diets deficient in vitamin C, a history of precancerous conditions and genetic predispositions (Roder and Wilson, 1983, Sugerman and Savage, 2002, Adair et al., 2011).

Risk factors for oropharyngeal, hypopharyngeal and laryngeal cancers are tobacco smoking, excess alcohol consumption and dietary deficiencies, particularly in fruit and vegetables (Roder and Wilson, 1983, Adair et al., 2011). Changes in sexual behaviours have

recently been associated with an increasing incidence of HPV-associated oropharyngeal cancers (Hocking et al., 2011). If this is true, the future incidence of this cancer may be affected by the recent introduction of the HPV vaccination programme in Australia for females aged 12 to 13 years (Shefer et al., 2008).

For nasopharyngeal cancers, the risk factors include the consumption of salted fish within Chinese culture, exposure to Epstein-Barr virus and inhalation of carcinogens such as formaldehyde, smoke, fumes, wood dust and wood-treatment chemicals (Roder and Wilson, 1983, Wong et al., 2007, Adair et al., 2011).

1.2.3 Management

The management of head and neck cancers remains a two-fold challenge: to cure and to preserve vital functions such as speech and swallowing (Fayette, 2011). The choice of treatment is highly dependent on the stage of the disease (Bessell et al., 2011). Surgery alone may be sufficient for early stage disease, but advanced stage disease may require radiotherapy (Glenny et al., 2010) with or without chemotherapy (Furness et al., 2011). More recently, the role of immunotherapy has been under investigation (De Costa and Young, 2011).

1.2.3.1 Surgery

Surgical treatment of the primary tumour includes traditional scalpel-based surgery, laser cutting or ablation, or harmonic scalpel (Bessell et al., 2011). Given that oral and oropharyngeal tumours frequently spread early to the cervical lymph nodes (Haddadin et al., 2000), surgical dissection of these lymph nodes may be undertaken as part of the management of the primary tumour (Robbins et al., 2002). Several clinical trials have found that elective neck dissection resulted in reduced locoregional recurrence as compared to therapeutic neck dissection (Fakih et al., 1989, Kligerman et al., 1994, Vandenbrouck et al., 1980, Yuen et al., 2009). Other clinical trials, which compared radical neck dissection with conservative neck

dissection found no difference in overall survival, disease free survival or recurrence (1998, Bier, 1994).

For laryngeal tumours, the well-accepted surgical treatment is partial laryngectomy. Both transcervical (Lefebvre, 2000) and transoral approaches (Zeitels et al., 2004) have proven to be successful, but the former remains more popular due to better tumour accessibility (Ceruse et al., 2011). However, the transoral approach is gradually regaining popularity due to the development of carbon dioxide lasers coupled with microscopes (Jako, 1972, Strong and Jako, 1972, Vaughan et al., 1978, Eckel and Thumfart, 1992) (Steiner, 1993) (Eckel, 1997) and the da Vinci robot (Ceruse et al., 2011).

Nevertheless, surgical treatment may be disfiguring. Patients who are unable to cope with altered appearance, speech, eating and drinking can become socially isolated, and this may result in a substantially reduced quality of life. As a consequence, there has been substantial research into non-surgical treatment options such as radiotherapy and chemotherapy (Furness et al., 2011).

1.2.3.2 Radiotherapy

Radiotherapy utilises high energy radiation to damage the deoxyribonucleic acid (DNA) of rapidly dividing cells so that the usual mechanisms of DNA repair (which are usually less effective in cancer cells compared to normal cells) fail and the cells die. Radiation affects cellular DNA through two main mechanisms, direct and indirect action (Hall, 2006). Direct action of radiation results from the interaction between radiation and DNA and consequently damage to chromosomes and to the mitotic apparatus of the cell. Indirect action involves the interaction of radiation with other atoms or molecules in the cell (particularly water) to produce free radicals. These free radicals, such as superoxide, hydrogen peroxide, and free hydroxyl radicals, are able to diffuse far enough to reach and damage critical DNA targets causing cell death.

Radiotherapy may be delivered via three methods (Glenny et al., 2010):

- External radiotherapy (also known as teletherapy)
- Internal radiotherapy (also known as internal radiotherapy, implant radiotherapy or brachytherapy)
- Systemic radiotherapy.

External radiotherapy is delivered by machine outside the body. Patients undergoing this therapy usually have to attend the clinic or hospital five days a week for several weeks. Internal radiotherapy involves the insertion of a radioactive material into the tissues near the cancer cells. These patients must stay at the hospital for the duration of the therapy. In systemic radiotherapy, a radioactive substance is administered either orally or parenterally to the patient (Glenny et al., 2010). The choice of radiotherapy prescribed by a radiation oncologist depends on several factors (National Cancer Institute, 2010):

- The type of cancer
- The size of the cancer
- The cancer's location in the body
- How close the cancer is to normal tissues that are sensitive to radiation
- How far within the body the radiation needs to travel
- The patient's general health and medical history
- Whether the patient will have other types of cancer treatment
- Other factors, such as the patient's age and other medical conditions.

Radiotherapy can be used as a monotherapy for small tumours or for patients who cannot undergo surgery (Glenny et al., 2010). When used as an adjuvant to surgery, post-operative radiotherapy has been shown to result in improved survival rates compared to pre-operative radiotherapy (Fanucchi et al., 2006). Radiotherapy can also be given during surgery

in cases where normal structures are too close to a tumour to allow the use of external radiotherapy (Calvo et al., 2006). In cases where the tumours are resistant to radiotherapy, chemotherapy can be added to enhance the action of radiotherapy (known as radiochemotherapy or chemoradiation) (Connell and Hellman, 2009).

1.2.3.2.1 Conventional fractionation

Conventional fractionation is based on experiments performed on rams in France in the 1920s and 1930s (Hall, 2006). It was discovered that rams could not be sterilised with a single dose of x-rays without extensive skin damage to the scrotum. However, if the radiation were delivered in daily fractions over a period of time, sterilisation was possible without skin damage. More than 80 years later, the rationale for fractionation can be explained by the 'four Rs" of radiobiology (Connell and Hellman, 2009, Hall, 2006):

- Repair of sublethal cellular damage (normal tissues are given time to repair themselves between dose fractions)
- Repopulation (healthy cells can migrate and repopulate areas damaged by radiation)
- Reassortment of cells within the cell cycle (tumour cells redistribute from a radio-resistant or late S phase to a radio-sensitive/G2-M phase)
- Reoxygenation (tumour cells reoxygenate and become more radio-sensitive).

Prolongation of treatment also has its benefits (Hall, 2006):

- Reduce risk of early reactions
- Allow better reoxygenation in tumours.

For the treatment of head and neck cancers, a typical conventional fractionation protocol consists of single daily fractions of 1.8 to 2.0 Gy, five days a week over 6½ to 7 weeks, giving a total dose of 60 to 70 Gy (Glenny et al., 2010).

1.2.3.2.2 Altered fractionation

Although the benefits of prolonging treatment is clear, excessive prolongation however has its disadvantages (Hall, 2006):

- Deceptively decreases acute reactions without sparing late injury
- Allows surviving tumour cells to proliferate during treatment.

With this increase in the understanding of the biology of normal tissue and tumour, the conventional fractionation protocol which uses one fraction per day has been altered to a protocol which uses multiple fractions per day (Connell and Hellman, 2009). There are two main types of altered fractionation (Glenny et al., 2010):

- Hyperfractionation
- Accelerated fractionation.

Hyperfractionation uses multiple, smaller daily doses (e.g. twice daily fractions of 1.1 to 1.2 Gy) over a similar or sometimes longer duration as conventional fractionation to give a higher total dose (e.g. a total dose of 74 to 80 Gy) (Glenny et al., 2010). The reduction of the dose per fraction is intended to reduce the risk of late toxicity (Bourhis et al., 2006b), while a higher total dose may provide better tumour control (Hall, 2006). In the 1990s, a large controlled clinical trial conducted by the European Cooperative Group (EORTC 22791) compared a hyperfractionation protocol which used 80.5 Gy/70 fractions/7 weeks (twice daily fractions of 1.15 Gy each) with the conventional fractionation protocol which used 70 Gy/35 fractions/7 weeks (single daily fractions of 2 Gy) in the treatment of head and neck cancer (Horiot et al., 1992). The results of this cooperative trial favoured the hyperfractionation protocol in several aspects:

- Local tumour control at 5 years increased from 40 to 59%, which also reflected in improved survival

- No increase in adverse effects.

The alternative strategy to hyperfractionation is accelerated fractionation. Accelerated fractionation uses the same total dose as conventional fractionation but in a reduced treatment time (e.g. less than 6 weeks) (Glenny et al., 2010). It is thought that by reducing the overall treatment time, the repopulation of tumour cells between sessions can be reduced, leading to improved locoregional control (Hall, 2006). The European Cooperative Group (EORTC 22851) conducted another large prospective clinical trial in the 1990s, this time comparing an accelerated fractionation protocol which used 72 Gy/45 fractions/5 weeks (1.6 Gy, three fractions per day) with the conventional fractionation protocol which used 70 Gy/35 fractions/7 weeks in the treatment of head and neck cancers, except oropharynx (Horiot et al., 1997). The results of this trial are summarised as follows:

- 15% increase in locoregional control but no survival benefit
- Increased acute effects (expected)
- Increase in late effects including lethal complications (unexpected).

Further variations in protocol have been attempted:

- Hyperfractionation/accelerated fractionation (Bourhis et al., 2006a, Dobrowsky and Naude, 2000, Marcial et al., 1987, Poulsen et al., 2001)
- Accelerated fractionation/boost (Ghoshal et al., 2008)
- Accelerated fractionation/split (Marcial et al., 1993)
- Hyperfractionation/accelerated fractionation/split (Bartelink et al., 2002, Horiot et al., 1997).

Today, altered fractionation has been considered to be superior to conventional fractionation in terms of locoregional control and survival benefit. Hyperfractionation may provide greater benefit than accelerated fractionation (Bourhis et al., 2006b, Glenny et al.,

2010). Despite its superiority, altered fractionation using multiple fractions per day was found to be associated with worse acute mucosal reactions than conventional fractionation using one fraction per day (Weissberg et al., 1983, Pinto et al., 1991, Horiot et al., 1992, Horiot et al., 1997, Bensadoun et al., 2001, Awwad et al., 2002, Zackrisson et al., 2003, Trotti et al., 2003). Severe oral mucositis may necessitate an interruption in the course of radiotherapy and thus can serve as a dose-limiting factor (Denham et al., 1999, Sonis, 1999, Sonis et al., 2004a, Vera-Llonch et al., 2006, Rosenthal, 2007).

1.2.3.2.3 Three-dimensional conformal radiotherapy (3-D CRT)

With the invention of computed tomography (CT) (Ambrose and Hounsfield, 1973, Hounsfield, 1973), three-dimensional planning became a possibility and this created a shift from 2-D to 3-D radiation delivery (Fanucchi et al., 2006). The three-dimensional conformal radiotherapy (3-D CRT) which is based on CT, delineates the targeted tumour and organs at risk to ensure dose coverage tightly conforms to the tumour (Thariat et al., 2011). In other words, this technique allows far more effective coverage of the tumour, while providing better protection of the adjacent normal tissues (Connell and Hellman, 2009).

1.2.3.2.4 Intensity-modulated radiation therapy (IMRT)

Unlike other types of radiotherapy, IMRT is planned in reverse (called inverse treatment planning) where the radiation oncologist selects the radiation doses to be administered to different areas of the tumour and surrounding tissue, then a high-powered computer program calculates the required number and angles of the radiation beam (Gaspar and Ding, 2008, Tribius and Bergelt, 2011). In contrast, traditional (forward) treatment planning involves advance selection of the number and angles of the radiation beam by the radiation oncologist, then computers calculate what dose will be delivered from each of the planned beams.

The goal of IMRT is to increase the radiation dose to the areas that need it and reduce radiation exposure to specific sensitive areas of surrounding normal tissue (Veldeman et al., 2008). In head and neck radiotherapy, IMRT has been associated with less damage to the salivary glands compared to full dose radiation (Chao et al., 2001, Saarilahti et al., 2006, Daly et al., 2007, Huang et al., 2008, Rusthoven et al., 2008, Chen et al., 2009, Jensen et al., 2010b, Jensen et al., 2010a, Marucci, 2011 #361).

1.2.3.2.5 Image-guided radiation therapy (IGRT)

Image-guided radiation therapy (IGRT) uses imaging scans in the treatment room that allow decisions related to dose adjustments, conformal sparing and non-uniform dose distributions to be made in real time. By allowing fractionation protocols to be revised accordingly, the accuracy of radiation treatment can be increased and the planned volume of tissue to be treated can be reduced, limiting the damaging effects of radiation on normal tissue (Noda et al., 2009).

1.2.3.3 Chemotherapy

Chemotherapy is the administration of cytotoxic drugs (most commonly intravenously, less commonly orally, or through intramuscular or intra-tumoural routes) to damage the deoxyribonucleic acid (DNA) of cancer cells so that they can no longer reproduce and subsequently die. Different types of chemotherapeutic agents interrupt different stages of the life cycle of cancer cells. Therefore, it is common to give two or more chemotherapy agents together (also known as combinational therapy) (Molin and Fayette, 2011, Furness et al., 2011). Over the past two decades, the standard chemotherapy regimen for oral cancer has been a combination of cisplatin and 5 fluorouracil (Specenier and Vermorken, 2007).

Clinical trials have shown that chemotherapy, in addition to locoregional treatment (either radiotherapy, surgery, or both) is associated with improved overall survival compared to locoregional treatment alone in patients with oral cavity and oropharyngeal cancers (Pignon

et al., 2000, Pignon et al., 2009, Furness et al., 2011). However, the timing of chemotherapy can vary (Pignon et al., 2009, Furness et al., 2011):

- Prior to surgery or radiotherapy (also known as induction chemotherapy)
- Concurrent radiotherapy (also known as concomitant or synchronous chemoradiotherapy)
- Following surgery or radiotherapy (also known as adjuvant chemotherapy)
- Induction chemotherapy followed by concurrent chemoradiotherapy (also known as sequential therapy).

For patients who are able to undergo surgery, induction chemotherapy (Richard et al., 1991, Paccagnella et al., 1994, Domenge et al., 2000, Licitra et al., 2003) and adjuvant concomitant chemoradiotherapy (Bernier et al., 2004, Cooper et al., 2004, Argiris et al., 2008, Tobias et al., 2010) can prolong survival up to 20% and 16% respectively. As for patients with unresectable tumours, the pooling of data from 26 clinical trials showed that concomitant or alternating chemoradiotherapy can prolong survival by 10 to 22% (Furness et al., 2011).

Chemotherapy is often associated with temporary but frequently severe adverse effects such as tiredness, anaemia, nausea/vomiting, diarrhoea or constipation, hair loss, mucositis and susceptibility to infections. These effects are the result of the chemotherapy agents targeting all dividing cells in the body including both cancer and normal cells (Furness et al., 2011). When chemotherapy is given concomitantly with radiotherapy, clinical trials have demonstrated an increased frequency in these severe acute adverse effects (Zackrisson et al., 2003).

1.2.3.4 Immunotherapy

In recent years, the use of immunotherapy to treat cancers has received much attention. Harnessing the immune system to treat malignant disease is a powerful tool, not only due to the specific nature of the immune response, but also due to the potential of establishing long-lasting tumour immunity via the capacity to exhibit memory.

Immunotherapy may also overtake surgery, radiotherapy and chemotherapy as the dominant and less toxic strategy for treating malignant disease in the future (Aldrich et al., 2010).

In head and neck cancers, certain tumour antigens are expressed selectively or in higher numbers on malignant tumours than on normal tissues (Gotte et al., 2002, Young et al., 2007). Immunotherapy hinges on the ability of the immune system to recognise these tumour antigens as foreign and develop a response, humoral and/or cellular, against the malignant tumour (De Costa and Young, 2011). Several immunotherapeutic approaches are available and a variety of agents have been developed (De Costa and Young, 2011):

- Cytokine therapy (e.g. interleukin-2, interferon-gamma, interleukin-12)
- Antibody therapy (e.g. anti-epidermal growth factor receptor/EGFR)
- Cellular therapy (e.g. adoptive T-cell immunotherapy, active vaccination therapy).

Immunotherapeutic agents can be administered via two modes (Pavitt et al., 2007):

- Locally (i.e. treatment is delivered to the affected area)
- Systemically (i.e. treatment is delivered to the entire body, often used in late stage cancers where metastasis has occurred).

The success of immunotherapy has been demonstrated in trials involving patients with solid tumours such as melanoma (Spitler et al., 2000, Maio et al., 2010), renal cell carcinoma (Schwaab et al., 2009) and colorectal carcinoma (Saltz et al., 2007). However, patients with head and neck cancer seem to respond poorly to immunotherapy because of the profound immune suppression that is induced by this type of cancer (Oddone et al., 2009). Two mechanisms have been proposed to explain this phenomenon (De Costa and Young, 2011):

- Production of soluble mediators that inhibit immune reactivity
- Induction of immune inhibitory cells.

It was then realised that the effectiveness of immunotherapy could be stimulated by alleviating the inhibitory environment established by the tumour, rather than attempting to use immune stimulatory strategies in an immune inhibitory environment. In a clinical trial involving twelve head and neck cancer patients, this strategy was found to be relatively safe and resulted in tumour regression, probably through the enlistment of macrophages and the activation of lymphocytes (Feinmesser et al., 2003). Larger clinical trials are warranted to confirm this, however.

1.3 Radiation-induced oral mucositis

Head and neck radiotherapy is associated with acute and chronic oral complications, affecting the oral mucosa, dentition, bone, adjacent salivary glands, as well as masticatory musculature and apparatus (Table 5) (Sciubba and Goldenberg, 2006, Kielbassa et al., 2006, Clarkson et al., 2007, Worthington et al., 2010). Acute complications develop during the early stages of radiotherapy and continue into the immediate post-treatment period (2–3 weeks), while chronic complications can manifest at any time thereafter, from weeks to years after treatment (Figure 3) (Sciubba and Goldenberg, 2006, Kielbassa et al., 2006).

Acute	Chronic
1. Altered taste sensation 2. Oral mucositis 3. Oral candidiasis 4. Salivary gland dysfunction: xerostomia	1. Mucosal fibrosis and atrophy 2. Salivary gland dysfunction: xerostomia 3. Taste dysfunction: dysgeusia, ageusia 4. Dental caries and radiation caries 5. Soft tissue necrosis 6. Osteoradionecrosis 7. Muscular fibrosis, cutaneous fibrosis, or trismus

Table 5: Acute and chronic oral complications of head and neck radiotherapy (modified from Sciubba and Goldenberg, 2006)

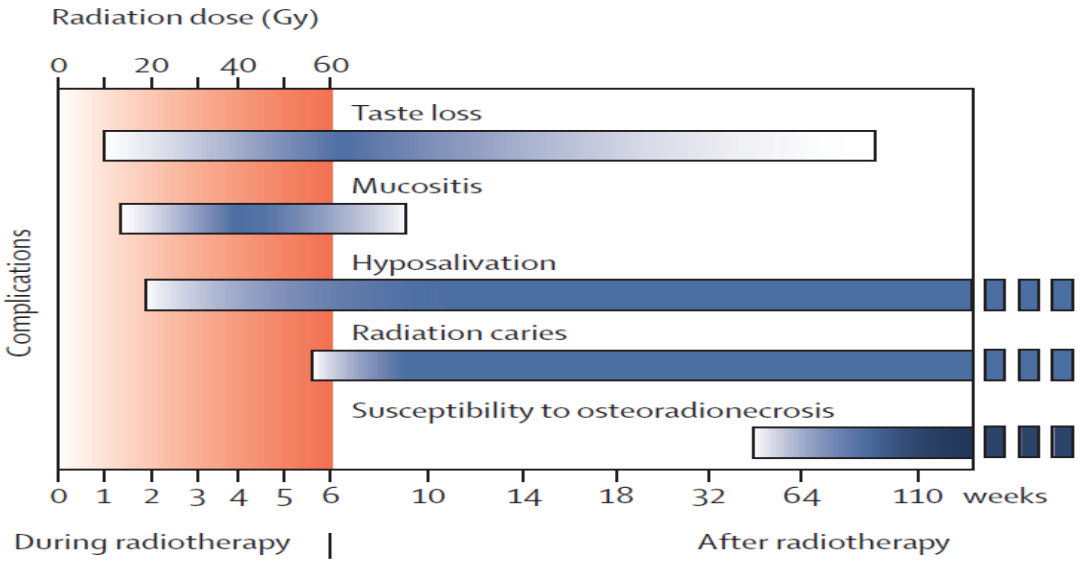


Figure 3: Schematic diagram of time, onset and duration of radiotherapy-induced oral complications (Kielbassa et al., 2006)

Acute complications range from merely uncomfortable to intensely painful. Although these effects generally resolve in time, they are highly significant because they can compromise the delivery of optimal cancer-therapy protocols and result in the need for treatment schedules to be modified so that oral lesions can resolve. In cases of severe oral morbidity, the patient might no longer be able to continue cancer therapy, after which treatment would have to be discontinued (Sciubba and Goldenberg, 2006).

Radiation-induced oral mucositis is defined as the reactive inflammation of the oral and oropharyngeal mucous membrane during head and neck radiotherapy (Vissink et al., 2003). Other than radiotherapy, oral mucositis is also a common acute complication following chemotherapy, chemoradiotherapy and haemopoietic stem cell transplant (Scully et al., 2006).

Virtually all patients who receive head and neck radiotherapy develop some degree of oral mucositis (Sonis, 2011). The mean incidence was found to be around 80% in patients receiving radiotherapy, and highest during altered fractionation radiotherapy (100%), followed by conventional radiotherapy (97%) and chemoradiotherapy (89%) (Trotti et al., 2003, Vera-Llonch et al., 2006).

The incidence of moderate and severe oral mucositis was reported as 63.8% (Vera-Llonch et al., 2006). Although the grading was based on the investigator's judgement rather than a standardised scoring system, these were performed by experienced clinicians (i.e. medical and radiation oncologists). The severity of oral mucositis was found to be dependent on the radiotherapy protocol used. Altered fractionation using multiple fractions per day was found to be associated with worse acute mucosal reactions than conventional fractionation using one fraction per day (Weissberg et al., 1983, Pinto et al., 1991, Horiot et al., 1992, Horiot et al., 1997, Bensadoun et al., 2001, Awwad et al., 2002, Zackrisson et al., 2003, Trotti et al., 2003). Mean cumulative radiation dosages >65 Gy were also found to be associated with severe oral mucositis (OR of 10.4; 95% CI, 2.9 –37.1)(Vera-Llonch et al., 2006). Apart from the radiotherapy protocol used, other factors such as nasopharyngeal tumour location (adjusted OR of 10.1; 95% CI, 2.1– 49.9), tumour location in the oral cavity (OR of 6.2; 95% CI, 2.3–16.8) and the receipt of concurrent chemotherapy (OR of 3.3; 95% CI, 1.4–8.0) were found to increase the risk of developing severe oral mucositis (Vera-Llonch et al., 2006).

1.3.1 Consequences

Radiation-induced oral mucositis has numerous consequences. In terms of the patient, oral mucositis causes severe pain (Wong et al., 2006) and dysphagia that can lead to anorexia, weight loss and weakness (Silverman, 2007). These problems are further complicated by associated altered taste sensation and xerostomia, which can lead to severe nutritional deficiencies (Vissink et al., 2003, Silverman, 2007). Severe cases often require artificial feeding which is very uncomfortable for the patient (Donaldson, 1977, Beumer et al., 1979b, Beumer et al., 1979a, Wood et al., 1989, Jansma et al., 1992b, Lees, 1999, Mekhail et al., 2001). Persistent inflammation and injury to the oral mucosa can also increase the likelihood of oral and systemic infections (Duncan and Grant, 2003) such as candidiasis (Clarkson et al., 2007, Worthington et al., 2010, Bensadoun et al., 2011). Some patients may even develop

depression (Silverman, 2007). Most patients report that oral mucositis is the most bothersome side effect of radiotherapy that they experience (Sonis, 2011).

In terms of cancer treatment, altered fractionation radiotherapy and chemoradiotherapy have been proven to be successful for the treatment of rapidly dividing tumours, but result in higher rates of acute toxicity, especially oral mucositis (Weissberg et al., 1983, Pinto et al., 1991, Horiot et al., 1992, Horiot et al., 1997, Bensadoun et al., 2001, Awwad et al., 2002, Zackrisson et al., 2003). Severe oral mucositis may necessitate an interruption of the course of radiotherapy and thus can serve as a dose-limiting factor (Denham et al., 1999, Sonis, 1999, Sonis et al., 2004a, Vera-Llonch et al., 2006, Rosenthal, 2007). It has been found that patients with oral mucositis were four-fold more likely to have had unplanned breaks in radiotherapy (Vera-Llonch et al., 2006). Such interruptions must be prevented as they may result in prolongation of treatment time and suboptimal cancer treatment (Fowler, 1986).

In terms of resource utilisation, oral mucositis increases the rate of hospitalisation, opioid use and the need for fluids and nutritional support (Elting et al., 2003, Vera-Llonch et al., 2006, Murphy, 2007). Vera- Llonch et al. (2006) found that patients with oral mucositis were more than three times as likely to have been hospitalised; and twice as likely to have received feeding tubes or total parenteral nutrition, or indwelling intravenous lines (Vera-Llonch et al., 2006). Elting et al. (2003) found the cost for treating patients without mucositis, with grade 1 or 2 oral mucositis and with grade 3 to 4 oral mucositis to be US$ 3,893, US$ 6,618 and US$ 9,458 respectively (Elting et al., 2003).

In light of the above, therapy designed to prevent the occurrence of oral mucositis or accelerate its resolution should result in a significant benefit to the patient, better cancer treatment as well as healthcare cost savings.

1.3.2 Clinical course

The clinical course of mucositis is relatively predictable in cancer patients undergoing head and neck radiotherapy using the conventional fractionation schedule. Concurrent chemotherapy using standard weekly or triweekly cisplatin seems to increase the severity of mucositis, but does not influence the course of the condition (Sonis, 2011).

As early as the end of the first week of treatment (cumulative radiation therapy dose of 10 Gy), adverse mucosal changes become apparent and patients complain of burning and intolerance of spicy foods (Scully et al., 2006, Sonis, 2007). At this stage, symptomatic relief is often achievable with topical palliative agents or non-steroidal anti-inflammatory drugs (NSAIDs) (Sonis, 2011).

By the end of the second week of treatment, early ulcerative changes are often seen and are accompanied by an increase in symptoms. Discomfort at this stage may require an increase in analgesic intensity and patients may have less tolerance of a regular diet (Sonis, 2011).

At cumulative radiation doses of 30 Gy or more (third week of treatment and beyond), diffuse mucosal ulceration is common, involving the movable mucosa of the cheeks, lips, ventral and lateral tongue, floor of the mouth, and soft palate (Stokman et al., 2006). The more heavily keratinised mucosa of the dorsal tongue, gingiva, or the hard palate may also be affected (Sonis, 2007). These ulcers may be associated with peripheral areas of erythema, and are often covered by a pseudomembrane consisting of fibrinous exudates and dead cells (Sonis, 2011). At this stage, mucositis is extremely painful and the patient may not be able to tolerate food orally (Wong et al., 2006). In severe cases, artificial feeding and treatment breaks may be required (Sonis, 2011).

For most patients, symptoms peak at 5–7 weeks, although occasionally patients will experience peak symptomatology after the completion of therapy (Murphy, 2007). These

ulcers are expected to resolve spontaneously 2–4 weeks after the completion of radiotherapy (Sonis, 2011). Chronic mucositis following radiotherapy is rare (Scully et al., 2006).

1.3.3 Diagnosis and scoring systems

Diagnosis of oral mucositis is often made clinically and based on the use of known stomatotoxic therapy (Vissink et al., 2003, Scully et al., 2006). The differential diagnoses of oral mucositis may include infections and graft-versus-host disease (Scully et al., 2006, Sciubba and Goldenberg, 2006). Viral infections can be distinguished clinically from mucositis in that they are typically localised, well-defined and involve keratinised mucosa of the hard palate, gingiva and dorsal tongue and their onset often coincides with fever. The diagnosis of viral infections can be confirmed by culture or exfoliative cytology (Scully et al., 2006). Graft-versus-host disease, on the other hand, is limited to patients who have received allogeneic haemopoietic stem cell transplants and develops following haematologic recovery, typically resulting in dramatic lichenoid-like oral lesions (Woo et al., 1997).

A number of scoring systems have been defined to assess the severity of oral mucositis, but no one scale is uniformly employed (Table 6) (Scully et al., 2006, Sonis et al., 1999). The major hurdle appears to be a lack of a definitive measurement method.

Some established guidelines are those proposed by the World Health Organization (WHO) in 1979 (Scully et al., 2006) and the National Cancer Institute (NCI)-Common Toxicity Criteria (CTC) Version 2, introduced in 1999 .

The WHO system (Scully et al., 2006) relies on a combination of clinician-based observations (erythema and ulceration) and functional outcomes (ability to eat) (Figure 4). Ulcerations are assessed simply as being absent or present, without size consideration. Ease of use is the strength of the WHO scale. A potential confounder of the WHO scale was the need for the evaluator to determine the patient's ability to eat. The evaluator, therefore had to be

certain that the subject's inability to eat was caused by mucositis and not by another cause such as nausea (Sonis, 2011).

The NCI-CTC version 2 system only relies on clinical-based observations (erythema and the extent of ulceration and pseudomembrane formation). It has been observed that the NCI-CTC version 2 scale tends to be lower than those reported using the WHO scale. For example, although a 1 cm ulcer of the soft palate would be scored as 2 on the NCI-CTC version 2 scale, it might be so symptomatic as to prevent the patient from eating normally and to limit intake of fluids (WHO 3) or even nothing at all orally (WHO 4) (Sonis, 2011). Therefore, an updated scale, the National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, was introduced in June 2010 using functional outcomes (ability to eat) to grade the severity of oral mucositis.

Clinical research scales such as the oral mucositis index (OMI) or the oral mucositis assessment scale (OMAS) (Sonis et al., 1999) tend to be sensitive, but due to their quantitative nature, impractical for routine clinical use. The Oral Mucositis Assessment Scale (OMAS) separates objective and subjective findings. Degrees of ulceration and redness measured at specific sites in the mouth are primary indicators of oral mucositis while oral pain, difficulty in swallowing, and the ability to eat are taken as secondary indicators. A single score is not produced from this scale, rather a combined score for ulceration and redness based on different locations in the mouth are used.

Scales used as nursing management tools tend to be comprehensively directed at describing overall oral health. As a result, they contain items related to dental hygiene, and dental and gingival health that may obscure the true level of mucosal damage (Scully et al., 2006).

Scales	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
WHO (Scully et al., 2006)	No changes	Soreness with erythema	Erythema, ulcers, can eat solids	Ulcers, only liquid diet	Alimentation not possible
NCI-CTC v2	No changes	Erythema	Patchy pseudomembranous reaction (patches generally ≤1.5cm in diameter)	Confluent pseudomembranous reaction (>1.5cm in diameter)	Necrosis or deep ulceration; may include bleeding not induced by minor trauma or abrasion
NCI-CTCAE v4.03	No changes	Asymptomatic or mild symptoms; intervention not indicated	Moderate pain; not interfering with oral intake; modified diet indicated	Severe pain; interfering with oral intake	Life-threatening consequences; urgent intervention indicated
OMAS ulceration/ erythema (Sonis et al., 1999)	Normal/ normal	Not severe/ < 1 square cm	Severe/ 1 – 3 square cm	NA/ >3 square cm	NA/ NA

(WHO, World Health Organization; NCI-CTC v2, National Cancer Institute-Common Toxicity Criteria version 2; NCI-CTCAE v4.03, National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.03; OMAS, Oral mucositis assessment scale)

Table 6: Oral mucositis scoring systems

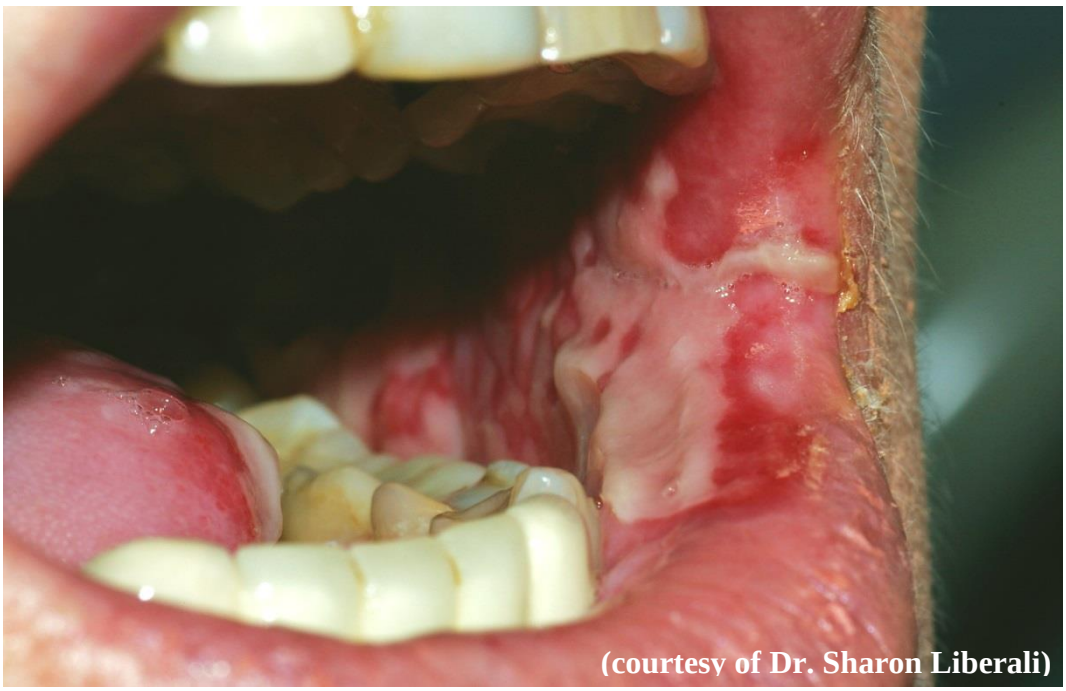


Figure 4: Clinical photograph showing grade 3 oral mucositis at the left buccal mucosa (according to the WHO system by Scully et al., 2006)

1.3.4 A multiple mechanism model of pathogenesis

It has become well accepted that oral mucositis develops as a consequence of a series of related and interacting biologic events occurring in the submucosa, culminating in injury and apoptosis of basal epithelial cells, in turn resulting in the loss of epithelial renewal, atrophy, and ulceration. As the initiating events occur almost immediately after the administration of radiotherapy, inhibition of the early molecular events may have a profound impact on the intensity of oral mucositis (Sonis et al., 2004a, Sonis, 2007, Silverman, 2007).

The following five-phase model describes the pathogenesis of oral mucositis (Figure 5, Table 7, Figure 6) (Sonis et al., 2004a, Sonis, 2007).

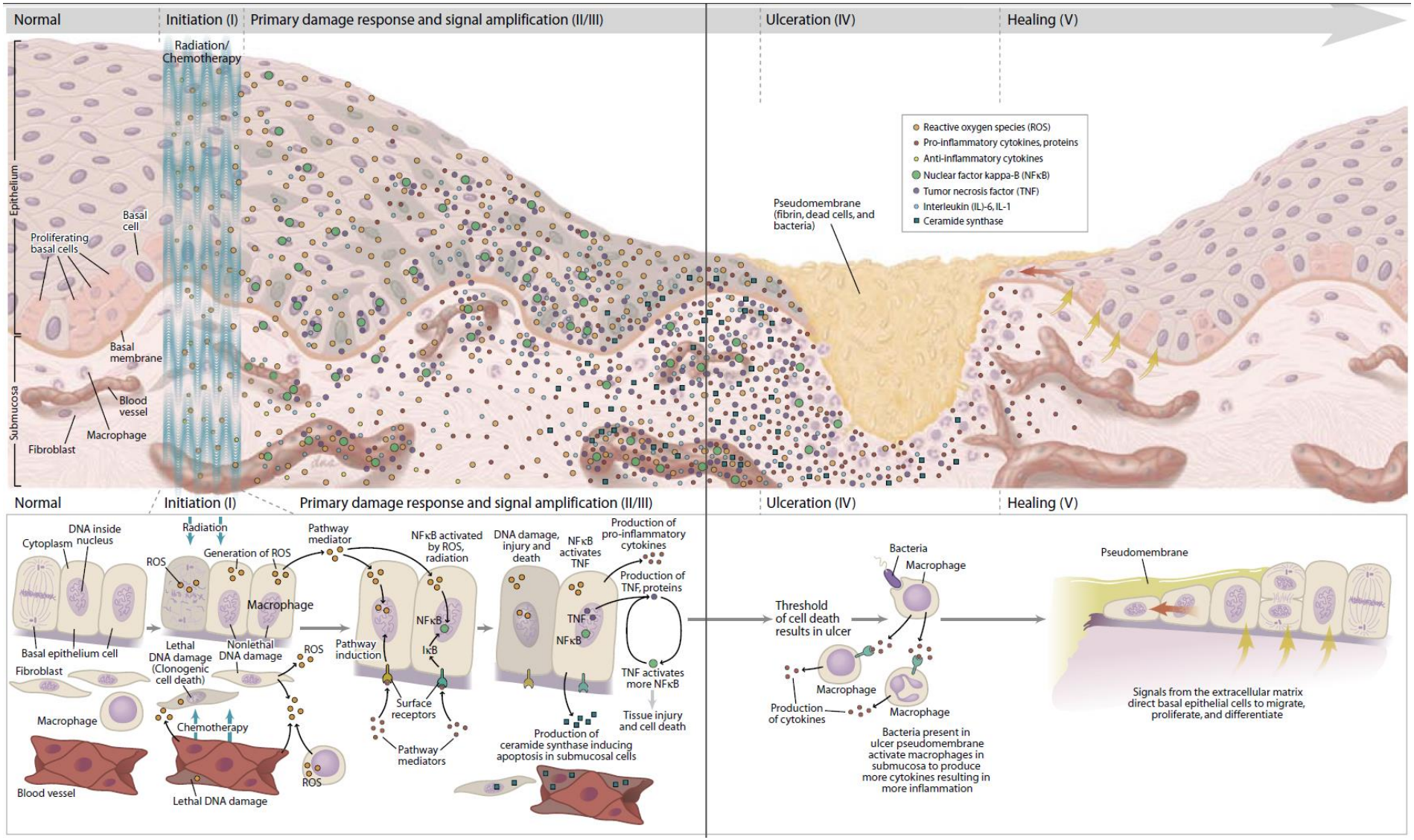


Figure 5: Multiple mechanism model of pathogenesis of mucositis (Sonis, 2007)

INITIATION	UPREGULATION AND MESSAGE GENERATION	SIGNALING AND AMPLIFICATION	ULCERATION	HEALING
X-rays or chemotherapy cause direct DNA damage.	The ceramide pathway signals cells to enter apoptosis.	NF-κB-activated COX-2 produces prostaglandins.	Submucosal cell death removes epithelial trophic factors such as KGF.	COX-2 activation promotes new angiogenesis.
X-rays or chemotherapy generate ROS.	Damaged cell membranes stimulate sphingo-myelinases.	TNF-α activates NF-κB and c-JUN.	MMPs degrade the ECM.	RM 2/3 macrophages downregulate inflamma-tory responses.
ROS damage lipids, DNA, connective tissue, and other biomolecules.	NF-κB modulates the pro-inflammatory cytokines.	Feedback loops re-initiate the damage response pathways.	The ECM swells with fluid, weakening the attach-ment between submucosa and epithelium.	Epithelial cells multiply and migrate to close the ulcerative wound.
Chemotherapy stimulates ceramide synthase.	NRF2 transcription factor activates antioxidant-related genes.	Damage responses are amplified in space and intensity.	Clonogenic cell death, reduced epithelial regeneration, and apop-tosis thin the epithelium.	Submucosal cells regenerate.
X-rays stimulate nuclear factor-kappaB (NF-κB).	NF-κB modulates apoptosis genes in the BCL family.	TNF-α stimulates apoptosis.	Opportunistic infections release bacterial cell wall components, which stimulate inflammatory responses.	Healing produces a new tissue that is not exactly the same as the old tissue.

Abbreviation: NF-κB = nuclear factor kappa-B; COX-2 = cyclooxygenase-2; KGF = keratinocyte growth factor; ROS = reactive oxygen species; TNF-α = tumor necrosis factor-alpha; MMPs = matrix metalloproteinases; ECM = extracellular matrix; NRF2 = nuclear factor erythroid-2 related factor 2

Table 7: Five-phase model describing the pathogenesis of oral mucositis (Sonis, 2007)

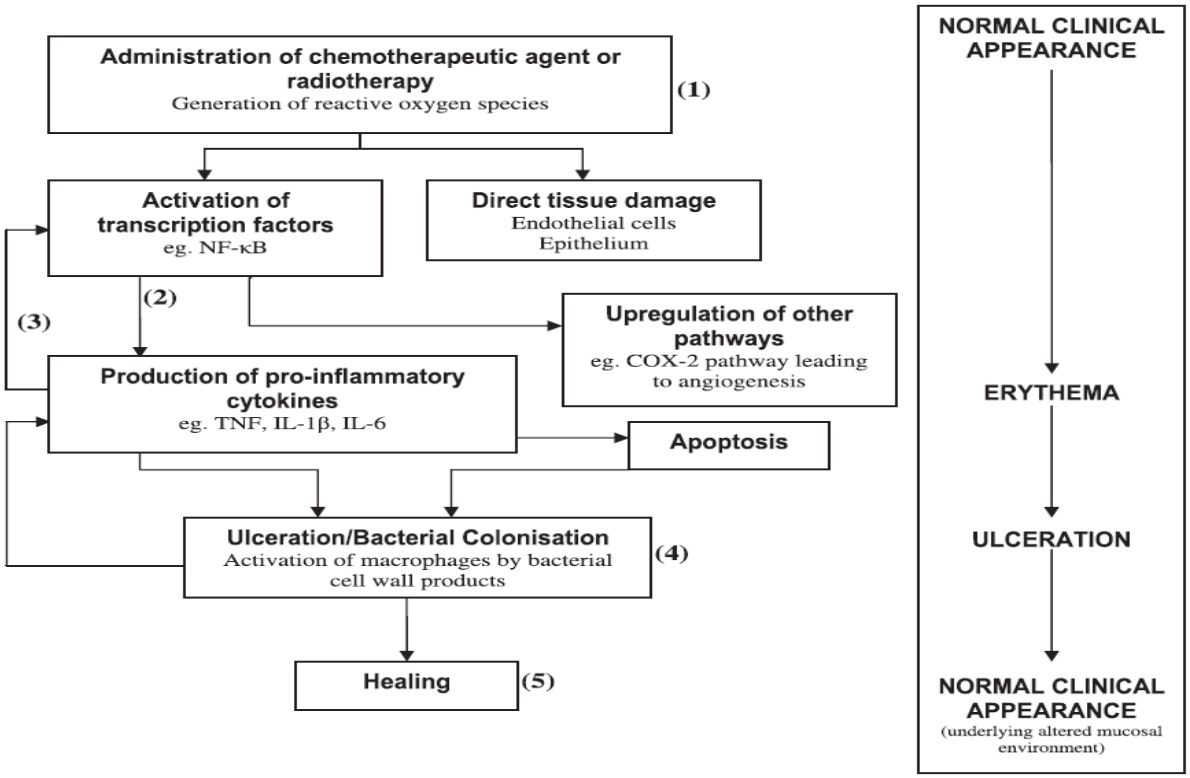


Figure 6: Diagram illustrating mucosal and clinical changes that occur leading to mucositis. The five overlapping stages are demonstrated (1) initiation; (2) upregulation and message generation; (3) signalling and amplification; (4) ulceration; (5) healing (Logan et al., 2007b)

1.3.4.1 Initiation (phase one)

Each dose of ionising radiation triggers events leading to oral mucositis (Denham and Hauer-Jensen, 2002). The first phase is termed initiation (Sonis et al., 2004a, Sonis, 2007).

The primary feature of this phase is the development of reactive oxygen species (ROS) and consequent oxidative stress (Sonis et al., 2004a, Sonis, 2007). Ionising radiation generates ROS which can activate a number of transcription factors of which nuclear factor kappa-B (NF-κB) is probably the best studied (Sonis, 2011).

The second component of the initiation phase involves the innate immune system of the host (Sonis, 2010). Cells made apoptotic or necrotic as a consequence of radiation may release endogenous radiation-associated pattern molecules that initiate a cascade of biological events similar to the danger-associated molecular pattern of the innate immune system. This involves the binding of these molecules to pattern recognition receptors which are located on epithelial and endothelial cells, and the activation of the transcription factor NF-κB that plays a role in mucosal injury.

1.3.4.2 Primary damage response (phase two)

The above events lead to the primary damage response which is the second phase (Sonis et al., 2004a, Sonis, 2007). This phase is extremely active despite preceding any visible clinical changes. It involves significant intercellular and intracellular signalling occurring in the connective tissue, endothelium and infiltrate of the submucosa. The consequences of this activity are directed toward the mucosal epithelium.

The activation of NF-κB leads to the upregulation of genes that result in the production of a range of destructive molecules and proteins that lead to apoptosis and tissue injury (Sonis, 2002). All of these culminate in apoptosis of cells of the basal epithelium. Factors that may be involved in the pathogenesis of oral mucositis include (Scully et al., 2006):

- Free radicals
    - Reactive oxygen species [ROS]
- Apoptosis, via activation of
    - Nuclear factor-κB (NF-κB)
    - AP1 family (c-FOS, c-JUN) and caspase 3
    - P53 tumour suppressor gene
    - NRF2 (NF-E2-related factor2)
    - BAX (pro-apoptotic protein)
    - Mitogen activated protein kinase: MAPK
    - Ceramide production from
        - Sphingomyelinase
        - Ceramide synthase
    - Nitric oxide [NO]
    - Cytokines
        - Interleukins (IL-1β, IL-6)
        - Tumour necrosis factor [TNF] (which also activates NF-κB, AP1, MAPK and matrix metalloproteinases [MMPs])
    - Cyclo-oxygenases [COX]
    - MMPs

1.3.4.3 Signal amplification (phase three)

The third phase, signal amplification, involves biological feedback (Sonis et al., 2004a, Sonis, 2007). The factors generated during the primary damage response are not only damaging but also provide a positive feedback loop (signal amplification) that drives the destructive process forward. For example, the pro-inflammatory cytokine, tumour necrosis factor (TNF), is a potent activator of NF-κB (Sonis, 2002), AP1, MAPK and matrix metalloproteinases [MMP](Scully et al., 2006). As a result, its presence can drive the NF-κB

and metalloproteinase pathways to produce and accelerate tissue injury by amplifying the original biological signals and magnifying the response of the initial injury. These loops also prolong damage by continuing to provide signals for days after the original irradiation insult (Sonis et al., 2004a, Sonis, 2007).

1.3.4.4 Ulceration (phase four)

In response to the apoptotic and necrotic chain of events elicited by radiotherapy, epithelial proliferation comes to a grinding halt. As a result, mucosal thinning begins, ultimately resulting in the fourth and most clinically significant phase, ulceration (Sonis et al., 2004a, Sonis, 2007). The ulceration that occurs transcends the entire epithelium and is usually covered by a pseudomembrane composed of dead cells and fibrin, a desirable environment for secondary bacterial colonisation. Both Gram-positive and Gram-negative organisms thrive within the pseudomembrane and may penetrate and invade the vessels of the submucosa to produce bacteremia, subsequently increasing the risk of sepsis. In addition, bacterial cell wall products can penetrate the disrupted mucosa and stimulate infiltrating macrophages, likely through innate immune system pathways, to produce additional pro-inflammatory cytokines and a release of additional destructive metalloproteinases. This phase is characterised by a robust inflammatory infiltrate containing macrophages, neutrophils and mast cells.

1.3.4.5 Healing (phase five)

During the final and probably least well-understood phase, healing occurs and is generally completed within 4 weeks after the final dose of radiation (Sonis et al., 2004a, Sonis, 2007).

Cyclooxygenase-2 (COX-2), expressed in fibroblasts and vascular endothelium, may play a role in the rebuilding of the submucosa as COX-2 potentiates angiogenesis, a hallmark of the end of the ulcerative phase (Sonis et al., 2004b). In addition, the extracellular matrix provides signals to the epithelium that impact its migration, proliferation, and differentiation.

Unfortunately, even after full replenishment of the epithelium, the structure of the reconstituted submucosa is not identical to its state prior to radiotherapy (Denham and Hauer-Jensen, 2002).

1.3.5 Risk factors

A patient's risk of developing radiation-induced oral mucositis is influenced by a number of factors. These factors can be grouped into two broad categories (Sonis, 2011):

- Treatment-related
- Patient-related.

1.3.5.1 Treatment-related factors

The aggressiveness of the focal tissue challenge in radiotherapy may be overwhelming enough to largely preclude other risk factors (Sonis, 2011). Important treatment-related factors include:

- Total cumulative dose of radiation exceeding 30 Gy directed to the mouth (Sonis, 2011)
- Radiotherapy to treat nasopharyngeal and oropharyngeal tumours (Vera-Llonch et al., 2006)
- Altered fractionation radiotherapy schedules (Weissberg et al., 1983, Pinto et al., 1991, Horiot et al., 1992, Horiot et al., 1997, Bensadoun et al., 2001, Awwad et al., 2002, Zackrisson et al., 2003, Trotti et al., 2003)
- Concomitant chemoradiotherapy (Zackrisson et al., 2003, Trotti et al., 2003, Vera-Llonch et al., 2006).

Despite the uniform risk of developing ulcerative mucositis, the time of onset and peak mucosal injury may be influenced by patient-related factors.

1.3.5.2 Patient-related factors

Several patient-related factors (Barasch and Peterson, 2003, Vera-Llonch et al., 2006) have been identified, but data supporting the significance of each factor is limited and uneven.

- Advanced age (sometimes found to confer a lower risk, possibly due to the lower intensity of treatment in these group of patients)
- Gender
- Body mass index
- Use of alcohol and tobacco
- Nutritional status
- Poor functional status
- Low leukocyte count
- Advanced disease and tumour stage
- Prior history of severe mucositis
- Various comorbid conditions
- Systemic inflammation (Pillsbury et al., 1986, Leborgne et al., 1998)
- Genetic polymorphism (Cho et al., 2010).

1.3.6 Evidence for the role of systemic inflammation

Nuclear factor-κB (NF-κB), pro-inflammatory cytokines, cyclo-oxygenases (COX) and matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of mucositis, following radiotherapy and/or chemotherapy (Scully et al., 2006).

These factors were found to be expressed at elevated levels in both serum and tissue following non-surgical cancer therapy. Using an established animal model (Dark Agouti rat), Logan et al. demonstrated the changes in expression of NF-κB, tumour necrosis factor (TNF), interleukin-1β (IL-1β), IL-6 in the mucosa (Logan et al., 2008a) and serum (Logan et al., 2008b) following chemotherapy. The pattern and timing of these changes were different

between tissue and serum, and were dependant on the type of mucotoxic drug administered (Logan et al., 2009). A human biopsy study by the same author found a statistically increased oral mucosal staining of NF-κB and COX-2 in 20 patients following cytotoxic chemotherapy (Logan et al., 2007a). Similar changes were also detected following radiotherapy. Local tissue levels of IL-1β and TNF have been shown to increase markedly in animal models concurrently with the development of radiation-induced mucositis (Sonis et al., 2000). Matrix metalloproteinases (MMPs) are also known to be up-regulated in mucositis and play a key role in tissue injury and inflammation. Using the same animal model (Dark Agouti rat), Al-Dasooqi et al. showed a significant alteration in both gene expression and tissue levels of MMPs and tissue inhibitor of metalloproteinase (TIMPs) following cytotoxic chemotherapy (Al-Dasooqi et al., 2010). The augmentation in the expression profiles of MMPs and their inhibitors was found to correlate with histopathological alterations observed in the tissue following chemotherapy.

To further support the role of systemic inflammation in the development of mucositis, studies have shown that therapy targeted to alter cytokine profiles were able to modify the course of mucositis. Animal (Lima et al., 2005) and human studies (Bianco et al., 1991, Ferra et al., 1997) demonstrated a decrease in the occurrence or severity of mucositis following the administration of TNF inhibitors.

1.3.7 Current prevention protocols

Two systematic reviews (Kowanko et al., 1998, Stokman et al., 2006) investigated the effectiveness of preventive protocols for radiation-induced oral mucositis.

Kowanko et al. (1998) found that there was insufficient evidence for most strategies reviewed, preventing them from drawing any conclusions regarding their effectiveness. Stockman et al. (2006) presented a figure showing possible pathways of intervention for prevention in relation to the 5-stage model of oral mucositis development (Figure 7) (Sonis et

al., 2004a). They performed a meta-analysis using 45 studies, in which eight different interventions were evaluated, and found four interventions showing a significant preventive effect on the development or severity of oral mucositis (Table 8). These were:

- Oral cooling using ice cubes (cryotherapy)
- Amifostine; a cytoprotective adjuvant used in cancer radiotherapy or chemotherapy
- Systemically-administered granulocyte macrophage-colony-stimulating factor/ granulocyte colony-stimulating factor (GM-CSF/G-CSF)
- Antibiotic and anti-fungal lozenges containing Polymyxin E, Tobramycine, and Amphotericin B (PTA).

However, these results should be interpreted with caution as studies included in their meta-analysis were fairly heterogeneous. For example, amifostine studies were based on variable doses of the drug and were used for various cancer types.

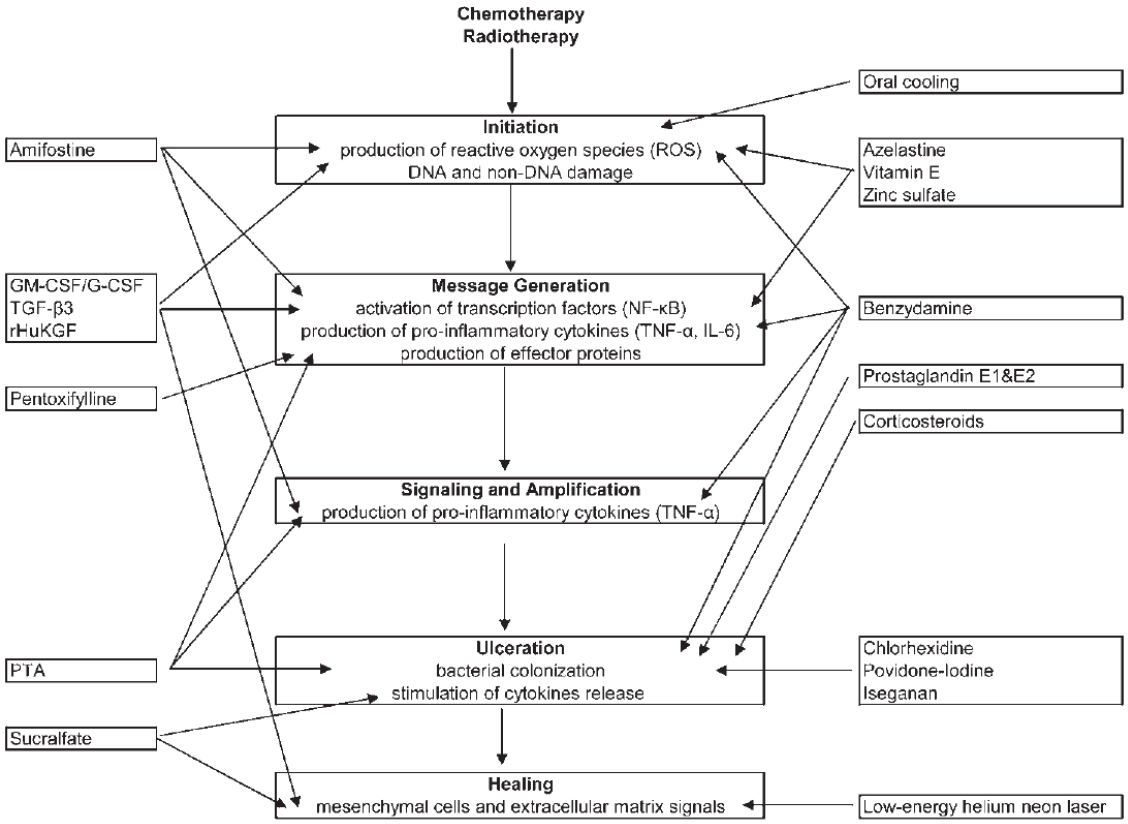


Figure 7: Possible pathways of intervention for oral mucositis prevention (Stokman et al., 2006)

A recently updated Cochrane review (Worthington et al., 2011), which included a total of 131 studies with 10,514 randomised participants, found two interventions (cryotherapy, keratinocyte growth factor[KGF]) which showed some benefit in preventing oral mucositis, and seven others which showed weaker evidence of benefit (Table 8). However, only 8% of these studies were assessed as being at low risk of bias.

Other than these reviews, clinical practice guidelines have been developed by the Mucositis Study Section of the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology for the prevention of radiation-induced oral mucositis (Table 8) (Rubenstein et al., 2004, Keefe et al., 2007). Their recommendations include:

- Oral care protocols (including the use of a soft toothbrush that is replaced on a regular basis)
- Use of midline radiation blocks and 3-dimensional radiotherapy
- Benzydamine hydrochloride, a locally-acting non-steroidal anti-inflammatory drug (for patients with head and neck cancer receiving moderate-dose radiotherapy).

It should be noted that no benzydamine hydrochloride ("Difflam") trials have been conducted since 2004. A study in the United States sponsored by McNeil had aimed to confirm the results of an earlier randomised, placebo-controlled American/Canadian multicentre trial (Epstein et al., 2001). However, the McNeil study was terminated early after the receipt of results from an interim analysis and the recommendations of the Data Monitoring Committee (Keefe et al., 2007).

In summary, current preventive protocols for radiation-induced oral mucositis are based on clinical experience or anecdotal evidence only. The main reason for this is the lack of well-designed and adequately powered randomised controlled trials (Worthington et al., 2011). As a result, great diversity exists in the preventive protocols practiced. Among these

trials, the majority (79%) were also found to be conducted primarily by medical teams and did not report the involvement of dental practitioners. Therefore recent clinical practice guidelines (McGuire et al., 2006, Keefe et al., 2007) have recommended the inclusion of dental professionals throughout active treatment and follow-up care of this group of patients.

Prevention strategies	Intervention	Evidence-based/ recommended	References
Non-pharmacological methods	Oral care protocols	Recommended	(Rubenstein et al., 2004, Keefe et al., 2007)
	Midline radiation blocks and 3D radiotherapy	Recommended	(Rubenstein et al., 2004, Keefe et al., 2007)
	Oral cooling using ice cubes (cryotherapy)	Preventive effect	(Stokman et al., 2006, Worthington et al., 2011)
	Laser	Weak preventive effect	(Worthington et al., 2011)
Radical scavengers	Amifostine	Preventive effect	(Stokman et al., 2006)
		Weak preventive effect	(Worthington et al., 2011)
Cytokines and/or growth factors	GM-CSF/G-CSF	Preventive effect (systemically-administered, not topical)	(Stokman et al., 2006)
		Weak preventive effect	(Worthington et al., 2011)
	KGF	Preventive effect	(Worthington et al., 2011)
Anti-inflammatory agents	Benzydamine	Recommended	(Rubenstein et al., 2004, Keefe et al., 2007)
Antiseptic and anti-microbial agents	PTA lozenges	Preventive effect	(Stokman et al., 2006)
		Weak preventive effect	(Worthington et al., 2011)
		Not recommended	(Keefe et al., 2007)
	Chlorhexidine	No preventive effect	(Stokman et al., 2006, Worthington et al., 2011)
		Not recommended	(Rubenstein et al., 2004, Keefe et al., 2007)
	Iseganan	No preventive effect	(Stokman et al., 2006)
Mouth-coating agents	Sucralfate	No preventive effect	(Stokman et al., 2006)
		Weak preventive effect	(Worthington et al., 2011)
Amino acids	Glutamine	No preventive effect	(Stokman et al., 2006)
		Weak preventive effect	(Worthington et al., 2011)
Herbal medications	Aloe vera	Weak preventive effect	(Worthington et al., 2011)
	Honey	Weak preventive effect	(Worthington et al., 2011)

Table 8: Evidence-based and recommended preventive strategies for radiation-induced oral mucositis (Stokman et al., 2006, Worthington et al., 2011, Rubenstein et al., 2004, Keefe et al., 2007)

1.3.7.1 Oral care protocols

Oral care protocols have been recommended for the prevention of radiation-induced oral mucositis (Kowanko et al., 1998, Rubenstein et al., 2004, Keefe et al., 2007) to achieve and maintain a clean oral cavity, and hence limit opportunistic infection to the damaged mucosa. It has also been recommended that caries and periodontitis be treated prior to commencing cancer therapy, and the patient should also be educated about implementing effective oral hygiene.

Two studies (Borowski et al., 1994, Shieh et al., 1997) compared an intense oral care protocol with routine care. The aim of both studies was to determine whether an intense oral care protocol would make a difference in the prevention of oral mucositis. Borowski et al. (1994) included 166 patients, both adults and children, undergoing bone marrow transplantation. Intensive oral care included an initial treatment of dental lesions and tooth and gum brushing during aplasia, while limited oral hygiene care excluded preventive dental treatment and tooth and gingival brushing. No evidence was found of a difference between groups with regard to the prevention of moderate and severe mucositis. However, Shieh et al. (1997) presented contrasting results. Thirty adults undergoing head and neck radiotherapy were included. The oral care protocol included mainly oral and written instructions in the Bass method of toothbrushing, the use of sterile water in mouth rinsing, and an emphasis on denture hygiene. A statistically significant difference was found favouring the oral care protocol in the prevention of any mucositis.

At present, there is a lack of consistent definition of which elements constitute basic oral care. Coupled with highly variable study designs, proper guidelines for basic oral care related to oral mucositis prevention have yet to be established (Rubenstein et al., 2004).

1.3.7.2 Anti-inflammatory drugs

Clarification of the complex biology of radiation-induced oral mucositis has resulted in the identification of numerous potential preventive strategies, one being the reduction of systemic inflammation during radiotherapy.

The concept that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the impact of radiation-induced mucositis has been suggested by Mennie and colleagues who treated radiation-induced gastrointestinal distress with oral aspirin in 28 women who were receiving treatment for uterine cancer (Mennie et al., 1975). Subsequently, Northway et al. reported dramatically milder radiation-induced esophagitis in opossums treated with indomethacin than the control animals (Northway et al., 1980). The benefits of using oral indomethacin to prevent oral mucositis in cancer patients undergoing head and neck radiotherapy was examined in one study by a group from the University of North Carolina (Pillsbury et al., 1986). In this study it was found that the onset of grade 3 mucositis was significantly delayed in the treatment group compared to the control group. This study was, however, under-powered due to the inclusion of only 20 patients.

Other than NSAIDs, oral corticosteroids have been used to prevent oral mucositis in cancer patients undergoing head and neck radiotherapy (Leborgne et al., 1998). Patients who received oral prednisolone throughout the duration of their radiotherapy experienced shorter treatment interruptions than the placebo group, but did not experience reduced severity or duration of oral mucositis.

These studies attempted to prevent radiation-induced oral mucositis by reducing systemic inflammation *during* radiotherapy. One study (Pillsbury et al., 1986) was under-powered, the other (Leborgne et al., 1998) showed modest benefits. Systemic inflammation should perhaps be managed *prior to* radiotherapy. One approach would be to treat existing inflammatory disease such as periodontitis before commencing radiotherapy.

1.4 Periodontitis

Periodontitis is well understood as a host-driven inflammatory response to a pathogenic bacterial biofilm in the subgingival environment, resulting in the progressive destruction of the tissues that support the teeth; specifically the gingiva, periodontal ligament, and alveolar bone (Kornman, 2008).

1.4.1 Prevalence

Not every individual is equally susceptible to the development of periodontitis (Lindhe et al., 1983, Löe et al., 1986, Papapanou et al., 1989, Albandar, 1990, Ismail et al., 1990, Hugoson et al., 2008, Eke et al., 2012). Severe periodontitis affects only 8.5-11% of the population, while 28-55.5% suffer from moderate disease (Table 9) (Hugoson et al., 2008, Eke et al., 2012). In Australia, moderate or severe periodontitis affects 61% of the population over 75 years of age (Table 9) (Roberts-Thomson, 2007). Given its high prevalence, periodontitis can be considered an important global health problem in terms of quality of life.

Country	Case definition	Findings
United States (Eke et al., 2012)	• Moderate: two sites between adjacent teeth with AL ≥4 mm, or two sites with PD ≥5 mm • Severe: two sites between adjacent teeth with AL ≥6 mm and at least one PD ≥5 mm	• ≥30 years: 38.5% with moderate or severe • ≥65 years: 64% with moderate or severe
Sweden (Hugoson and Norderyd, 2008)	• Moderate: radiographic bone loss around most teeth <1/3 of the length of the roots • Severe: radiographic bone loss around most teeth ranging between 1/3 and 2/3 of the length of the roots • Advanced: radiographic bone loss around most teeth > 2/3 of the length of the roots; presence of angular bony defects and/or furcation defects	• 20-70 years: 39% with moderate, severe or advanced
Australia (Roberts-Thomson, 2007)	• Moderate: two sites between adjacent teeth with AL ≥4 mm, or two sites with PD ≥5 mm • Severe: two sites between adjacent teeth with AL ≥6 mm and at least one PD ≥5 mm	• 35–54 years: 25% with moderate or severe • 55–74 years: 44% with moderate or severe • ≥75 years: 61% with moderate or severe

Table 9: Prevalence of periodontitis according to different case definitions in the United States, Sweden and Australia

1.4.2 Diagnosis

1.4.2.1 Clinical examination & radiographic imaging

Diagnosis of periodontitis is usually made clinically. It has been recommended that the severity of periodontitis be categorised on the basis of the amount of clinical attachment loss (Table 10) (Armitage, 1999). Clinical attachment loss is measured with a periodontal probe, and is the distance from the cemento-enamel junction to the base of the probeable crevice (Armitage, 1995).

Criteria	Mild	Moderate	Severe
Clinical attachment loss (Armitage, 1999)	1-2 mm	3-4 mm	≥5 mm
Radiographic marginal bone loss (Hugoson and Jordan, 1982)	< 1/3 root length	1/3 – 2/3 root length	>2/3 root length

Table 10: Measurements of the severity of periodontitis (Armitage, 1999, Hugoson and Jordan, 1982)

Nevertheless, it should be noted that probing depth measurements, while not used as the primary criterion for establishing the severity of periodontitis, should be recorded as part of a comprehensive periodontal examination (Figure 8) (Armitage, 2004b). Probing depth is measured with a periodontal probe, and is the distance from the gingival margin to the base of the probeable crevice. It is not used as a main criterion for severity because the gingival margin is not a fixed reference point from which to measure. The gingival margin can be considerably coronal to the cemento-enamel junction in cases where there is gingival enlargement or swelling. In cases of gingival recession, the gingival margin is apical to the cemento-enamel junction. Most importantly, the gingival margin can move coronally or apically over time and therefore is not a good reference point from which to assess longitudinal changes in clinical attachment. On the other hand, probing depth measurements provide useful information about the location and size of the principal habitat (i.e. periodontal pockets) of subgingival bacteria. Deep pockets are of concern because they are difficult to clean, both for the patient and therapist (Armitage, 1995). It is because of this that one of the goals of periodontal therapy is probing depth reduction (Heitz-Mayfield et al., 2002).

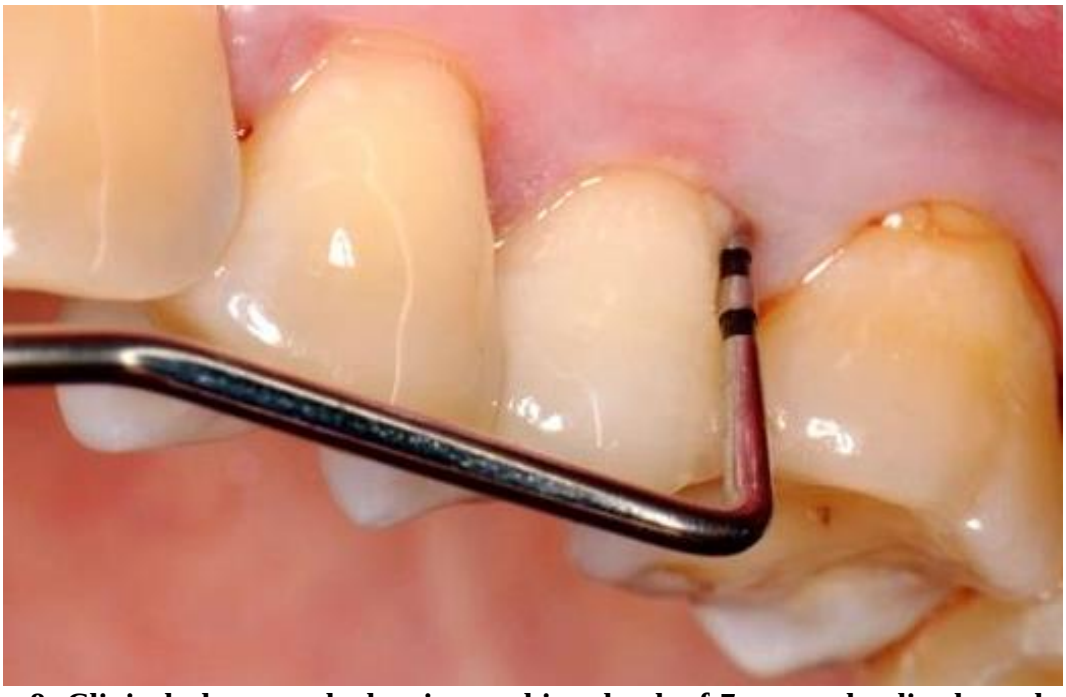


Figure 8: Clinical photograph showing probing depth of 7mm at the distobuccal aspect of a left maxillary premolar measured with a Michigan O probe with Williams markings

Bleeding on probing (BOP) is a widely used criterion to diagnose gingival inflammation. It is generally well-accepted that the inflamed gingiva bleed when gently probed because of the presence of minute ulcerations in the pocket epithelium and the fragility of the underlying vasculature (Page and Schroeder, 1976). Patients with a mean BOP of ≤ 20% have been shown to possess a significantly lower risk of disease progression (Joss et al., 1994).

Radiographic imaging can be useful in assessing alveolar bone support (Figure 9) (Mol, 2004, Brägger, 2005). Hugoson & Jordan (1982) proposed a classification of the severity of periodontitis based on the location of the alveolar crest in relation to root length as seen on intraoral radiographs (Table 10) (Hugoson and Jordan, 1982).

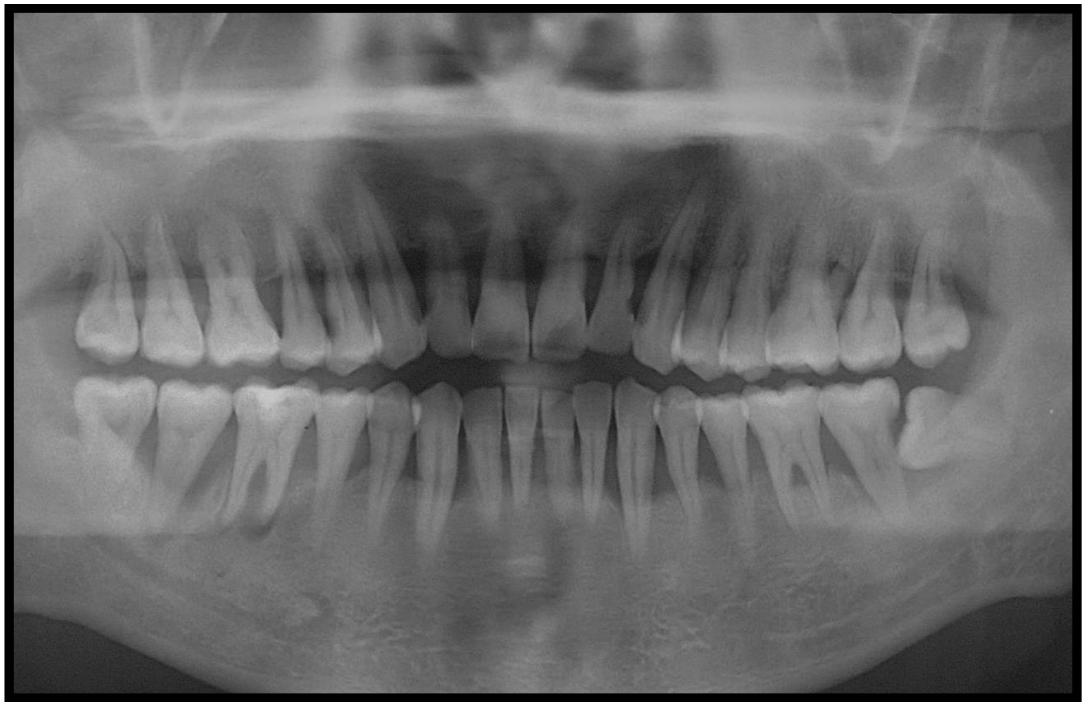


Figure 9: Panoramic radiograph demonstrating severe marginal bone loss

1.4.2.2 Biochemical analysis of gingival crevicular fluid (GCF)

Although clinical attachment loss and radiographic marginal bone loss are often used to establish the severity of periodontitis in a clinical setting as well as in research, they are measures of past periodontal tissue destruction and do not elucidate the current state of disease activity. To overcome these limitations, supplemental diagnostic tests have been

developed. One of these tests includes the biochemical analysis of gingival crevicular fluid (GCF).

GCF can be collected from the gingival sulcus surrounding the teeth, and exists as either a serum transudate or more commonly as an inflammatory exudate. The most commonly employed technique is by inserting an absorbent filter paper strip into the entrance of the gingival crevice until minimum resistance is felt (Figure 10) (Brill, 1959). A short collection time of thirty seconds is commonly used to decrease the probability of blood contamination and to prevent the collection of excessive volumes (>1.0µl) usually found in diseased sites, which cannot to be measured with the Periotron 6000®. However, the minimal detection limit (≥0.1µl) of the Periotron must also be reached which can be difficult in healthy sites sampled over short durations. Regardless, this technique is relatively simple and can be used to assay selected sites with minimal tissue trauma (Griffiths, 2003).

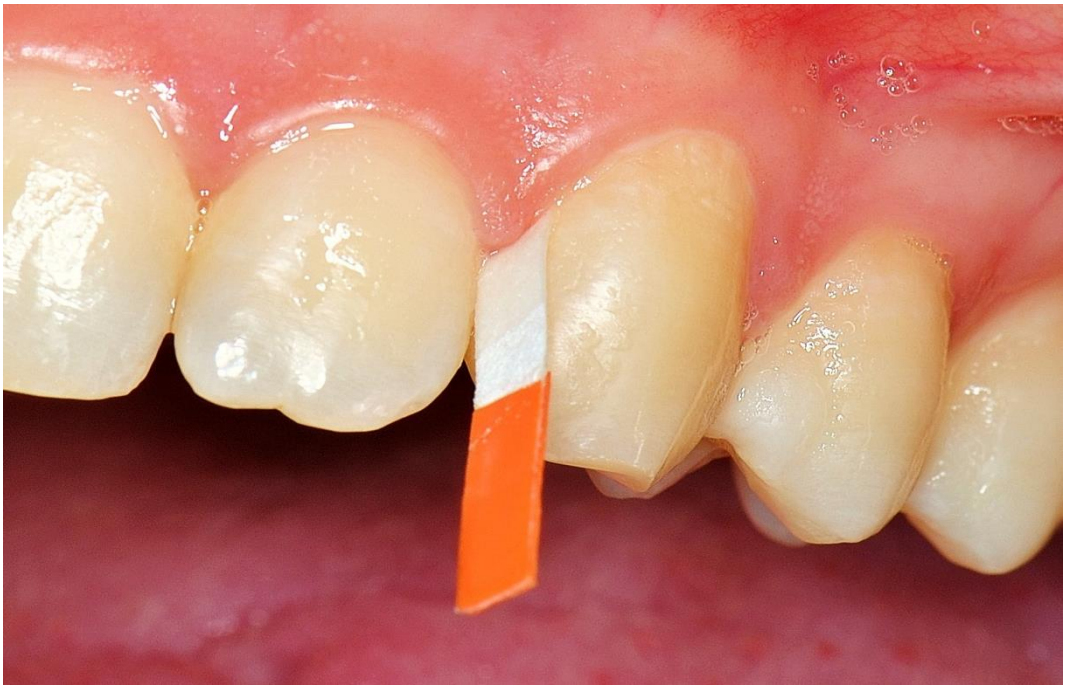


Figure 10: Clinical photograph showing gingival crevicular fluid collection using an absorbent filter paper strip

The amount of GCF produced at a given site was found to significantly increase with the severity of gingival inflammation (Egelberg, 1967). This occurs because of the increase in

vascular permeability and ulceration of the pocket epithelium at inflamed sites. Quantitative assessments of GCF volumes has been extensively used in research studies, but has no practical clinical application in the management of patients due to its wide variation.

More recently, GCF has been analysed qualitatively. Over 65 GCF components have been preliminarily examined as possible markers for the progression of periodontitis (Table 11) (Armitage, 2004a). These components fall into three general categories:

- Inflammatory mediators and host-response modifiers
- Host-derived enzymes and their inhibitors
- Tissue breakdown products.

Categories	GCF components
Inflammatory mediators and host-response modifiers	• Cytokines • RANTES • Prostaglandin E2 • Leukotriene B4 • Acute phase proteins (Lactoferrin, Transferrin, α2-macroglobulin α1-proteinase inhibitor, C-reactive protein) • Autoantibodies (Anti-desmosomal antibody) • Antibacterial antibodies (IgG, IgM, IgA) • Plasminogen activator/ PA • PA inhibitor-2/ PAI-2 • Substance P • Vasoactive intestinal peptide • Neurokinin A • Neopterin • Platelet-activating factor • CD14 • Cystatins • Calgranulin A/ MRP-8
Host-derived enzymes and their inhibitors	• Aspartate aminotransferase • Alkaline phosphatase • Acid phosphatase • β-glucuronidase • Elastase • Elastase inhibitors (α2-macroglobulin, α1-proteinase inhibitor) • Cathepsins (Cysteine proteinase, Serine proteinase, Cathepsin D) • Trypsin-like enzymes • Immunoglobulin-degrading enzymes • Glycosidases • Dipeptidyl peptidases • Non-specific neutral proteinases

	• Collagenases (Matrix metalloproteinases-1,3,8,13) • Gelatinases (Matrix metalloproteinases-2,9) • Tissue inhibitor of matrix metalloproteinase-1/ TIMP-1 • Stromelysins • Myeloperoxidases • Lactate dehydrogenase • Arylsulfatase • Creatinine kinase • β-N-acetyl-hexosaminidase
Tissue breakdown products	• Glycosaminoglycans (Hyaluronic acid, Chondroitin-4-sulfate, Chondroitin-6-sulfate, Dermatan sulfate) • Hydroxyproline • Connective tissue and bone proteins (Osteonectin, Osteocalcin, Type 1 collagen peptides, Osteopontin) • Laminin • Calprotectin • Hemoglobin β-chain peptides • Pyridinoline crosslinks/ ICTP

Table 11: GCF components examined as possible markers for the progression of periodontitis (Armitage, 2004a)

1.4.2.2.1 Cytokines

Cytokines are low-molecular-weight proteins involved in the initiation and effector stages of inflammation and immunity, in which they regulate the amplitude and duration of the response (Gemmell et al., 1997). These (are):

- Usually produced transiently, mainly but not exclusively by macrophages and T-cells
- Extremely potent; generally acting at picomolar concentrations and interact with specific cell surface receptors, which are usually expressed in relatively low numbers
- May target restricted or very diverse cells
- Pleiotropic; having multiple activities on different target cells and/or overlapping cell regulatory actions
- Interact in a network; first by inducing each other, second by transmodulating cell surface receptors and third by synergistic, additive or antagonistic interactions on cell function.

Certain cytokines have been proposed as potentially useful diagnostic or prognostic markers for periodontitis activity and wound healing. These include interleukin (IL)-1α, IL-

1β, IL-2, IL-4, IL-6, IL-8, tumour necrosis factor (TNF) and interferon (IFN)-γ, which have been shown to function in concert with other members of the cytokine network in order to regulate the cellular inflammatory response in the periodontium. Evidence demonstrating an association between periodontitis and eight cytokines detected in GCF samples is summarised in Table 12.

Cytokines	References	Study design	Study groups	Method of detection	Results
IL-1α	(Holzhausen et al., 2010)	Cross-sectional	40 moderate CP 40 advanced CP 40 healthy controls	ELISA	Significantly higher in CP than healthy controls, significantly higher in advanced CP than moderate CP
IL-1β	(Fitzsimmons et al., 2010)	Cross-sectional, population based	430 periodontitis, 509 healthy controls	ELISA	Higher levels in periodontitis, may indicate higher susceptibility
	(Rescala et al., 2010)	Cross-sectional	20 CP, 17 GAgP, 10 gingivitis	Multiplexed bead immunoassay (Luminex)	Significantly higher in deep sites from both periodontitis groups than gingivitis group and shallow sites
	(Teles et al., 2009)	Cross-sectional	20 CP, 20 healthy controls	ELISA	Higher mean levels in CP than healthy controls
	(Golub et al., 2008)	Controlled trial with 2 year follow-up	64 SDD, 64 placebo (CP, postmenopausal women)	ELISA	Significantly reduced in SDD group compared to placebo group, for subjects who had been post-menopausal for > 5 years
	(Kardesler et al., 2008)	Cross-sectional	17 DM + periodontal disease, 17 healthy + periodontal disease, 17 healthy controls	ELISA	Significantly higher in DM than healthy controls, significantly lower in DM than periodontal disease, levels not significantly influenced by DM alone
	(Toker et al., 2008)	Intervention (SRP)	15 GAgP	ELISA	Significantly higher at moderate and deep pocket sites than shallow sites, significantly reduced in moderate and deep pocket sites after SRP
	(Yoshinari et al., 2004)	Intervention (SRP)	7 CP	ELISA	Mean amount and concentration slightly increased during the month after SRP although clinical parameters improved, IL-1 is effective for evaluating in detail the state of subgingival inflammation
	(Oringer et al., 2002)	Controlled trial with 6 months follow-up	23 SRP + minocycline, 25 SRP + placebo (CP), 8 healthy controls	ELISA	Significantly lower in healthy controls than CP, significantly higher in deep sites than shallow sites in treated CP, significant reduction in SRP + minocycline group than CRP + placebo group, IL-1β correlated with clinical data and may aid in assessing disease status and response to periodontal therapy
IL-2	(Rescala et al., 2010)	Cross-sectional	20 CP, 17 GAgP, 10 gingivitis	Multiplexed bead immunoassay (Luminex)	Significantly higher in deep sites than shallow sites in GAgP group
IL-4	(Rescala et al., 2010)	Cross-sectional	20 CP, 17 GAgP, 10 gingivitis	Multiplexed bead immunoassay (Luminex)	Significantly higher in shallow sites of gingivitis group than shallow sites of periodontitis groups, indicate protective role

(Continued)

IL-6	(Holzhausen et al., 2010)	Cross-sectional	40 moderate CP 40 advanced CP 40 healthy controls	ELISA	Significantly higher in advanced CP than moderate CP and healthy controls
	(Lin et al., 2005)	Cross-sectional	14 patients (62 sites divided into 4 severity groups by PD, BOP)	ELISA	Total amounts but not concentrations correlated with disease severity
	(Kurtis et al., 1999)	Cross-sectional	24 CP, 24 healthy controls	ELISA	Higher in CP than healthy controls, but no correlation with clinical parameters
IL-8	(Holzhausen et al., 2010)	Cross-sectional	40 moderate CP, 40 advanced CP, 40 healthy controls	ELISA	Significantly higher in advanced CP than healthy controls
	(Rescala et al., 2010)	Cross-sectional	20 CP, 17 GAgP, 10 gingivitis	Multiplexed bead immunoassay (Luminex)	Detected in all samples tested, but no significant finding
	(Teles et al., 2009)	Cross-sectional	20 CP, 20 healthy controls	ELISA	Higher mean levels in CP than healthy controls
TNF	(Holzhausen et al., 2010)	Cross-sectional	40 moderate CP 40 advanced CP 40 healthy controls	ELISA	Significantly higher in CP than healthy controls
	(Ikezawa-Suzuki et al., 2008)	Intervention (SRP)	35 CP (12 smokers, 23 non-smokers)	ELISA	Levels increased slightly in smoking CP, while levels decreased in non-smoking CP after treatment, differences between baseline and after treatment insignificant
IFN-γ	(Rescala et al., 2010)	Cross-sectional	20 CP, 17 GAgP, 10 gingivitis	Multiplexed bead immunoassay (Luminex)	Detected in only 57% of the samples, no significant finding
	(Dutzan et al., 2009)	Cross-sectional	106 moderate to advanced CP	ELISA	Total amount and concentration significantly higher in progressive periodontal lesions

Table 12: Association between periodontitis and cytokines IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF and IFN-γ detected in GCF samples (CP, chronic periodontitis; ELISA, Enzyme-Linked Immuno-Sorbant Assay; GAgP, generalised aggressive periodontitis; SDD, subantimicrobial dose doxycycline; DM, diabetes mellitus; SRP, scaling and root planing; PD, probing depth; BOP, bleeding on probing)

The most widely used and best validated method to measure cytokine levels in GCF is Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Holzhausen et al., 2010, Fitzsimmons et al., 2010, Teles et al., 2009, Dutzan et al., 2009, Golub et al., 2008, Kardesler et al., 2008, Toker et al., 2008, Ikezawa-Suzuki et al., 2008, Lin et al., 2005, Yoshinari et al., 2004, Oringer et al., 2002, Kurtis et al., 1999). This approach is highly quantitative and generally reproducible, but is associated with several shortcomings (Leng et al., 2008):

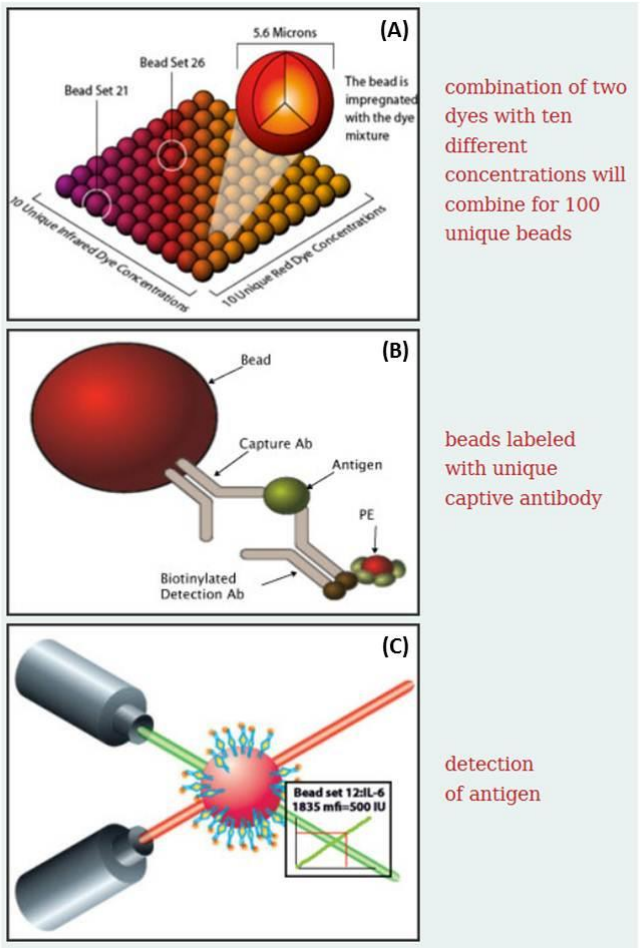
- Performance is largely dependent on antibody quality, kit manufacturer, as well as operator skill and experience
- Permits the measurement of only one cytokine at a time in a given sample
- The number of tests is limited by the volume of GCF obtained
- Comparisons between two cytokine levels measured by two different ELISA assays is difficult as both procedures are conducted under different conditions
- Narrow dynamic range (i.e. range over which there is a linear relationship between the cytokine concentration and the absorbance reading). Samples with cytokine concentrations above this range have to be diluted for the assay, making comparison with samples with cytokine levels within this range (that do not require dilution) difficult.

To overcome the shortcomings of ELISA, multiplex assays have been recently developed with the purpose of measuring multiple cytokines in the same sample at the same time. Bead-based multiplex assays which utilise the analysis platform Luminex xMAP (Milliplex) represent the most commonly used format presently (Lash and Pinto, 2010). The principle of Luminex bead technology is the use of polystyrene beads that have been impregnated with differing combinations of two different fluorescent dyes, creating a hundred bead set that can be discriminated by flow cytometry (Figure 11A). Each bead is labelled with a unique capture antibody and binding of the antibody can be quantified using detection antibodies which are also conjugated with a fluorescent tag with different properties to the

beads (Figure 11B). This assay is performed in a 96-well plate format, of which commonly 80 wells are available for sample analysis (16 wells are used for internal standards and controls). This plate is then placed in the Luminex xMAP (Milliplex) analyser containing two laser channels (Figure 11C); one to detect the different bead sets, defining the analyte that is being measured, and the other to detect the detection antibody, thereby quantifying the amount of each particular analyte. Subsequently, data analysis is performed using specialised software to generate various standard curves (Wallace et al., 2012).

Compared with traditional ELISA, multiplex assays have a number of advantages (Leng et al., 2008):

- High throughput multiplex analysis; therefore less labour-intensive and cheaper
- Require less sample volume
- Able to compare levels between different cytokines
- Able to perform repeated measures of the same cytokine panels in the same subjects under the same experimental assay condition
- Able to reliably detect different proteins across a broad dynamic range of concentrations.



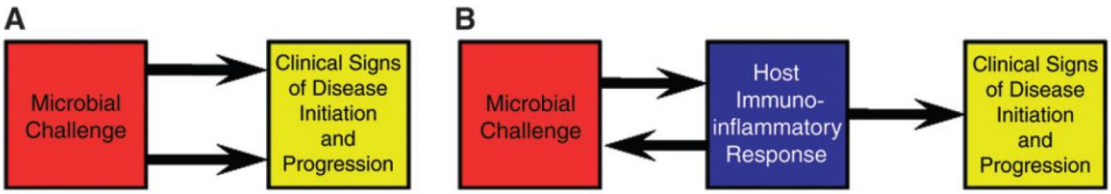
Figures 11A-C: Principles of Luminex bead technology (Wallace et al., 2012)

Full assay kits containing a predetermined set of analytes are available for purchase. Alternatively researchers can select individual bead/analyte sets that they can mix themselves. The ability to easily prepare custom-made assays has made this technique a very attractive tool for researchers, including those in the field of periodontics (Rescala et al., 2010).

1.4.3 Pathogenesis

In the mid-1960s human and animal experimental studies showed the critical role of bacteria in the initiation of gingivitis and periodontitis (Löe et al., 1965, Lindhe et al., 1973), leading to a simple concept of pathogenesis, i.e. bacteria cause periodontitis (Figure 12A). Extensive research through the mid-1980s led to critical refinements in the pathogenesis concept. One of the most important features is the distinction between the role of the microbial challenge and host immune-inflammatory responses (Figure 12B) (Page and Schroeder, 1976, Seymour et al., 1979, Page and Schroeder, 1981, Seymour et al., 1993). The

understanding was that bacteria initiated the disease process by activating host responses which could be both protective and destructive.



Figures 12A-B: Early models of pathogenesis of periodontitis. A) A linear model depicting the principal etiologic role for bacteria in the initiation and progression of periodontitis. B) Circa 1980s model emphasising a central role for the host immune-inflammatory response in the clinical development and progression of periodontitis (Kornman, 2008)

These early models were subsequently challenged when significant variations were observed in host responses and in the clinical expression of disease. In a classic study in beagle dogs (Lindhe et al., 1975), plaque accumulation was associated with a progression to periodontitis, but two of eight dogs failed to develop periodontitis despite substantial plaque and calculus accumulations and extensive gingivitis. Perhaps most striking were the published reports from longitudinal studies of Sri Lankan tea labourers who had limited access to dental care (Löe et al., 1986). Based on interproximal loss of attachment and tooth loss, three subpopulations were identified; rapid, moderate and no disease progression.

At the same time, evidence began to emerge showing that environmental and acquired risk factors such as smoking (Preber and Bergstrom, 1985, Preber and Bergstrom, 1986, Bergstrom, 1989) and diabetes (Belting et al., 1964, Glavind et al., 1979, Löe, 1993) were powerful determinants of periodontitis severity. Heavy smoking was associated with greater risk of attachment and alveolar bone loss than light smoking (Grossi et al., 1994, Grossi et al., 1995), while poorly controlled diabetes showed a significantly higher odds ratio of developing severe periodontitis than those with better-controlled diabetes (2.90 versus 1.56)

(Tsai et al., 2002). Extensive laboratory and clinical studies demonstrated that these risk factors were most likely influencing disease expression by altering host protective and destructive mechanisms (Zee, 2009, Mealey and Ocampo, 2007).

To add further complexity, there was a growing appreciation of the importance of genetic variations in determining the development and severity of periodontitis (Kornman et al., 1997). The profound influence of genotype on periodontitis susceptibility became apparent when twin studies showed that monozygotic twins were more similar in both the severity and extent of disease than dizygotic twins (Michalowicz et al., 1991a, Michalowicz et al., 1991b). It was estimated that adult periodontitis have approximately 50% heritability, unaltered following adjustments for behavioural variables including smoking (Michalowicz et al., 2000).

The acknowledgment of the role of multiple environmental and acquired risk factors, including genetics, as modifiers of the immune-inflammatory response and in resulting connective tissue and bone metabolism, lead to revision of the model of pathogenesis of periodontitis in 1997 (Figure 13) (Page et al., 1997).

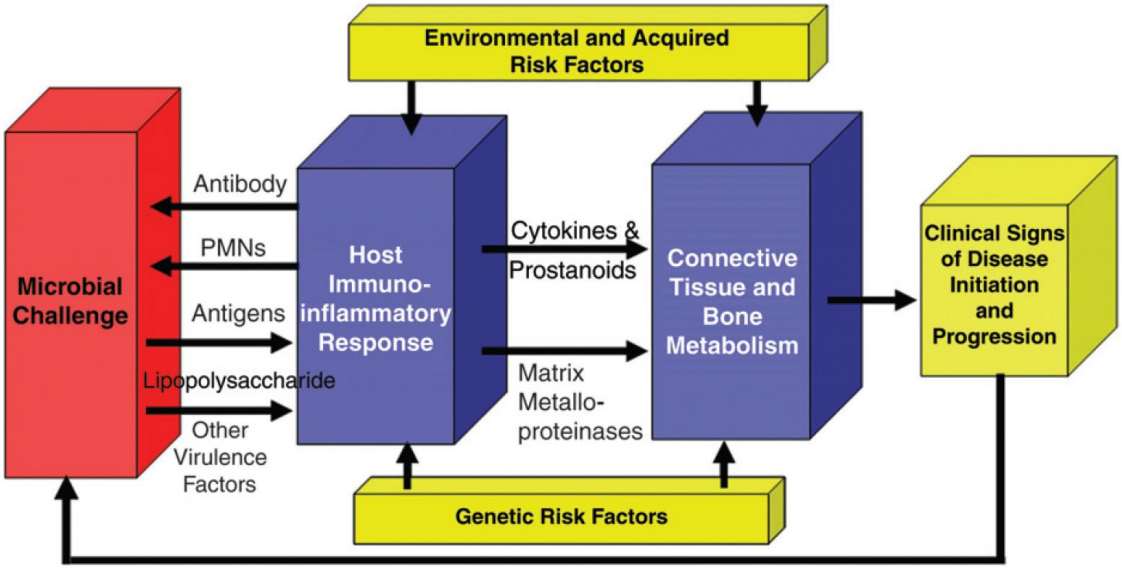


Figure 13: The 1997 model demonstrating various factors contributing to the pathogenesis of human periodontitis (Kornman, 2008)

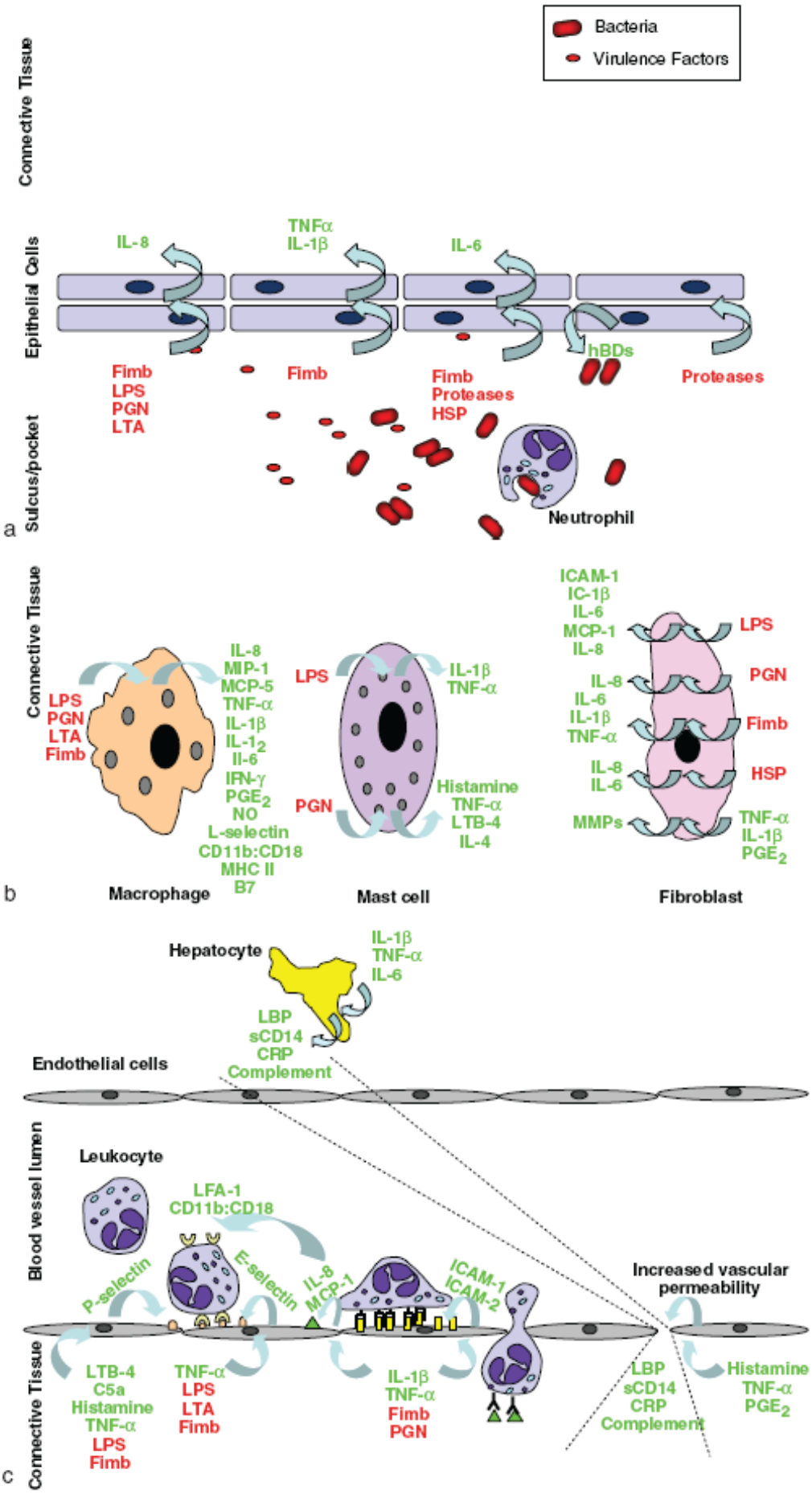
1.4.3.1 Inflammation at the periodontium level

The initiation of inflammation at the gingival margin by bacteria has been described in a model by Madianos and colleagues (Madianos et al., 2005). Epithelial cells are the first cells to be challenged by bacteria in the sulcus/pocket (Figure 14a). Bacterial adhesion through fimbriae and the release of their virulence factors activate epithelial cells to secrete inflammatory mediators (IL-8, TNF, IL-1β and IL-6) into the connective tissue compartment. Additionally, neutrophils are recruited to the crevice.

Following the diffusion of bacterial virulence factors and inflammatory mediators, host cells such as monocytes/macrophages, mast cells and fibroblasts produce and release pro-inflammatory cytokines (TNF, IL-1β, IL-6), chemotactic molecules (MIP-1, MCP-1, MCP-5, IL-8), PGE2, histamine, LTB-4, as well as MMPs that degrade collagen (Figure 14b). In addition, macrophages also express MHC class II molecules and co-stimulatory molecules (B7), and dendritic cells engulf bacteria and their products and process them for antigen presentation at the local lymph nodes. Hence, while the inflammatory response is getting organised, the host prepares for the adaptive immune response.

Subsequently endothelial cells which express surface molecules, such as P- and E-selectins and ICAMs, that are important for leucocyte extravasation, become activated (Figure 14c). Leucocytes then migrate through the tissues in response to a concentration gradient of chemoattractants derived from the host (IL-8, MCP-1) towards the focus of infection, where they start phagocytosing bacteria and their virulence factors. Histamine, TNF and PGE2 increase vascular permeability, which leads to efflux of plasma proteins and fluid in the connective tissue, and subsequently into the crevice, forming part of the gingival crevicular fluid. Finally, locally produced cytokines (IL-1β, TNF and IL-6) enter circulation and activate hepatocytes to synthesise acute-phase proteins (LBP, sCD14, CRP, complement), which help the host in the elimination of the infection.

The future of this complex process is determined by the ability of the inflammatory response, initiated by the host, to clear the infection. If the infection is not eliminated and contained, more periodontal destruction will occur and the host will launch the more effective adaptive immune response. On the other hand, if the infection is resolved, the inflammatory process ceases and repair mechanisms are activated in order to repair damage and bring the tissues to a healthy state free of inflammation. Such ideal conditions are extremely rare and may be achieved only in experimental settings. Hence, in reality, histological signs of inflammation such as neutrophils in the crevice are present even in ‘‘healthy’’ gingiva (Page et al., 1997).


Bacteria
Virulence Factors
Connective Tissue
Epithelial Cells
Sulcus/pocket
IL-8
TNFα
IL-1β
IL-6
hBDs
Proteases
Fimb
LPS
PGN
LTA
Fimb
Fimb
Proteases
HSP
Neutrophil
a
Connective Tissue
LPS
PGN
LTA
Fimb
IL-8
MIP-1
MCP-5
TNF-α
IL-1β
IL-12
Il-6
IFN-γ
PGE2
NO
L-selectin
CD11b:CD18
MHC II
B7
Macrophage
LPS
PGN
IL-1β
TNF-α
Histamine
TNF-α
LTB-4
IL-4
Mast cell
ICAM-1
IC-1β
IL-6
MCP-1
IL-8
IL-8
IL-6
IL-1β
TNF-α
IL-8
IL-6
MMPs
LPS
PGN
Fimb
HSP
TNF-α
IL-1β
PGE2
Fibroblast
b
Hepatocyte
IL-1β
TNF-α
IL-6
LBP
sCD14
CRP
Complement
Endothelial cells
Blood vessel lumen
Leukocyte
LFA-1
CD11b:CD18
P-selectin
E-selectin
IL-8
MCP-1
ICAM-1
ICAM-2
Increased vascular permeability
Connective Tissue
LTB-4
C5a
Histamine
TNF-α
LPS
Fimb
TNF-α
LPS
LTA
Fimb
IL-1β
TNF-α
Fimb
PGN
LBP
sCD14
CRP
Complement
Histamine
TNF-α
PGE2
c

(Fimb, fimbriae; LPS, lipopolysaccharides; PGN, peptidoglycans; LTA, lipotechoic acids; HSP, heat-shock proteins; IL-8, interleukin-8; TNF, tumour necrosis factor; IL-1β, interleukin-1β; IL-6, interleukin-6; hBDs, human β-defensins; MIP-1, macrophage inflammatory protein-1; MCP-5, monocyte chemotactic protein-5; IFN-γ, interferon-γ; PGE2, prostaglandin-E2; NO, nitrous oxide; MHC II, major histocompatibility complex-II; LTB-4, leukotriene B4; IL-4, interleukin-4; ICAM, intracellular adhesion molecule; MCP-1, monocyte chemotactic protein-1; MMPs, matrix metalloproteinase; LBP, lipopolysaccharide binding protein; sCD14, soluble CD14; CRP, C-reactive proteins)

Figures 14a-c: Initiation of inflammation at the gingival level by bacteria: a schematic representation including bacterial (red) and host (green) components (Madianos et al., 2005)

1.4.3.2 Systemic inflammation due to periodontitis

Despite the localised nature of periodontitis, a plethora of systemic inflammatory markers of this disease have been reported. It is speculated that these contribute to systemic diseases such as cardiovascular disease, diabetes mellitus and rheumatoid arthritis (Loos, 2005, Madianos et al., 2010). The systemic response includes:

- Increased number of peripheral leukocytes
- Elevated levels of plasma pro-inflammatory cytokines (IL-1β, IL-6 and TNF)
- Elevated levels of acute-phase proteins (LBP, sCD14, CRP, complement)
- Higher concentration of antibodies against periodontopathogens.

C-reactive protein (CRP) has especially been the focus of attention. A systematic review and meta-analysis was performed in 2008 (Paraskevas et al., 2008) to investigate the robustness of observations that plasma/serum levels of CRP are elevated in patients with destructive periodontitis, in comparison to subjects without periodontitis. It was found that the majority of studies showed that CRP levels are higher in periodontitis patients than in

controls. Often studies showed that periodontitis patients had CRP levels above 2.1mg/l. A meta-analysis of 10 cross-sectional studies showed that the weighted mean difference of CRP between periodontitis patients and controls was 1.56mg/l, providing strong evidence that periodontitis is associated with elevated plasma CRP levels.

With regard to cytokine levels, these are often present in plasma in extremely low concentrations. If present at high levels, they would have profound effects such as fever, wasting and severe overall illness, not normal symptoms of periodontitis. The previously used method to measure plasma levels of cytokines is the enzyme-linked immunosorbent assays (ELISA). This is often not sensitive enough and may yield both false-positive and false-negative results. Despite methodological difficulties, several groups have reported on plasma levels of IL-6 (Loos et al., 2000, Buhlin et al., 2009). Loos et al. (2000) and Buhlin et al. (2009) found that periodontitis patients showed significantly higher levels of IL-6 compared with healthy controls. The IL-6 levels, like CRP, also showed a positive relation to the extent of disease. Plasma levels of other cytokines such as IL-1 and TNF have also been studied in periodontitis patients, but their levels were often below the detection limit of ELISA, preventing meaningful interpretation. This area of research however will evolve with gradual transition from ELISA methods to multiplex assays which are more sensitive.

1.5 Association between radiation-induced oral mucositis and periodontitis

1.5.1 A hypothetical "two-hit" model

Radiation-induced oral mucositis and periodontitis may be associated through a primed inflammatory response as proposed by the "two-hit" model (Golub et al., 2006). This model suggests that inflammation associated with periodontitis (first "hit") followed by radiation (second "hit") can lead to an exacerbated response in the form of oral mucositis. The converse may also hold true in that radiation-induced oral mucositis (first "hit") exacerbates

the inflammatory response of developing periodontitis (second "hit") (Figure 15). If this hypothesis holds, then targeting both "hits" provides the optimal therapeutic strategy, with benefits for both local periodontal tissues and the experience of radiation-induced oral mucositis. Additionally, this concept could facilitate the incorporation of systemic/medical/pharmacologic treatment with the established local approach in the management of chronic destructive periodontitis.

This hypothesis is strengthened by evidence demonstrating that radiotherapy can worsen the periodontal condition of cancer patients, by increasing attachment loss and gingival recession (Markitziu et al., 1992, Epstein et al., 1998, Marques and Dib, 2004, Schuurhuis et al., 2011). There are several explanations for this. Firstly, radiotherapy directed at the jaws leads to changes in the oral equilibrium, including hyposalivation and increased bacterial counts. These modifications, along with dietary changes and oral hygiene difficulties, favour the development of dental caries and periodontal disease (Fischer and Epstein, 2008, Lerman et al., 2008, Hong et al., 2010, Bensadoun et al., 2011). Secondly, radiation causes hyperemia, inflammation, thrombosis, cytopenia, hypovascularisation, and fibrosis of the periodontal tissues (Joyston-Bechal, 1992, Marx, 1983, Marx and Johnson, 1987). Such changes increase the risk of periodontal disease and alter the healing process, thus decreasing the repair and remodelling capacity of the periodontium (Epstein and Stevenson-Moore, 2001). Thirdly, as mentioned above, radiation-induced oral mucositis (first "hit") may exacerbate the inflammatory response of developing periodontitis (second "hit").

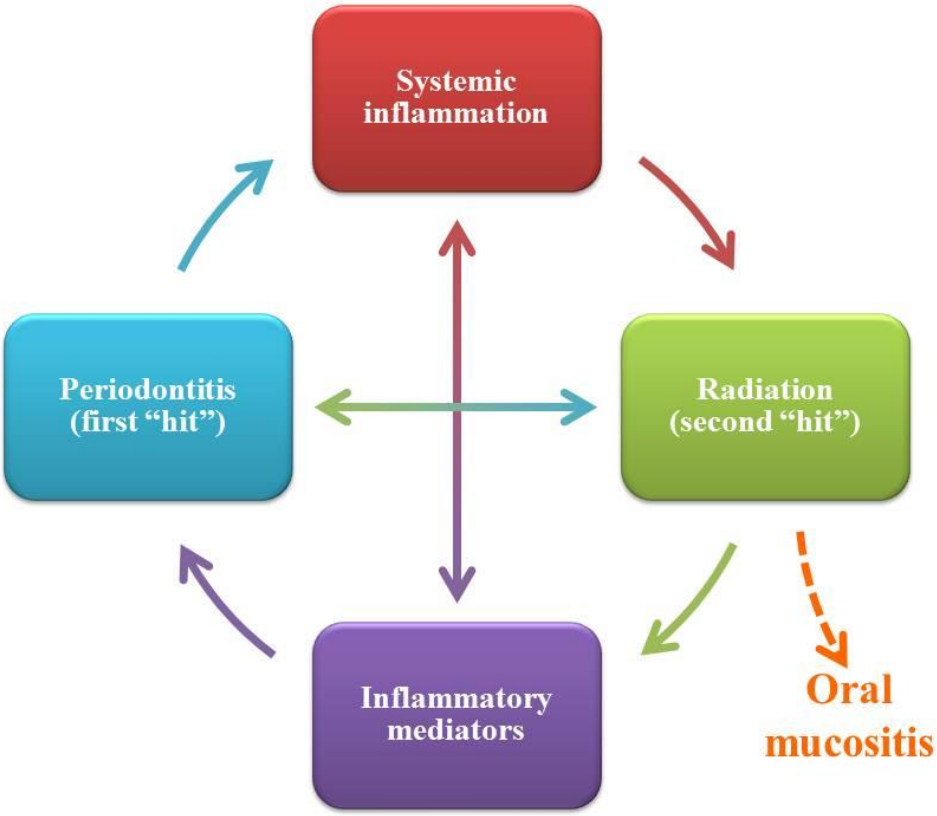


Figure 15: A hypothetical "two-hit" model demonstrating the association between radiation-induced oral mucositis and periodontitis

The "two-hit" model has previously been used to explain the pathogenesis of acute respiratory distress syndrome (ARDS) following cardiopulmonary bypass (Carney et al., 1999). In the first "hit", the animal, a Yorkshire pig, was subjected to cardiopulmonary bypass which induced ventilator-induced lung injury. This procedure did not predictably result in lung tissue destruction, but did promote the accumulation of polymorphonuclear leukocytes (PMNL) in the lung alveoli. However, the second "hit", generated by injection of a relatively low dose of endotoxin, induced PMNL degranulation and irreversible lung destruction mediated by MMPs and elastase (ARDS) (Figure 16). Treatment with a potent MMP and elastase inhibitor, a chemically modified tetracycline (CMT-3), was found to prevent the pathological changes typical of ARDS after cardiopulmonary bypass.

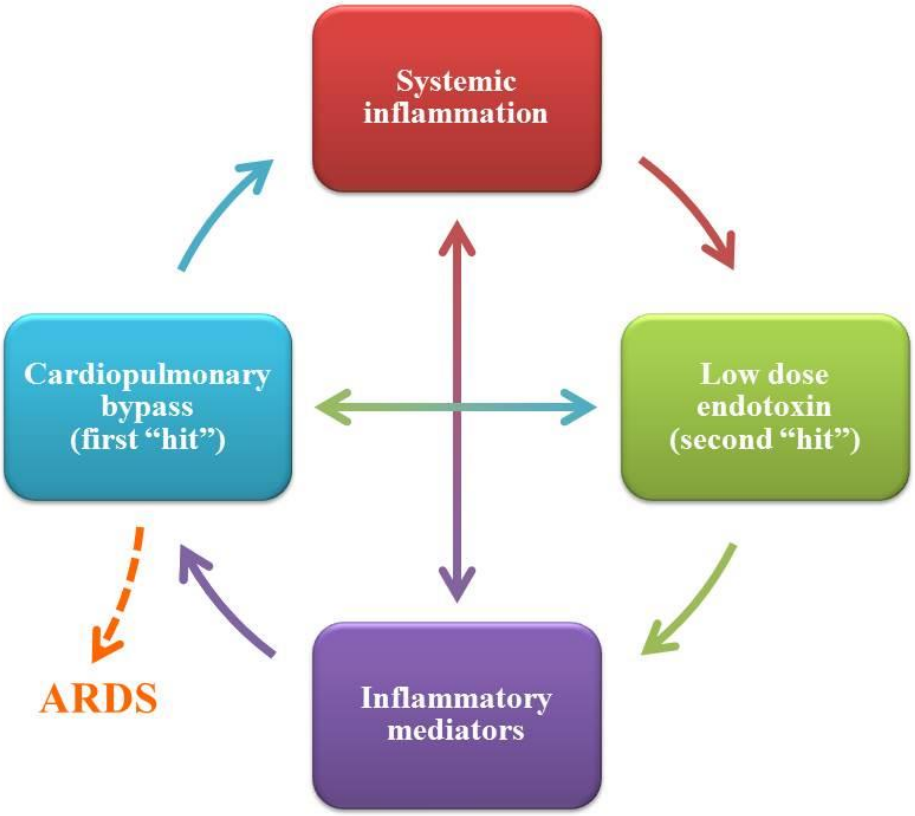


Figure 16: A "two-hit" model demonstrating the pathogenesis of acute respiratory distress syndrome (ARDS) following cardiopulmonary bypass

Subsequently a group of researchers in the United States designed a "two-hit" model of ischemia/reperfusion (I/R) injury to the gut followed by faecal peritonitis (FC) in Yorkshire pigs to create a clinically applicable model of ARDS (Steinberg et al., 2005b). The group of animals subjected to intraperitoneal placement of a faecal blood clot to induce faecal peritonitis (FC) (first "hit") showed signs of sepsis but no physiologic or histologic evidence of lung injury. However, another group of animals that underwent superior mesenteric artery occlusion for 30 minutes (I/R) (second "hit") followed by FC demonstrated more severe sepsis and signs of progressive pulmonary injury, both physiologically and histologically (Figure 17). The same group (Steinberg et al., 2005a) also showed that treatment with a modified tetracycline, which blocks MMP-2, MMP-9 and elastase production, prevented the development of ARDS and unexpectedly also prevented septic shock in these animals.

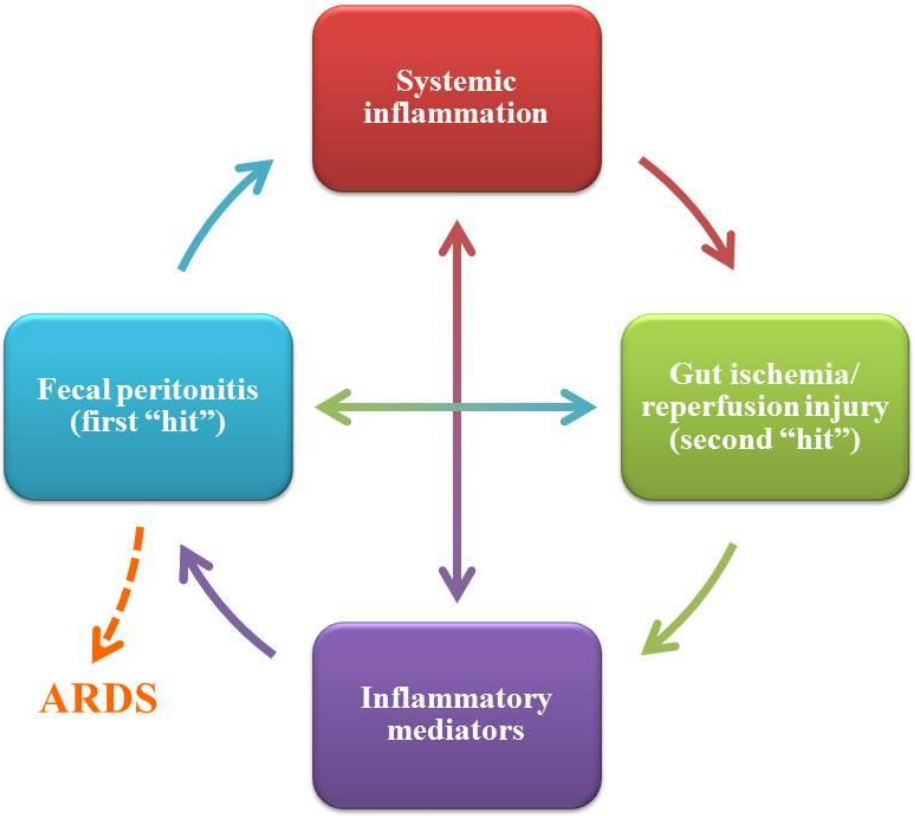


Figure 17: A "two-hit" model of ischemia/reperfusion injury to the gut followed by faecal peritonitis in Yorkshire pigs to create a clinically applicable model of ARDS

In recent years, the "two-hit" model has been used to explain the association between periodontitis and systemic diseases such as rheumatoid arthritis (Figure 18).

Using a rat model, Ramamurthy et al. demonstrated that experimental arthritis can induce periodontitis, evidenced by the presence of elevated inflammatory markers in the gingival tissues, periodontal bone loss and tooth mobility (Ramamurthy et al., 2005). The changes in both the joint and periodontal tissues were also successfully reversed through gene therapy with tissue inhibitor of matrix metalloproteinases (TIMP-4). Subsequently, a group from the University of Adelaide have shown that Porphyromonas gingivalis-induced inflammation (Bartold et al., 2010) and pre-existing periodontitis (Cantley et al., 2011) can exacerbate experimental arthritis in a mouse model. Recent clinical reports have also indicated that the control of periodontal inflammation can lead to a reduction in the severity

of active arthritis (Ribeiro et al., 2005, Al-Katma et al., 2007, Ortiz et al., 2009, Erciyas et al., 2012).

The association between periodontitis and osteoporosis can also be explained using the "two-hit" model. Using the standard animal model of post-menopausal osteoporosis, an ovariectomised (OVX) aged rat, Golub et al. observed that ovariectomy and oestrogen deficiency lead to increased MMP (collagenase and gelatinase) activity in the gingiva, alveolar bone loss locally, and trabecular bone density loss in the long bones systemically (Golub et al., 1999). This group also showed that treatment with CMT-8 (a chemically modified non-antimicrobial analogue of doxycycline) can reduce the severity of both periodontitis and osteoporosis. Another group demonstrated that ageing, ovariectomy, malnutrition and glucocorticoid application can lead to periodontal destruction in a sheep model (Dvorak et al., 2009).

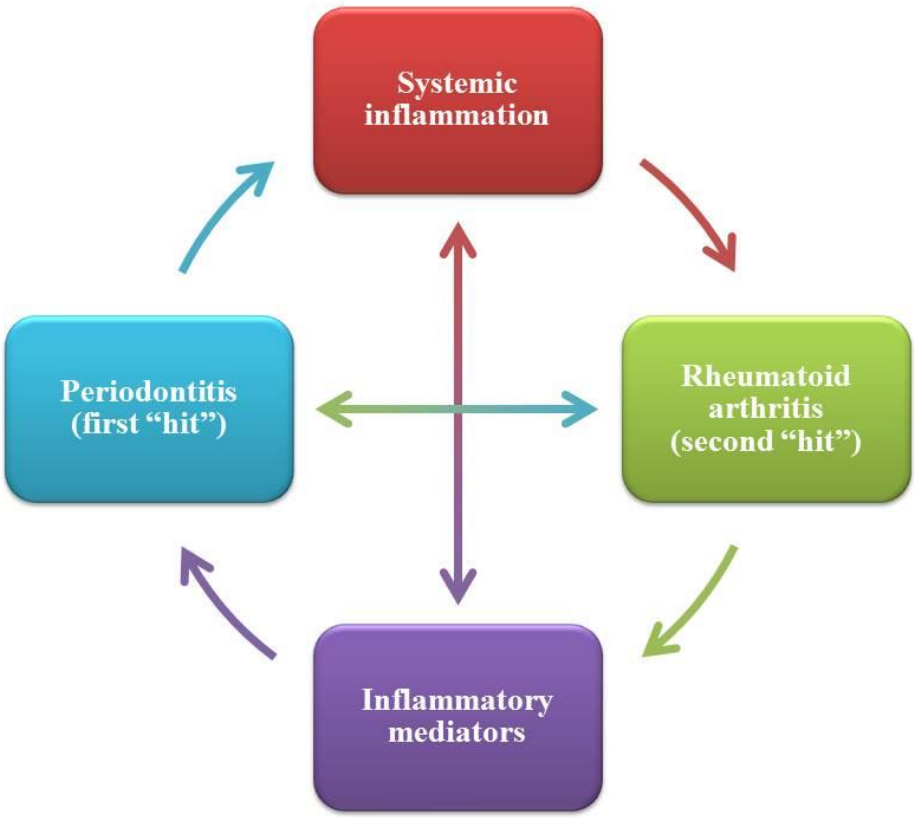


Figure 18: A "two-hit" model demonstrating the association between periodontitis and systemic diseases such as rheumatoid arthritis

1.5.2 Dysregulation of the inflammatory response

The lack of control (dysregulation) of the inflammatory response is thought to contribute to the pathogenesis of chronic inflammatory diseases (Bartold et al., 2010). One example is chronic destructive periodontitis. In the periodontal tissues, subgingival bacteria constantly challenge the host to maintain an effective defence that resists periodontitis (Darveau et al., 1997). The host defence response is generally considered to be protective, whereby innate and acquired mechanisms are activated to dilute, destroy or negate damaging agents and initiate tissue repair (Dennison and Van Dyke, 1997). This response involves coordination between numerous biologic pathways of inflammation, resolution and repair. When appropriately regulated, tissue repair ensues; however, when dysregulated, the inflammatory response becomes chronic and persistent, leading to further tissue destruction and progression of disease.

For cancer patients undergoing head and neck radiotherapy, radiation induces the formation of ROS, which activates an important transcription factor, NF-κB. NF-κB which is also directly activated by radiation and several chemotherapeutic drugs, is responsible for the upregulation of transcription of over 200 genes involved in mucositis. This results in the production of pro-inflammatory cytokines such as TNF, IL-1β, and IL-6 (Sonis, 2004). NF-κB is also thought to have both pro-apoptotic and anti-apoptotic effects, thus making it a significant factor in determining the fate of normal tissues following radiation or chemotherapy (Sonis, 2002). Cytokines produced earlier may activate NF-κB in other cells, resulting in transcription of genes generating biologically active proteins such as cyclooxygenase-2 (COX-2). COX-2 plays a key role in initiating the inflammatory cascade, and in signalling the activation of matrix metalloproteinases (MMPs) 1 and 3 (Sonis et al., 2004b). Collectively, these processes lead ultimately to the destruction and breakdown of the oral mucosa.

In addition to the “two-hit” model therefore, periodontitis and radiation-induced oral mucositis may be correlated as both conditions represent a dysregulation of the inflammatory response.

1.6 Potential benefits of treating periodontitis

1.6.1 Prevention of osteoradionecrosis

Pre-radiation dental screening in head and neck cancer patients is a well-accepted practice. Although head and neck radiotherapy is associated with both acute and chronic oral complications, the main purpose of pre-radiation dental screening is to prevent chronic oral complications such as osteoradionecrosis (Sennhenn-Kirchner et al., 2009, Nabil and Samman, 2011). This approach however is based on clinical experience and is not particularly evidence-based (Jansma et al., 1992b, Sulaiman et al., 2003). As a result, a wide variability exists in the level of oral care that is provided to head and neck cancer patients (Jansma et al., 1992a).

Jansma et al. have presented an evidence-based protocol for the prevention and treatment of oral sequelae resulting from head and neck radiation therapy (Jansma et al., 1992b). This protocol is especially applicable in centres operating with a dental team that is devoted to a wide range of preventive and treatment measures. The authors proposed that this team should ideally consist of an oral and maxillofacial surgeon, a hospital dentist and a dental hygienist. According to this protocol, periodontal examination should be performed and periodontitis should be treated, either by extraction or dental prophylaxis, prior to radiotherapy (Table 13).

- Physical and radiographic examination
  - Dentition (caries, restorations, calculus, vitality)
  - Periodontium (bleeding index, pocket depth, furcation)
  - Oral hygiene (plaque, bleeding index, denture hygiene)
  - Dental awareness and motivation
  - Oral mucosa and alveolar process (infection, irritation fibroma, hyperplasia, exostosis)
  - Dentures (fit of partial or full dentures)
  - Mouth opening (on indication)
  - Radiographic examination
    - Panoramic radiograph (intraoral radiographs when indicated)
    - Detection of foci (periapical infections, periodontal disease, unerupted or partially erupted teeth, residual root tips, cysts)
- Treatment and prophylaxis
  - Extraction of non-salvageable teeth and surgical removal of foci (alveolotomy, primary wound closure, 3 weeks of wound healing)
  - Dental prophylaxis (polishing, scaling, root planing, curettage)
  - Restorative dental procedures (restorations, endodontics)
  - Dentures (correction of ill-fitting dentures, no soft lining)
  - Initiation of preventive regimen
    - Plaque removal (tooth brushing, interdental plaque removal)
    - Topical fluoride (application of neutral 1% sodium fluoride gel every second day, custom-made fluoride carriers)
    - Oral rinses (salt-soda rinses at least 8-10 times daily)
    - Selective oral flora elimination (lozenges containing polymyxin E, tobramycin, and amphotericin B four times daily)
  - Denture wearing discouraged after the start of radiation therapy
  - Trismus prevention (exercises from the start of radiation therapy, when indicated)
  - Nutritional advises (instructions, counselling, ideally by a dietician)

Table 13: Care of patient before radiotherapy (Jansma et al., 1992b)

A retrospective study based at the University Medical Centre Groningen, the Netherlands, found that 76% of patients presented with oral foci, and these lesions were predominantly periodontal in origin (Schuurhuis et al., 2011). This has also been reported in other studies (Bonan et al., 2006, Jham et al., 2008). Patients in Schuurhuis et al. (2011) were managed according to the standard protocol described above (Jansma et al., 1992b). It was found that those presenting with periodontal pockets ≥ 6mm at dental screening had an increased risk (19%) of developing osteoradionecrosis compared to the total group of patients. Interestingly, if existing periodontitis was not aggressively treated before radiotherapy (i.e. extraction), osteoradionecrosis developed in 33% of these patients. The ineffectiveness of

initial periodontal therapy performed by the dental hygienist and hospital dentist was attributed to the period between diagnosis of periodontal disease and start of radiotherapy being too short to allow for proper evaluation of the effects of treatment. In addition, prosthodontic considerations lead to the preservation of periodontally-compromised teeth to enhance retention of prosthetic devices thought essential for oral functioning. Some patients also refused extractions due to increasing dental awareness. This demonstrates the complexity of treatment needs required by these patients. There may therefore be a niche for the involvement of other dental specialties, such as periodontics and prosthodontics, in the management of these patients.

To summarise, the benefits of initial periodontal therapy for teeth with periodontal pockets ≥ 6mm in order to prevent osteoradionecrosis in patients scheduled for radiotherapy are unclear. The literature on this topic is also scarce (Epstein and Stevenson-Moore, 2001, Marques and Dib, 2004).

1.6.2 Prevention of periodontal disease progression

Periodontal therapy, based on infection control by non-surgical and surgical approaches, has proven to be effective in the treatment of chronic periodontitis in terms of attachment level gain and reduction in gingival inflammation in the general population (Heitz-Mayfield et al., 2002).

The evidence supporting the benefits of periodontal therapy in cancer patients undergoing head and neck radiotherapy is limited however. A recent study evaluated periodontal changes following periodontal therapy in cancer patients undergoing radiotherapy to the head and neck region with or without chemotherapy (Bueno et al., 2013). Periodontal therapy was reported to be effective in improving periodontal status, evidenced by decreases in probing depths and the maintenance of attachment levels. It was unclear however in this study who performed the periodontal treatment. Nevertheless, this study suggested that

periodontal disease in these patients can be controlled, provided they are closely monitored by dental professionals. The authors also recommended the involvement of periodontists in the management of irradiated patients. In addition, the importance of controlling periodontal disease has also been emphasised in studies demonstrating an increase in attachment loss and gingival recession following radiotherapy (Markitziu et al., 1992, Epstein et al., 1998, Marques and Dib, 2004, Schuurhuis et al., 2011).

1.6.3 Prevention of oral mucositis

Whether or not periodontal therapy can influence the experience of oral mucositis in cancer patients undergoing head and neck radiotherapy remains to be explored. A beneficial effect, however, may be anticipated by analysing evidence from other studies.

Several studies have investigated the effects of periodontal therapy on serum markers of inflammation (e.g. CRP, IL-6 and fibrinogen), or on surrogate markers of subclinical cardiovascular disease (e.g. endothelial dysfunction, carotid intima media thickness). These studies have found a beneficial effect of periodontal therapy at least in the short term (Lockhart et al., 2012). Periodontal therapy performed in these studies commonly included the use of adjunctive local (D'Aiuto et al., 2005, D'Aiuto et al., 2006, Tonetti et al., 2007) or systemic antibiotics (Seinost et al., 2005, Lopez et al., 2012).

For patients suffering both type 2 diabetes mellitus and periodontitis, periodontal therapy consistently lead to a reduction in systemic inflammatory markers (e.g. CRP) and an improvement in metabolic control measured by the levels of glycated haemoglobin (HbA1c). Some studies performed purely mechanical subgingival debridement (Chen et al., 2012, Koromantzos et al., 2012), while others included the use of local (Katagiri et al., 2009, Lin et al., 2012) or systemic antibiotics (Sun et al., 2010). One study even reported an increase in serum anti-inflammatory cytokine (i.e. adiponectin) following intensive periodontal therapy and extraction of hopeless teeth (Sun et al., 2010).

Non-surgical periodontal therapy has also been shown to reduce the severity of rheumatoid arthritis measured by the Health Assessment Questionnaire (HAQ) (Ribeiro et al., 2005) and Disease Activity Score 28 joints (DAS28) (Al-Katma et al., 2007, Pinho Mde et al., 2009, Ortiz et al., 2009, Erciyas et al., 2012). Furthermore, these studies also reported a reduction in serum levels of TNF and CRP, as well as erythrocyte sedimentation rate.

A recent prospective study conducted in Thailand (Siribamrungwong and Puangpanngam, 2012) found non-surgical and surgical periodontal therapy lead to a reduction in systemic inflammation (i.e. reduction in CRP), improved nutritional status (i.e. increase in blood urea nitrogen and serum levels of albumin) and a reduction in erythropoietin dosage requirements, in maintenance haemodialysis patients.

Collectively these studies indicate a general trend toward a periodontal therapy–induced suppression of systemic inflammation. This outcome could potentially be useful for patients who are scheduled for head and neck radiotherapy, in order to prevent or reduce the severity of radiation-induced oral mucositis. Nevertheless, the magnitude of its effect has not been consistent across studies, and their sustainability over time has not been established convincingly. The determinants of variability in these responses are also poorly understood.

Clearly further investigations are required before periodontal therapy can be recommended to all periodontitis patients prior to head and neck radiotherapy to prevent oral mucositis. Periodontal therapy has, however, some added benefits over current preventive protocols for radiation-induced oral mucositis. Firstly, non-surgical periodontal therapy can be performed within 24 hours over one or two sessions. This approach has been shown to result in similar outcomes to conventional quadrant scaling and root planing which is performed in discrete sessions over a period of several weeks (Eberhard et al., 2008). A potential concern would be systemic inflammation observed 24 hours following the procedure due to bacteraemia and tissue damage following subgingival instrumentation (Graziani et al.,

2010). This spike however is transient and has been shown to drop below baseline levels after one week. The potential of completing therapy within 24 hours, as well as observing encouraging results within one week, is clearly beneficial as a short time period often elapses between dental screening and the start of radiotherapy in this group of patients. It is generally accepted that patients have to be free of dental foci 10–14 days before radiotherapy starts in order to ensure initial healing of the oral tissues before radiotherapy (Schuurhuis et al., 2011).

Secondly, periodontal therapy is a very safe procedure and is well-tolerated by the majority of patients. Cross-sectional studies have reported an improvement in patients' Oral-Health-Related Quality of Life (OHQoL) immediately after the delivery of non-surgical periodontal therapy (Needleman et al., 2004, D'Avila et al., 2005, Bajwa et al., 2007, Ozcelik et al., 2007, Saito et al., 2010, Tsakos et al., 2010). A recent longitudinal study (Wong et al., 2012) found that the improvement in patients' satisfaction level can lasts up to 12 months after non-surgical periodontal therapy. These positive changes were largely associated with reduced physical pain, psychological discomfort and psychological disability.

Thirdly, successful periodontal therapy followed by long-term maintenance can enhance tooth retention (Hirschfeld and Wasserman, 1978, Wilson et al., 1987, Nabers et al., 1988), precluding the need for tooth extraction and subsequent replacement using removable dentures or endo-osseous implants. A clinical practice guideline aimed to prevent osteoradionecrosis of the jaw, a chronic complication of head and neck radiotherapy (Marx and Johnson, 1987), recommended against tooth extraction during radiotherapy. Should a need arise for tooth extraction during or after radiotherapy, 20 sessions of pre-extraction hyperbaric oxygen therapy and 10 sessions of post-surgical hyperbaric therapy was recommended. Tooth extraction is not the only cumbersome situation to manage in this group of patients. Oral rehabilitation with removable dentures or endo-osseous implants is complex in patients with a history of head and neck radiotherapy. The use of removable dentures,

especially those that are ill-fitting, has been discouraged during radiotherapy to alleviate symptoms of oral mucositis and prevent oral candidiasis (Curtis et al., 1976, Beumer and Brady, 1978). Some authors have even recommended avoidance of the use of removable dentures after radiotherapy to prevent osteoradionecrosis of the jaw (Kielbassa et al., 2006). This group of patients are also not ideal candidates for implant therapy. A systematic review of animal and human studies (Ihde et al., 2009) concluded that implants placed in irradiated bone exhibited a 2–3 times greater failure rate compared with non-irradiated bone, regardless of whether the implants were placed before or after radiotherapy. Radiation doses above 50 Gy were found to be associated with a higher failure rate. Therefore, the importance of treating and maintaining teeth with reasonable periodontal prognosis cannot be over-emphasised.

1.7 Conclusions

To date, no studies have looked into the association between radiation-induced oral mucositis and periodontitis.

The aim of the study described in Chapter 2 is to determine whether the severity of oral mucositis is associated with the severity of periodontitis in cancer patients undergoing head and neck radiotherapy. To achieve this, the relationship between clinical signs of oral mucositis, and the clinical, radiological and immunological signs of periodontitis were analysed. The hypothesis is that patients with higher severity of periodontitis will suffer from a higher severity of oral mucositis following head and neck radiotherapy.

If this hypothesis is true, this study will justify routine screening and treatment of periodontitis patients before undergoing head and neck radiotherapy. Perhaps preventive protocols for radiation-induced oral mucositis should aim to reduce systemic inflammation *prior to* radiotherapy, rather than during radiotherapy. Additionally, if medical and radiation oncologists could predict which patients on standard-dose treatments will suffer oral

mucositis (e.g. severe periodontitis patients), newer, more expensive agents such as palifermin could be targeted in a cost-effective manner.

1.8 References

1. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03: June 14 2010.
2. National Cancer Institute Common Toxicity Criteria. Version 2.0, April 30, 1999.
3. Adair, T., Hoy, D., Dettrick, Z. & Lopez, A. D. (2011) Trends in oral, pharyngeal and oesophageal cancer mortality in Australia: the comparative importance of tobacco, alcohol and other risk factors. *Aust N Z J Public Health* **35,** 212-219.
4. Adelstein, D. J., Ridge, J. A., Gillison, M. L., Chaturvedi, A. K., D'Souza, G., Gravitt, P. E., Westra, W., Psyrri, A., Kast, W. M., Koutsky, L. A., Giuliano, A., Krosnick, S., Trotti, A., Schuller, D. E., Forastiere, A. & Ullmann, C. D. (2009) Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9-10, 2008, Washington, D.C. *Head Neck* **31,** 1393-1422.
5. Al-Dasooqi, N., Gibson, R. J., Bowen, J. M., Logan, R. M., Stringer, A. M. & Keefe, D. M. (2010) Matrix metalloproteinases are possible mediators for the development of alimentary tract mucositis in the dark agouti rat. *Exp Biol Med (Maywood)* **235,** 1244-1256.
6. Al-Katma, M. K., Bissada, N. F., Bordeaux, J. M., Sue, J. & Askari, A. D. (2007) Control of periodontal infection reduces the severity of active rheumatoid arthritis. *J Clin Rheumatol* **13,** 134-137.
7. Albandar, J. M. (1990) A 6-year study on the pattern of periodontal disease progression. *J Clin Periodontol* **17,** 467-471.
8. Aldrich, J. F., Lowe, D. B., Shearer, M. H., Winn, R. E., Jumper, C. A. & Kennedy, R. C. (2010) Vaccines and immunotherapeutics for the treatment of malignant disease. *Clin Dev Immunol* **2010,** Article ID 697158, 12 pages

9. Ambrose, J. & Hounsfield, G. (1973) Computerized transverse axial tomography. *Br J Radiol* **46,** 148-149.

10. Argiris, A., Karamouzis, M. V., Johnson, J. T., Heron, D. E., Myers, E., Eibling, D., Cano, E., Urba, S., Gluckman, J., Grandis, J. R., Wang, Y. & Agarwala, S. S. (2008) Long-term results of a phase III randomized trial of postoperative radiotherapy with or without carboplatin in patients with high-risk head and neck cancer. *Laryngoscope* **118,** 444-449.

11. Armitage, G. C. (1995) Clinical evaluation of periodontal diseases. *Periodontology 2000* **7,** 39-53.

12. Armitage, G. C. (1999) Development of a Classification System for Periodontal Diseases and Conditions. *Annals of Periodontology* **4,** 1-6.

13. Armitage, G. C. (2004a) Analysis of gingival crevice fluid and risk of progression of periodontitis. *Periodontology 2000* **34,** 109-119.

14. Armitage, G. C. (2004b) Periodontal diagnoses and classification of periodontal diseases. *Periodontology 2000* **34,** 9-21.

15. Attner, P., Du, J., Nasman, A., Hammarstedt, L., Ramqvist, T., Lindholm, J., Marklund, L., Dalianis, T. & Munck-Wikland, E. (2010) The role of human papillomavirus in the increased incidence of base of tongue cancer. *Int J Cancer* **126,** 2879-2884.

16. Australian Institute of Health and Welfare (2011) ACIM (Australian Cancer Incidence and Mortality) Books. AIHW: Canberra.

17. Australian Institute of Health and Welfare (2012) Cancer incidence projections: Australia, 2011 to 2020. Cancer Series no. 66. Cat. No. CAN 62. Canberra: AIHW.

18. Awwad, H. K., Lotayef, M., Shouman, T., Begg, A. C., Wilson, G., Bentzen, S. M., Abd El-Moneim, H. & Eissa, S. (2002) Accelerated hyperfractionation (AHF) compared to conventional fractionation (CF) in the postoperative radiotherapy of locally advanced head and neck cancer: influence of proliferation. *Br J Cancer* **86,** 517-523.

19. Bajwa, A., Watts, T. L. & Newton, J. T. (2007) Health control beliefs and quality of life considerations before and during periodontal treatment. *Oral Health Prev Dent* **5,** 101-104.

20. Barasch, A. & Peterson, D. E. (2003) Risk factors for ulcerative oral mucositis in cancer patients: unanswered questions. *Oral Oncol* **39,** 91-100.

21. Bartelink, H., Van den Bogaert, W., Horiot, J. C., Jager, J. & van Glabbeke, M. (2002) Concomitant cisplatin and radiotherapy in a conventional and modified fractionation schedule in locally advanced head and neck cancer: a randomised phase II EORTC trial. *Eur J Cancer* **38,** 667-673.

22. Bartold, P. M., Marino, V., Cantley, M. & Haynes, D. R. (2010) Effect of Porphyromonas gingivalis-induced inflammation on the development of rheumatoid arthritis. *J Clin Periodontol* **37,** 405-411.

23. Belting, C. M., Hiniker, J. J. & Dummett, C. O. (1964) Influence of diabetes on severity of periodontal disease. *Journal of periodontology* **35,** 476-480.

24. Bensadoun, R. J., Magne, N., Marcy, P. Y. & Demard, F. (2001) Chemotherapy- and radiotherapy-induced mucositis in head and neck cancer patients: new trends in pathophysiology, prevention and treatment. *Eur Arch Otorhinolaryngol* **258,** 481-487.

25. Bensadoun, R. J., Patton, L. L., Lalla, R. V. & Epstein, J. B. (2011) Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. *Support Care Cancer* **19,** 737-744.

26. Benson, J. & Skull, S. (2007) Hiding from the sun - vitamin D deficiency in refugees. *Aust Fam Physician* **36,** 355-357.

27. Bergstrom, J. (1989) Cigarette smoking as risk factor in chronic periodontal disease. *Community Dent Oral Epidemiol* **17,** 245-247.

28. Bernier, J., Domenge, C., Ozsahin, M., Matuszewska, K., Lefebvre, J. L., Greiner, R. H., Giralt, J., Maingon, P., Rolland, F., Bolla, M., Cognetti, F., Bourhis, J., Kirkpatrick,

A. & van Glabbeke, M. (2004) Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. *N Engl J Med* **350,** 1945-1952.

29. Bessell, A., Glenny, A. M., Furness, S., Clarkson, J. E., Oliver, R., Conway, D. I., Macluskey, M., Pavitt, S., Sloan, P. & Worthington, H. V. (2011) Interventions for the treatment of oral and oropharyngeal cancers: surgical treatment. *Cochrane Database Syst Rev* **9,** CD006205.
30. Beumer, J., 3rd & Brady, F. A. (1978) Dental management of the irradiated patient. *Int J Oral Surg* **7,** 208-220.
31. Beumer, J., 3rd, Curtis, T. & Harrison, R. E. (1979a) Radiation therapy of the oral cavity: sequelae and management, part 1. *Head Neck Surg* **1,** 301-312.
32. Beumer, J., 3rd, Curtis, T. & Harrison, R. E. (1979b) Radiation therapy of the oral cavity: sequelae and management, part 2. *Head Neck Surg* **1,** 392-408.
33. Bianco, J. A., Appelbaum, F. R., Nemunaitis, J., Almgren, J., Andrews, F., Kettner, P., Shields, A. & Singer, J. W. (1991) Phase I-II trial of pentoxifylline for the prevention of transplant-related toxicities following bone marrow transplantation. *Blood* **78,** 1205-1211.
34. Bier, J. (1994) Radical neck dissection versus conservative neck dissection for squamous cell carcinoma of the oral cavity. *Recent Results Cancer Res* **134,** 57-62.
35. Bonan, P. R., Lopes, M. A., Pires, F. R. & Almeida, O. P. (2006) Dental management of low socioeconomic level patients before radiotherapy of the head and neck with special emphasis on the prevention of osteoradionecrosis. *Braz Dent J* **17,** 336-342.
36. Borowski, B., Benhamou, E., Pico, J. L., Laplanche, A., Margainaud, J. P. & Hayat, M. (1994) Prevention of oral mucositis in patients treated with high-dose chemotherapy and bone marrow transplantation: a randomised controlled trial comparing two protocols of dental care. *Eur J Cancer B Oral Oncol* **30B,** 93-97.

37. Bourhis, J., Lapeyre, M., Tortochaux, J., Rives, M., Aghili, M., Bourdin, S., Lesaunier, F., Benassi, T., Lemanski, C., Geoffrois, L., Lusinchi, A., Verrelle, P., Bardet, E., Julieron, M., Wibault, P., Luboinski, M. & Benhamou, E. (2006a) Phase III randomized trial of very accelerated radiation therapy compared with conventional radiation therapy in squamous cell head and neck cancer: a GORTEC trial. *J Clin Oncol* **24,** 2873-2878.

38. Bourhis, J., Overgaard, J., Audry, H., Ang, K. K., Saunders, M., Bernier, J., Horiot, J. C., Le Maitre, A., Pajak, T. F., Poulsen, M. G., O'Sullivan, B., Dobrowsky, W., Hliniak, A., Skladowski, K., Hay, J. H., Pinto, L. H., Fallai, C., Fu, K. K., Sylvester, R. & Pignon, J. P. (2006b) Hyperfractionated or accelerated radiotherapy in head and neck cancer: a meta-analysis. *Lancet* **368,** 843-854.

39. Brägger, U. (2005) Radiographic parameters: biological significance and clinical use. *Periodontology 2000* **39,** 73-90.

40. Brazilian Head and Neck Cancer Study Group (1998) Results of a prospective trial on elective modified radical classical versus supraomohyoid neck dissection in the management of oral squamous carcinoma. *Am J Surg* **176,** 422-427.

41. Brill, N. (1959) The gingival pocket fluid. Studies of its occurrence, composition, and effects. *Acta Odontol Scand* **20(supplement),** 32.

42. Bueno, A. C., Ferreira, R. C., Barbosa, F. I., Jham, B. C., Magalhaes, C. S. & Moreira, A. N. (2013) Periodontal care in patients undergoing radiotherapy for head and neck cancer. *Support Care Cancer* **21,** 969-975.

43. Buhlin, K., Hultin, M., Norderyd, O., Persson, L., Pockley, A. G., Rabe, P., Klinge, B. & Gustafsson, A. (2009) Risk factors for atherosclerosis in cases with severe periodontitis. *J Clin Periodontol* **36,** 541-549.

44. Calvo, F. A., Meirino, R. M. & Orecchia, R. (2006) Intraoperative radiation therapy first part: rationale and techniques. *Crit Rev Oncol Hematol* **59,** 106-115.

45. Cancer Council Australia (2011) Head and neck cancers. Available at: http://www.cancer.org.au/about-cancer/types-of-cancer/head-and-neck-cancer.html. Accessed August 16, 2013.

46. Cantley, M. D., Haynes, D. R., Marino, V. & Bartold, P. M. (2011) Pre-existing periodontitis exacerbates experimental arthritis in a mouse model. *J Clin Periodontol* **38,** 532-541.

47. Carney, D. E., Lutz, C. J., Picone, A. L., Gatto, L. A., Ramamurthy, N. S., Golub, L. M., Simon, S. R., Searles, B., Paskanik, A., Snyder, K., Finck, C., Schiller, H. J. & Nieman, G. F. (1999) Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* **100,** 400-406.

48. Ceruse, P., Lallemant, B., Moriniere, S., Vergez, S., Benlyazid, A., Ramade, A., Buiret, G. & Mallet, Y. (2011) Transoral minimally invasive robotic surgery for carcinoma of the pharynx and the larynx: a new approach. *Anti-Cancer Drugs* **22,** 591-595.

49. Chao, K. S., Majhail, N., Huang, C. J., Simpson, J. R., Perez, C. A., Haughey, B. & Spector, G. (2001) Intensity-modulated radiation therapy reduces late salivary toxicity without compromising tumor control in patients with oropharyngeal carcinoma: a comparison with conventional techniques. *Radiother Oncol* **61,** 275-280.

50. Chen, L., Luo, G., Xuan, D., Wei, B., Liu, F., Li, J. & Zhang, J. (2012) Effects of non-surgical periodontal treatment on clinical response, serum inflammatory parameters, and metabolic control in patients with type 2 diabetes: a randomized study. *J Periodontol* **83,** 435-443.

51. Chen, W. C., Hwang, T. Z., Wang, W. H., Lu, C. H., Chen, C. C., Chen, C. M., Weng, H. H., Lai, C. H. & Chen, M. F. (2009) Comparison between conventional and intensity-modulated post-operative radiotherapy for stage III and IV oral cavity cancer in terms of treatment results and toxicity. *Oral Oncol* **45,** 505-510.

52. Cho, H. J., Eom, H. S., Kim, H. J., Kim, I. S., Lee, G. W. & Kong, S. Y. (2010) Glutathione-S-transferase genotypes influence the risk of chemotherapy-related toxicities and prognosis in Korean patients with diffuse large B-cell lymphoma. *Cancer Genet Cytogenet* **198,** 40-46.

53. Clarkson, J. E., Worthington, H. V. & Eden, O. B. (2007) Interventions for preventing oral candidiasis for patients with cancer receiving treatment. *Cochrane Database Syst Rev,* CD003807.

54. Connell, P. P. & Hellman, S. (2009) Advances in radiotherapy and implications for the next century: a historical perspective. *Cancer Res* **69,** 383-392.

55. Cooper, J. S., Pajak, T. F., Forastiere, A. A., Jacobs, J., Campbell, B. H., Saxman, S. B., Kish, J. A., Kim, H. E., Cmelak, A. J., Rotman, M., Machtay, M., Ensley, J. F., Chao, K. S., Schultz, C. J., Lee, N. & Fu, K. K. (2004) Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med* **350,** 1937-1944.

56. Curtis, T. A., Griffith, M. R. & Firtell, D. N. (1976) Complete denture prosthodontics for the radiation patient. *J Prosthet Dent* **36,** 66-76.

57. D'Aiuto, F., Nibali, L., Parkar, M., Suvan, J. & Tonetti, M. S. (2005) Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *J Dent Res* **84,** 269-273.

58. D'Aiuto, F., Parkar, M., Nibali, L., Suvan, J., Lessem, J. & Tonetti, M. S. (2006) Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. *Am Heart J* **151,** 977-984.

59. D'Avila, G. B., Carvalho, L. H., Feres-Filho, E. J., Feres, M. & Leao, A. (2005) Oral health impacts on daily living related to four different treatment protocols for chronic periodontitis. *J Periodontol* **76,** 1751-1757.

60. D'Souza, G., Kreimer, A. R., Viscidi, R., Pawlita, M., Fakhry, C., Koch, W. M., Westra, W. H. & Gillison, M. L. (2007) Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* **356,** 1944-1956.

61. Daly, M. E., Lieskovsky, Y., Pawlicki, T., Yau, J., Pinto, H., Kaplan, M., Fee, W. E., Koong, A., Goffinet, D. R., Xing, L. & Le, Q. T. (2007) Evaluation of patterns of failure and subjective salivary function in patients treated with intensity modulated radiotherapy for head and neck squamous cell carcinoma. *Head Neck* **29,** 211-220.

62. Darveau, R. P., Tanner, A. & Page, R. C. (1997) The microbial challenge in periodontitis. *Periodontology 2000* **14,** 12-32.

63. De Costa, A.-M. & Young, M. R. I. (2011) Immunotherapy for head and neck cancer: advances and deficiencies. *Anti-Cancer Drugs* **22,** 674-681.

64. Denham, J. W. & Hauer-Jensen, M. (2002) The radiotherapeutic injury--a complex 'wound'. *Radiother Oncol* **63,** 129-145.

65. Denham, J. W., Peters, L. J., Johansen, J., Poulsen, M., Lamb, D. S., Hindley, A., O'Brien, P. C., Spry, N. A., Penniment, M., Krawitz, H., Williamson, S., Bear, J. & Tripcony, L. (1999) Do acute mucosal reactions lead to consequential late reactions in patients with head and neck cancer? *Radiother Oncol* **52,** 157-164.

66. Dennison, D. K. & Van Dyke, T. E. (1997) The acute inflammatory response and the role of phagocytic cells in periodontal health and disease. *Periodontology 2000* **14,** 54-78.

67. Dobrowsky, W. & Naude, J. (2000) Continuous hyperfractionated accelerated radiotherapy with/without mitomycin C in head and neck cancers. *Radiother Oncol* **57,** 119-124.

68. Domenge, C., Hill, C., Lefebvre, J. L., De Raucourt, D., Rhein, B., Wibault, P., Marandas, P., Coche-Dequeant, B., Stromboni-Luboinski, M., Sancho-Garnier, H. & Luboinski, B. (2000) Randomized trial of neoadjuvant chemotherapy in oropharyngeal

carcinoma. French Groupe d'Etude des Tumeurs de la Tete et du Cou (GETTEC). *Br J Cancer* **83,** 1594-1598.

69. Donaldson, S. S. (1977) Nutritional consequences of radiotherapy. *Cancer Res* **37,** 2407-2413.

70. Duncan, M. & Grant, G. (2003) Oral and intestinal mucositis - causes and possible treatments. *Aliment Pharmacol Ther* **18,** 853-874.

71. Dutzan, N., Vernal, R., Hernandez, M., Dezerega, A., Rivera, O., Silva, N., Aguillon, J. C., Puente, J., Pozo, P. & Gamonal, J. (2009) Levels of interferon-gamma and transcription factor T-bet in progressive periodontal lesions in patients with chronic periodontitis. *J Periodontol* **80,** 290-296.

72. Dvorak, G., Reich, K., Tangl, S., Lill, C. A., Gottschalk-Baron, M., Watzek, G., Gruber, R. & Haas, R. (2009) Periodontal histomorphometry and status of aged sheep subjected to ovariectomy, malnutrition and glucocorticoid application. *Arch Oral Biol* **54,** 857-863.

73. Eberhard, J., Jepsen, S., Jervoe-Storm, P. M., Needleman, I. & Worthington, H. V. (2008) Full-mouth disinfection for the treatment of adult chronic periodontitis. *Cochrane Database Syst Rev***,** CD004622.

74. Eckel, H. E. (1997) Endoscopic laser resection of supraglottic carcinoma. *Otolaryngol Head Neck Surg* **117,** 681-687.

75. Eckel, H. E. & Thumfart, W. F. (1992) Laser surgery for the treatment of larynx carcinomas: indications, techniques, and preliminary results. *Ann Otol Rhinol Laryngol* **101,** 113-118.

76. Egelberg J. (1967) The topography and permeability of vessels at the dento-gingival junction in dogs. J Periodontal Res Suppl **1**:1–39.

77.]Eke, P. I., Dye, B. A., Wei, L., Thornton-Evans, G. O. & Genco, R. J. (2012) Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* **91,** 914-920.

78. Elting, L. S., Cooksley, C., Chambers, M., Cantor, S. B., Manzullo, E. & Rubenstein, E. B. (2003) The burdens of cancer therapy. Clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer* **98,** 1531-1539.

79. Epstein, J. B., Lunn, R., Le, N. & Stevenson-Moore, P. (1998) Periodontal attachment loss in patients after head and neck radiation therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **86,** 673-677.

80. Epstein, J. B., Silverman, S., Jr., Paggiarino, D. A., Crockett, S., Schubert, M. M., Senzer, N. N., Lockhart, P. B., Gallagher, M. J., Peterson, D. E. & Leveque, F. G. (2001) Benzydamine HCl for prophylaxis of radiation-induced oral mucositis: results from a multicenter, randomized, double-blind, placebo-controlled clinical trial. *Cancer* **92,** 875-885.

81. Epstein, J. B. & Stevenson-Moore, P. (2001) Periodontal disease and periodontal management in patients with cancer. *Oral Oncol* **37,** 613-619.

82. Erciyas, K., Sezer, U., Ustun, K., Pehlivan, Y., Kisacik, B., Senyurt, S., Tarakcioglu, M. & Onat, A. (2012) Effects of periodontal therapy on disease activity and systemic inflammation in rheumatoid arthritis patients. *Oral Dis*.

83. Fakih, A. R., Rao, R. S., Borges, A. M. & Patel, A. R. (1989) Elective versus therapeutic neck dissection in early carcinoma of the oral tongue. *Am J Surg* **158,** 309-313.

84. Fanucchi, M., Khuri, F. R., Shin, D., Johnstone, P. A. S. & Chen, A. (2006) Update in the management of head and neck cancer. *Update on Cancer Therapeutics* **1,** 211-219.

85. Fayette, J. (2011) Editorial. *Anti-Cancer Drugs* **22,** 585.

86. Feinmesser, R., Hardy, B., Sadov, R., Shwartz, A., Chretien, P. & Feinmesser, M. (2003) Report of a clinical trial in 12 patients with head and neck cancer treated intratumorally and peritumorally with multikine. *Arch Otolaryngol Head Neck Surg* **129,** 874-881.

87. Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. & Parkin, D. M. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*.

88. Ferra, C., de Sanjose, S., Lastra, C. F., Marti, F., Marino, E. L., Sureda, A., Brunet, S., Gallardo, D., Berlanga, J. J., Garcia, J. & Granena, A. (1997) Pentoxifylline, ciprofloxacin and prednisone failed to prevent transplant-related toxicities in bone marrow transplant recipients and were associated with an increased incidence of infectious complications. *Bone Marrow Transplant* **20,** 1075-1080.

89. Fischer, D. J. & Epstein, J. B. (2008) Management of patients who have undergone head and neck cancer therapy. *Dent Clin North Am* **52,** 39-60, viii.

90. Fitzsimmons, T. R., Sanders, A. E., Bartold, P. M. & Slade, G. D. (2010) Local and systemic biomarkers in gingival crevicular fluid increase odds of periodontitis. *J Clin Periodontol* **37,** 30-36.

91. Fowler, J. F. (1986) Potential for increasing the differential response between tumors and normal tissues: can proliferation rate be used? *Int J Radiat Oncol Biol Phys* **12,** 641-645.

92. Furness, S., Glenny, A. M., Worthington, H. V., Pavitt, S., Oliver, R., Clarkson, J. E., Macluskey, M., Chan, K. K. & Conway, D. I. (2011) Interventions for the treatment of oral cavity and oropharyngeal cancer: chemotherapy. *Cochrane Database Syst Rev***,** CD006386.

93. Gaspar, L. E. & Ding, M. (2008) A review of intensity-modulated radiation therapy. *Curr Oncol Rep* **10,** 294-299.

94. Gemmell, E., Marshall, R. I. & Seymour, G. J. (1997) Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontology 2000* **14,** 112-143.

95. Ghoshal, S., Goda, J. S., Mallick, I., Kehwar, T. S. & Sharma, S. C. (2008) Concomitant boost radiotherapy compared with conventional radiotherapy in squamous cell carcinoma of the head and neck--a phase III trial from a single institution in India. *Clin Oncol (R Coll Radiol)* **20,** 212-220.

96. Glavind, L., Lund, B. & Löe, H. (1979) The relationship between periodontal state and diabetes duration, insulin dosage and retinal changes. *Journal of periodontology* **39,** 341-347.

97. Glenny, A. M., Furness, S., Worthington, H. V., Conway, D. I., Oliver, R., Clarkson, J. E., Macluskey, M., Pavitt, S., Chan, K. K. & Brocklehurst, P. (2010) Interventions for the treatment of oral cavity and oropharyngeal cancer: radiotherapy. *Cochrane Database Syst Rev***,** CD006387.

98. Golub, L. M., Lee, H. M., Stoner, J. A., Sorsa, T., Reinhardt, R. A., Wolff, M. S., Ryan, M. E., Nummikoski, P. V. & Payne, J. B. (2008) Subantimicrobial-dose doxycycline modulates gingival crevicular fluid biomarkers of periodontitis in postmenopausal osteopenic women. *J Periodontol* **79,** 1409-1418.

99. Golub, L. M., Payne, J. B., Reinhardt, R. A. & Nieman, G. (2006) Can systemic diseases co-induce (not just exacerbate) periodontitis? A hypothetical "two-hit" model. *J Dent Res* **85,** 102-105.

100. Golub, L. M., Ramamurthy, N. S., Llavaneras, A., Ryan, M. E., Lee, H. M., Liu, Y., Bain, S. & Sorsa, T. (1999) A chemically modified nonantimicrobial tetracycline (CMT-8) inhibits gingival matrix metalloproteinases, periodontal breakdown, and extra-oral bone loss in ovariectomized rats. *Ann N Y Acad Sci* **878,** 290-310.

101. Gotte, K., Usener, D., Riedel, F., Hormann, K., Schadendorf, D. & Eichmuller, S. (2002) Tumor-associated antigens as possible targets for immune therapy in head and neck cancer: comparative mRNA expression analysis of RAGE and GAGE genes. *Acta Otolaryngol* **122,** 546-552.

102. Graziani, F., Cei, S., Tonetti, M., Paolantonio, M., Serio, R., Sammartino, G., Gabriele, M. & D'Aiuto, F. (2010) Systemic inflammation following non-surgical and surgical periodontal therapy. *J Clin Periodontol* **37,** 848-854.

103. Griffiths, G. S. (2003) Formation, collection and significance of gingival crevice fluid. *Periodontology 2000* **31,** 32-42.

104. Grossi, S. G., Genco, R. J., Machtei, E. E., Ho, A. W., Koch, G., Dunford, R., Zambon, J. J. & Hausmann, E. (1995) Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* **66,** 23-29.

105. Grossi, S. G., Zambon, J. J., Ho, A. W., Koch, G., Dunford, R. G., Machtei, E. E., Norderyd, O. M. & Genco, R. J. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* **65,** 260-267.

106. Haddadin, K. J., Soutar, D. S., Webster, M. H., Robertson, A. G., Oliver, R. J. & MacDonald, D. G. (2000) Natural history and patterns of recurrence of tongue tumours. *Br J Plast Surg* **53,** 279-285.

107. Hall, E. J. (2006) *Radiobiology for the Radiologist*. Lippincott Williams and Wilkins.

108. Hammarstedt, L., Lindquist, D., Dahlstrand, H., Romanitan, M., Dahlgren, L. O., Joneberg, J., Creson, N., Lindholm, J., Ye, W., Dalianis, T. & Munck-Wikland, E. (2006) Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer* **119,** 2620-2623.

109. Hansson, B. G., Rosenquist, K., Antonsson, A., Wennerberg, J., Schildt, E. B., Bladstrom, A. & Andersson, G. (2005) Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: a

population-based case-control study in southern Sweden. *Acta Otolaryngol* **125,** 1337-1344.

110. Heitz-Mayfield, L. J., Trombelli, L., Heitz, F., Needleman, I. & Moles, D. (2002) A systematic review of the effect of surgical debridement vs non-surgical debridement for the treatment of chronic periodontitis. *J Clin Periodontol* **29 Suppl 3,** 92-102; discussion 160-102.

111. Hirschfeld, L. & Wasserman, B. (1978) A long-term survey of tooth loss in 600 treated periodontal patients. *J Periodontol* **49,** 225-237.

112. Hocking, J. S., Stein, A., Conway, E. L., Regan, D., Grulich, A., Law, M. & Brotherton, J. M. (2011) Head and neck cancer in Australia between 1982 and 2005 show increasing incidence of potentially HPV-associated oropharyngeal cancers. *Br J Cancer* **104,** 886-891.

113. Holzhausen, M., Cortelli, J. R., da Silva, V. A., Franco, G. C., Cortelli, S. C. & Vergnolle, N. (2010) Protease-activated receptor-2 (PAR(2)) in human periodontitis. *J Dent Res* **89,** 948-953.

114. Hong, C. H., Napenas, J. J., Hodgson, B. D., Stokman, M. A., Mathers-Stauffer, V., Elting, L. S., Spijkervet, F. K. & Brennan, M. T. (2010) A systematic review of dental disease in patients undergoing cancer therapy. *Support Care Cancer* **18,** 1007-1021.

115. Horiot, J. C., Bontemps, P., van den Bogaert, W., Le Fur, R., van den Weijngaert, D., Bolla, M., Bernier, J., Lusinchi, A., Stuschke, M., Lopez-Torrecilla, J., Begg, A. C., Pierart, M. & Collette, L. (1997) Accelerated fractionation (AF) compared to conventional fractionation (CF) improves loco-regional control in the radiotherapy of advanced head and neck cancers: results of the EORTC 22851 randomized trial. *Radiother Oncol* **44,** 111-121.

116. Horiot, J. C., Le Fur, R., N'Guyen, T., Chenal, C., Schraub, S., Alfonsi, S., Gardani, G., Van Den Bogaert, W., Danczak, S., Bolla, M. & et al. (1992) Hyperfractionation versus

conventional fractionation in oropharyngeal carcinoma: final analysis of a randomized trial of the EORTC cooperative group of radiotherapy. *Radiother Oncol* **25,** 231-241.

117. Hounsfield, G. N. (1973) Computerized transverse axial scanning (tomography). 1. Description of system. *Br J Radiol* **46,** 1016-1022.
118. Howlader, N., Noone, A. M., Krapcho, M., Neyman, N., Aminou, R., Waldron, W., Altekruse, S. F., Kosary, C. L., Ruhl, J., Tatalovich, Z., Cho, H., Mariotto, A., Eisner, M. P., Lewis, D. R., Chen, H. S., Feuer, E. J., Cronin, K. A. & Edwards, B. K. (2011) SEER Cancer Statistics Review, 1975-2008, National Cancer Institute. Bethesda, MD, based on November 2010 SEER data submission, posted to the SEER web site, 2011.
119. Huang, K., Xia, P., Chuang, C., Weinberg, V., Glastonbury, C. M., Eisele, D. W., Lee, N. Y., Yom, S. S., Phillips, T. L. & Quivey, J. M. (2008) Intensity-modulated chemoradiation for treatment of stage III and IV oropharyngeal carcinoma: the University of California-San Francisco experience. *Cancer* **113,** 497-507.
120. Hugoson, A. & Jordan, T. (1982) Frequency distribution of individuals aged 20-70 years according to severity of periodontal disease. *Community Dent Oral Epidemiol* **10,** 187-192.
121. Hugoson, A. & Norderyd, O. (2008) Has the prevalence of periodontitis changed during the last 30 years? *J Clin Periodontol* **35,** 338-345.
122. Hugoson, A., Sjodin, B. & Norderyd, O. (2008) Trends over 30 years, 1973-2003, in the prevalence and severity of periodontal disease. *J Clin Periodontol* **35,** 405-414.
123. IARC (WHO) (2011) About INHANCE. Available at: http://inhance.iarc.fr/about.php. Accessed August 16, 2013.
124. Ihde, S., Kopp, S., Gundlach, K. & Konstantinovic, V. S. (2009) Effects of radiation therapy on craniofacial and dental implants: a review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **107,** 56-65.

125. Ikezawa-Suzuki, I., Shimada, Y., Tai, H., Komatsu, Y., Tanaka, A. & Yoshie, H. (2008) Effects of treatment on soluble tumour necrosis factor receptor type 1 and 2 in chronic periodontitis. *J Clin Periodontol* **35,** 961-968.

126. Ismail, A. I., Morrison, E. C., Burt, B. A., Caffesse, R. G. & Kavanagh, M. T. (1990) Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Disease Study, 1959-87. *J Dent Res* **69,** 430-435.

127. Jako, G. J. (1972) Laser surgery of the vocal cords. An experimental study with carbon dioxide lasers on dogs. *Laryngoscope* **82,** 2204-2216.

128. Jansma, J., Vissink, A., Bouma, J., Vermey, A., Panders, A. K. & Gravenmade, E. J. (1992a) A survey of prevention and treatment regimens for oral sequelae resulting from head and neck radiotherapy used in Dutch radiotherapy institutes. *Int J Radiat Oncol Biol Phys* **24,** 359-367.

129. Jansma, J., Vissink, A., Spijkervet, F. K., Roodenburg, J. L., Panders, A. K., Vermey, A., Szabo, B. G. & Gravenmade, E. J. (1992b) Protocol for the prevention and treatment of oral sequelae resulting from head and neck radiation therapy. *Cancer* **70,** 2171-2180.

130. Jensen, S. B., Pedersen, A. M., Vissink, A., Andersen, E., Brown, C. G., Davies, A. N., Dutilh, J., Fulton, J. S., Jankovic, L., Lopes, N. N., Mello, A. L., Muniz, L. V., Murdoch-Kinch, C. A., Nair, R. G., Napenas, J. J., Nogueira-Rodrigues, A., Saunders, D., Stirling, B., von Bultzingslowen, I., Weikel, D. S., Elting, L. S., Spijkervet, F. K. & Brennan, M. T. (2010a) A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: management strategies and economic impact. *Support Care Cancer* **18,** 1061-1079.

131. Jensen, S. B., Pedersen, A. M., Vissink, A., Andersen, E., Brown, C. G., Davies, A. N., Dutilh, J., Fulton, J. S., Jankovic, L., Lopes, N. N., Mello, A. L., Muniz, L. V., Murdoch-Kinch, C. A., Nair, R. G., Napenas, J. J., Nogueira-Rodrigues, A., Saunders, D., Stirling, B., von Bultzingslowen, I., Weikel, D. S., Elting, L. S., Spijkervet, F. K. &

Brennan, M. T. (2010b) A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: prevalence, severity and impact on quality of life. *Support Care Cancer* **18,** 1039-1060.

132. Jham, B. C., Reis, P. M., Miranda, E. L., Lopes, R. C., Carvalho, A. L., Scheper, M. A. & Freire, A. R. (2008) Oral health status of 207 head and neck cancer patients before, during and after radiotherapy. *Clin Oral Investig* **12,** 19-24.
133. Joss, A., Adler, R. & Lang, N. P. (1994) Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. *J Clin Periodontol* **21,** 402-408.
134. Joyston-Bechal, S. (1992) Prevention of dental diseases following radiotherapy and chemotherapy. *Int Dent J* **42,** 47-53.
135. Kardesler, L., Buduneli, N., Biyikoglu, B., Cetinkalp, S. & Kutukculer, N. (2008) Gingival crevicular fluid PGE2, IL-1beta, t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease. *Clin Biochem* **41,** 863-868.
136. Katagiri, S., Nitta, H., Nagasawa, T., Uchimura, I., Izumiyama, H., Inagaki, K., Kikuchi, T., Noguchi, T., Kanazawa, M., Matsuo, A., Chiba, H., Nakamura, N., Kanamura, N., Inoue, S., Ishikawa, I. & Izumi, Y. (2009) Multi-center intervention study on glycohemoglobin (HbA1c) and serum, high-sensitivity CRP (hs-CRP) after local anti-infectious periodontal treatment in type 2 diabetic patients with periodontal disease. *Diabetes Res Clin Pract* **83,** 308-315.
137. Keefe, D. M., Schubert, M. M., Elting, L. S., Sonis, S. T., Epstein, J. B., Raber-Durlacher, J. E., Migliorati, C. A., McGuire, D. B., Hutchins, R. D. & Peterson, D. E. (2007) Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer* **109,** 820-831.
138. Kielbassa, A. M., Hinkelbein, W., Hellwig, E. & Meyer-Luckel, H. (2006) Radiation-related damage to dentition. *Lancet Oncol* **7,** 326-335.

139. Kligerman, J., Lima, R. A., Soares, J. R., Prado, L., Dias, F. L., Freitas, E. Q. & Olivatto, L. O. (1994) Supraomohyoid neck dissection in the treatment of T1/T2 squamous cell carcinoma of oral cavity. *Am J Surg* **168,** 391-394.

140. Kornman, K. S. (2008) Mapping the pathogenesis of periodontitis: a new look. *J Periodontol* **79,** 1560-1568.

141. Kornman, K. S., Crane, A., Wang, H. Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., Wilson, T. G., Jr., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* **24,** 72-77.

142. Koromantzos, P. A., Makrilakis, K., Dereka, X., Offenbacher, S., Katsilambros, N., Vrotsos, I. A. & Madianos, P. N. (2012) Effect of non-surgical periodontal therapy on C-reactive protein, oxidative stress, and matrix metalloproteinase (MMP)-9 and MMP-2 levels in patients with type 2 diabetes: a randomized controlled study. *J Periodontol* **83,** 3-10.

143. Kowanko, I., Long, L., Hodgkinson, B. & Evans, D. (1998) The effectiveness of strategies for preventing and treating chemotherapy and radiation induced oral mucositis in patients with cancer. *Joanna Briggs Institute for Evidence Based Nursing and Midwifery,* 1-84.

144. Kurtis, B., Develioglu, H., Taner, I. L., Balos, K. & Tekin, I. O. (1999) IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. *J Oral Sci* **41,** 163-167.

145. La Vecchia, C., Tavani, A., Franceschi, S., Levi, F., Corrao, G. & Negri, E. (1997) Epidemiology and prevention of oral cancer. *Oral Oncol* **33,** 302-312.

146. Lash, G. E. & Pinto, L. A. (2010) Multiplex cytokine analysis technologies. *Expert Rev Vaccines* **9,** 1231-1237.

147. Leborgne, J. H., Leborgne, F., Zubizarreta, E., Ortega, B. & Mezzera, J. (1998) Corticosteroids and radiation mucositis in head and neck cancer. A double-blind placebo-controlled randomized trial. *Radiother Oncol* **47,** 145-148.

148. Lees, J. (1999) Incidence of weight loss in head and neck cancer patients on commencing radiotherapy treatment at a regional oncology centre. *Eur J Cancer Care (Engl)* **8,** 133-136.

149. Lefebvre, J. L. (2000) What is the role of primary surgery in the treatment of laryngeal and hypopharyngeal cancer? Hayes Martin Lecture. *Arch Otolaryngol Head Neck Surg* **126,** 285-288.

150. Leng, S. X., McElhaney, J. E., Walston, J. D., Xie, D., Fedarko, N. S. & Kuchel, G. A. (2008) ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *J Gerontol A Biol Sci Med Sci* **63,** 879-884.

151. Lerman, M. A., Laudenbach, J., Marty, F. M., Baden, L. R. & Treister, N. S. (2008) Management of oral infections in cancer patients. *Dent Clin North Am* **52,** 129-153.

152. Licitra, L., Grandi, C., Guzzo, M., Mariani, L., Lo Vullo, S., Valvo, F., Quattrone, P., Valagussa, P., Bonadonna, G., Molinari, R. & Cantu, G. (2003) Primary chemotherapy in resectable oral cavity squamous cell cancer: a randomized controlled trial. *J Clin Oncol* **21,** 327-333.

153. Lima, V., Brito, G. A., Cunha, F. Q., Reboucas, C. G., Falcao, B. A., Augusto, R. F., Souza, M. L., Leitao, B. T. & Ribeiro, R. A. (2005) Effects of the tumour necrosis factor-alpha inhibitors pentoxifylline and thalidomide in short-term experimental oral mucositis in hamsters. *Eur J Oral Sci* **113,** 210-217.

154. Lin, S. J., Chen, Y. L., Kuo, M. Y., Li, C. L. & Lu, H. K. (2005) Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine* **30,** 160-167.

155. Lin, S. J., Tu, Y. K., Tsai, S. C., Lai, S. M. & Lu, H. K. (2012) Non-surgical periodontal therapy with and without subgingival minocycline administration in patients with poorly controlled type II diabetes: a randomized controlled clinical trial. *Clin Oral Investig* **16,** 599-609.

156. Lindel, K., Beer, K. T., Laissue, J., Greiner, R. H. & Aebersold, D. M. (2001) Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer* **92,** 805-813.

157. Lindhe, J., Haffajee, A. D. & Socransky, S. S. (1983) Progression of periodontal disease in adult subjects in the absence of periodontal therapy. *J Clin Periodontol* **10,** 433-442.

158. Lindhe, J., Hamp, S. & Löe, H. (1973) Experimental periodontitis in the beagle dog. *J Periodontal Res* **8,** 1-10.

159. Lindhe, J., Hamp, S. E. & Löe, H. (1975) Plaque induced periodontal disease in beagle dogs. A 4-year clinical, roentgenographical and histometrical study. *J Periodontal Res* **10,** 243-255.

160. Lockhart, P. B., Bolger, A. F., Papapanou, P. N., Osinbowale, O., Trevisan, M., Levison, M. E., Taubert, K. A., Newburger, J. W., Gornik, H. L., Gewitz, M. H., Wilson, W. R., Smith, S. C., Jr. & Baddour, L. M. (2012) Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: a scientific statement from the American Heart Association. *Circulation* **125,** 2520-2544.

161. Löe, H. (1993) Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care* **16,** 329-334.

162. Löe, H., Anerud, A., Boysen, H. & Morrison, E. (1986) Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. *J Clin Periodontol* **13,** 431-445.

163. Löe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *J Periodontol* **36,** 177-187.

164. Logan, R. M. (2009) Advances in understanding of toxicities of treatment for head and neck cancer. *Oral Oncol* **45,** 844-848.

165. Logan, R. M., Gibson, R. J., Bowen, J. M., Stringer, A. M., Sonis, S. T. & Keefe, D. M. (2008a) Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis. *Cancer Chemother Pharmacol* **62,** 33-41.

166. Logan, R. M., Gibson, R. J., Sonis, S. T. & Keefe, D. M. (2007a) Nuclear factor-kappaB (NF-kappaB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. *Oral Oncol* **43,** 395-401.

167. Logan, R. M., Stringer, A. M., Bowen, J. M., Gibson, R. J., Sonis, S. T. & Keefe, D. M. (2008b) Serum levels of NFkappaB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biol Ther* **7,** 1139-1145.

168. Logan, R. M., Stringer, A. M., Bowen, J. M., Gibson, R. J., Sonis, S. T. & Keefe, D. M. (2009) Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered? *Cancer Chemother Pharmacol* **63,** 239-251.

169. Logan, R. M., Stringer, A. M., Bowen, J. M., Yeoh, A. S., Gibson, R. J., Sonis, S. T. & Keefe, D. M. (2007b) The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. *Cancer Treat Rev* **33,** 448-460.

170. Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *J Periodontol* **76,** 2106-2115.

171. Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. & van der Velden, U. (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* **71,** 1528-1534.

172. Lopez, N. J., Quintero, A., Casanova, P. A., Ibieta, C. I., Baelum, V. & Lopez, R. (2012) Effects of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: a controlled clinical trial. *J Periodontol* **83,** 267-278.

173. Madianos, P. N., Bobetsis, Y. A. & Kinane, D. F. (2005) Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. *J Clin Periodontol* **32 Suppl 6,** 57-71.

174. Madianos, P. N., Bobetsis, Y. A. & Van Dyke, T. E. (2010) Chapter 3: Infection and inflammation. In: *Periodontal disease and overall health: a clinician's guide*, (eds.) R. J. Genco & R. C. Williams. Professional Audience Communications, Inc.

175. Maio, M., Mackiewicz, A., Testori, A., Trefzer, U., Ferraresi, V., Jassem, J., Garbe, C., Lesimple, T., Guillot, B., Gascon, P., Gilde, K., Camerini, R. & Cognetti, F. (2010) Large randomized study of thymosin alpha 1, interferon alfa, or both in combination with dacarbazine in patients with metastatic melanoma. *J Clin Oncol* **28,** 1780-1787.

176. Marcial, V. A., Pajak, T. F., Chang, C., Tupchong, L. & Stetz, J. (1987) Hyperfractionated photon radiation therapy in the treatment of advanced squamous cell carcinoma of the oral cavity, pharynx, larynx, and sinuses, using radiation therapy as the only planned modality: (preliminary report) by the Radiation Therapy Oncology Group (RTOG). *Int J Radiat Oncol Biol Phys* **13,** 41-47.

177. Marcial, V. A., Pajak, T. F., Rotman, M., Brady, L. W. & Amato, D. (1993) "Compensated" split-course versus continuous radiation therapy of carcinoma of the tonsillar fossa. Final results of a prospective randomized clinical trial of the Radiation Therapy Oncology Group. *Am J Clin Oncol* **16,** 389-396.

178. Markitziu, A., Zafiropoulos, G., Tsalikis, L. & Cohen, L. (1992) Gingival health and salivary function in head and neck-irradiated patients. A five-year follow-up. *Oral Surg Oral Med Oral Pathol* **73,** 427-433.

179. Marques, M. A. & Dib, L. L. (2004) Periodontal changes in patients undergoing radiotherapy. *J Periodontol* **75,** 1178-1187.

180. Marx, R. E. (1983) Osteoradionecrosis: a new concept of its pathophysiology. *J Oral Maxillofac Surg* **41,** 283-288.

181. Marx, R. E. & Johnson, R. P. (1987) Studies in the radiobiology of osteoradionecrosis and their clinical significance. *Oral Surg Oral Med Oral Pathol* **64,** 379-390.

182. McGuire, D. B., Correa, M. E., Johnson, J. & Wienandts, P. (2006) The role of basic oral care and good clinical practice principles in the management of oral mucositis. *Support Care Cancer* **14,** 541-547.

183. Mealey, B. L. & Ocampo, G. L. (2007) Diabetes mellitus and periodontal disease. *Periodontol 2000* **44,** 127-153.

184. Mekhail, T. M., Adelstein, D. J., Rybicki, L. A., Larto, M. A., Saxton, J. P. & Lavertu, P. (2001) Enteral nutrition during the treatment of head and neck carcinoma: is a percutaneous endoscopic gastrostomy tube preferable to a nasogastric tube? *Cancer* **91,** 1785-1790.

185. Mennie, A. T., Dalley, V. M., Dinneen, L. C. & Collier, H. O. (1975) Treatment of radiation-induced gastrointestinal distress with acetylsalicylate. *Lancet* **2,** 942-943.

186. Michalowicz, B. S., Aeppli, D., Virag, J. G., Klump, D. G., Hinrichs, J. E., Segal, N. L., Bouchard, T. J., Jr. & Pihlstrom, B. L. (1991a) Periodontal findings in adult twins. *J Periodontol* **62,** 293-299.

187. Michalowicz, B. S., Aeppli, D. P., Kuba, R. K., Bereuter, J. E., Conry, J. P., Segal, N. L., Bouchard, T. J., Jr. & Pihlstrom, B. L. (1991b) A twin study of genetic variation in proportional radiographic alveolar bone height. *J Dent Res* **70,** 1431-1435.

188. Michalowicz, B. S., Diehl, S. R., Gunsolley, J. C., Sparks, B. S., Brooks, C. N., Koertge, T. E., Califano, J. V., Burmeister, J. A. & Schenkein, H. A. (2000) Evidence of a substantial genetic basis for risk of adult periodontitis. *J Periodontol* **71,** 1699-1707.

189. Mol, A. (2004) Imaging methods in periodontology. *Periodontology 2000* **34,** 34-48.

190. Molin, Y. & Fayette, J. (2011) Current chemotherapies for recurrent/metastatic head and neck cancer. *Anti-Cancer Drugs* **22,** 621-625.

191. Mork, J., Lie, A. K., Glattre, E., Hallmans, G., Jellum, E., Koskela, P., Moller, B., Pukkala, E., Schiller, J. T., Youngman, L., Lehtinen, M. & Dillner, J. (2001) Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* **344,** 1125-1131.

192. Murphy, B. A. (2007) Clinical and economic consequences of mucositis induced by chemotherapy and/or radiation therapy. *J Support Oncol* **5,** 13-21.

193. Nabers, C. L., Stalker, W. H., Esparza, D., Naylor, B. & Canales, S. (1988) Tooth loss in 1535 treated periodontal patients. *J Periodontol* **59,** 297-300.

194. Nabil, S. & Samman, N. (2011) Incidence and prevention of osteoradionecrosis after dental extraction in irradiated patients: a systematic review. *Int J Oral Maxillofac Surg* **40,** 229-243.

195. National Cancer Institute (2010) Radiation Therapy for Cancer. Available at: http://www.cancer.gov/cancertopics/factsheet/Therapy/radiation. Accessed August 16, 2013.

196. Needleman, I., McGrath, C., Floyd, P. & Biddle, A. (2004) Impact of oral health on the life quality of periodontal patients. *J Clin Periodontol* **31,** 454-457.

1. Noda, S. E., Lautenschlaeger, T., Siedow, M. R., Patel, D. R., El-Jawahri, A., Suzuki, Y., Loeffler, J. S., Bussiere, M. R. & Chakravarti, A. (2009) Technological advances in radiation oncology for central nervous system tumors. *Semin Radiat Oncol* **19,** 179-186.

197. Northway, M. G., Libshitz, H. I., Osborne, B. M., Feldman, M. S., Mamel, J. J., West, J. H. & Szwarc, I. A. (1980) Radiation esophagitis in the opossum: radioprotection with indomethacin. *Gastroenterology* **78,** 883-892.

198. Oddone, N., Morgan, G. J., Palme, C. E., Perera, L., Shannon, J., Wong, E., Gebski, V. & Veness, M. J. (2009) Metastatic cutaneous squamous cell carcinoma of the head and neck: the Immunosuppression, Treatment, Extranodal spread, and Margin status (ITEM) prognostic score to predict outcome and the need to improve survival. *Cancer* **115,** 1883-1891.

199. Oringer, R. J., Al-Shammari, K. F., Aldredge, W. A., Iacono, V. J., Eber, R. M., Wang, H. L., Berwald, B., Nejat, R. & Giannobile, W. V. (2002) Effect of locally delivered minocycline microspheres on markers of bone resorption. *J Periodontol* **73,** 835-842.

200. Ortiz, P., Bissada, N. F., Palomo, L., Han, Y. W., Al-Zahrani, M. S., Panneerselvam, A. & Askari, A. (2009) Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. *J Periodontol* **80,** 535-540.

201. Ozcelik, O., Haytac, M. C. & Seydaoglu, G. (2007) Immediate post-operative effects of different periodontal treatment modalities on oral health-related quality of life: a randomized clinical trial. *J Clin Periodontol* **34,** 788-796.

202. Paccagnella, A., Orlando, A., Marchiori, C., Zorat, P. L., Cavaniglia, G., Sileni, V. C., Jirillo, A., Tomio, L., Fila, G., Fede, A. & et al. (1994) Phase III trial of initial chemotherapy in stage III or IV head and neck cancers: a study by the Gruppo di Studio sui Tumori della Testa e del Collo. *J Natl Cancer Inst* **86,** 265-272.

203. Page, R. C., Offenbacher, S., Schroeder, H. E., Seymour, G. J. & Kornman, K. S. (1997) Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontology 2000* **14,** 216-248.

204. Page, R. C. & Schroeder, H. E. (1976) Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* **34,** 235-249.

205. Page, R. C. & Schroeder, H. E. (1981) Current status of the host response in chronic marginal periodontitis. *J Periodontol* **52,** 477-491.

206. Papapanou, P. N., Wennstrom, J. L. & Grondahl, K. (1989) A 10-year retrospective study of periodontal disease progression. *J Clin Periodontol* **16,** 403-411.

207. Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* **35,** 277-290.

208. Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. (2001) Estimating the world cancer burden: Globocan 2000. *Int J Cancer* **94,** 153-156.

209. Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. (2005) Global cancer statistics, 2002. *CA Cancer J Clin* **55,** 74-108.

210. Pavitt, S., Clarkson, J. E., Conway, D., Glenny, A. M., Macluskey, M., Oliver, R., Sloan, P., The CSROC Expert Panel, Warnakulasuriya, S. & Worthington, H. V. (2007) Interventions for the treatment of oral and oropharyngeal cancers: immunotherapy/biotherapy (Protocol). *Cochrane Database of Systematic Reviews 2007*.

211. Pignon, J. P., Bourhis, J., Domenge, C. & Designe, L. (2000) Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data. MACH-NC Collaborative Group. Meta-Analysis of Chemotherapy on Head and Neck Cancer. *Lancet* **355,** 949-955.

212. Pignon, J. P., le Maitre, A., Maillard, E. & Bourhis, J. (2009) Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiother Oncol* **92,** 4-14.

213. Pillsbury, H. C., 3rd, Webster, W. P. & Rosenman, J. (1986) Prostaglandin inhibitor and radiotherapy in advanced head and neck cancers. *Arch Otolaryngol Head Neck Surg* **112,** 552-553.

214. Pinho Mde, N., Oliveira, R. D., Novaes, A. B., Jr. & Voltarelli, J. C. (2009) Relationship between periodontitis and rheumatoid arthritis and the effect of non-surgical periodontal treatment. *Braz Dent J* **20,** 355-364.

215. Pinto, L. H., Canary, P. C., Araujo, C. M., Bacelar, S. C. & Souhami, L. (1991) Prospective randomized trial comparing hyperfractionated versus conventional radiotherapy in stages III and IV oropharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* **21,** 557-562.

216. Poulsen, M. G., Denham, J. W., Peters, L. J., Lamb, D. S., Spry, N. A., Hindley, A., Krawitz, H., Hamilton, C., Keller, J., Tripcony, L. & Walker, Q. (2001) A randomised trial of accelerated and conventional radiotherapy for stage III and IV squamous carcinoma of the head and neck: a Trans-Tasman Radiation Oncology Group Study. *Radiother Oncol* **60,** 113-122.

217. Preber, H. & Bergstrom, J. (1985) Occurrence of gingival bleeding in smoker and non-smoker patients. *Acta Odontol Scand* **43,** 315-320.

218. Preber, H. & Bergstrom, J. (1986) Cigarette smoking in patients referred for periodontal treatment. *Scand J Dent Res* **94,** 102-108.

219. Psyrri, A., Boutati, E. & Karageorgopoulou, S. (2011) Human papillomavirus in head and neck cancers: biology, prognosis, hope of treatment, and vaccines. *Anti-Cancer Drugs* **22,** 586-590.

220. Ramamurthy, N. S., Greenwald, R. A., Celiker, M. Y. & Shi, E. Y. (2005) Experimental arthritis in rats induces biomarkers of periodontitis which are ameliorated by gene therapy with tissue inhibitor of matrix metalloproteinases. *J Periodontol* **76,** 229-233.

221. Ramos, M., Benavente, S. & Giralt, J. (2010) Management of squamous cell carcinoma of the head and neck: updated European treatment recommendations. *Expert Rev Anticancer Ther* **10,** 339-344.

222. Rescala, B., Rosalem, W., Jr., Teles, R. P., Fischer, R. G., Haffajee, A. D., Socransky, S. S., Gustafsson, A. & Figueredo, C. M. (2010) Immunologic and microbiologic profiles of chronic and aggressive periodontitis subjects. *J Periodontol* **81,** 1308-1316.

223. Ribeiro, J., Leao, A. & Novaes, A. B. (2005) Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol* **32,** 412-416.

224. Richard, J. M., Kramar, A., Molinari, R., Lefebvre, J. L., Blanchet, F., Jortay, A. & Sancho-Garnier, H. (1991) Randomised EORTC head and neck cooperative group trial of preoperative intra-arterial chemotherapy in oral cavity and oropharynx carcinoma. *Eur J Cancer* **27,** 821-827.

225. Robbins, K. T., Clayman, G., Levine, P. A., Medina, J., Sessions, R., Shaha, A., Som, P. & Wolf, G. T. (2002) Neck dissection classification update: revisions proposed by the American Head and Neck Society and the American Academy of Otolaryngology-Head and Neck Surgery. *Arch Otolaryngol Head Neck Surg* **128,** 751-758.

226. Roberts-Thomson, K. F. (2007) Chapter 5. Oral health status. In: *Australia's Dental Generations: The National Survey of Adult Oral Health 2004–06. Canberra: Australian Institute of Health and Welfare*, (eds.) G. D. Slade, A. J. Spencer & K. F. Roberts-Thomson, pp. 81-142.

227. Roder, D. & Wilson, D. (1983) Oral cancer in South Australia--incidence and case survival. *Aust Dent J* **28,** 312-315.

228. Rosenthal, D. I. (2007) Consequences of mucositis-induced treatment breaks and dose reductions on head and neck cancer treatment outcomes. *J Support Oncol* **5,** 23-31.

229. Rubenstein, E. B., Peterson, D. E., Schubert, M., Keefe, D., McGuire, D., Epstein, J., Elting, L. S., Fox, P. C., Cooksley, C. & Sonis, S. T. (2004) Clinical practice guidelines

for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. *Cancer* **100,** 2026-2046.

230. Rusthoven, K. E., Raben, D., Ballonoff, A., Kane, M., Song, J. I. & Chen, C. (2008) Effect of radiation techniques in treatment of oropharynx cancer. *Laryngoscope* **118,** 635-639.

231. Ryerson, A. B., Peters, E. S., Coughlin, S. S., Chen, V. W., Gillison, M. L., Reichman, M. E., Wu, X., Chaturvedi, A. K. & Kawaoka, K. (2008) Burden of potentially human papillomavirus-associated cancers of the oropharynx and oral cavity in the US, 1998-2003. *Cancer* **113,** 2901-2909.

232. Saarilahti, K., Kouri, M., Collan, J., Kangasmaki, A., Atula, T., Joensuu, H. & Tenhunen, M. (2006) Sparing of the submandibular glands by intensity modulated radiotherapy in the treatment of head and neck cancer. *Radiother Oncol* **78,** 270-275.

233. Saito, A., Hosaka, Y., Kikuchi, M., Akamatsu, M., Fukaya, C., Matsumoto, S., Ueshima, F., Hayakawa, H., Fujinami, K. & Nakagawa, T. (2010) Effect of initial periodontal therapy on oral health-related quality of life in patients with periodontitis in Japan. *J Periodontol* **81,** 1001-1009.

234. Saltz, L. B., Lenz, H. J., Kindler, H. L., Hochster, H. S., Wadler, S., Hoff, P. M., Kemeny, N. E., Hollywood, E. M., Gonen, M., Quinones, M., Morse, M. & Chen, H. X. (2007) Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study. *J Clin Oncol* **25,** 4557-4561.

235. Schuurhuis, J. M., Stokman, M. A., Roodenburg, J. L., Reintsema, H., Langendijk, J. A., Vissink, A. & Spijkervet, F. K. (2011) Efficacy of routine pre-radiation dental screening and dental follow-up in head and neck oncology patients on intermediate and late radiation effects. A retrospective evaluation. *Radiother Oncol* **101,** 403-409.

236. Schwaab, T., Schwarzer, A., Wolf, B., Crocenzi, T. S., Seigne, J. D., Crosby, N. A., Cole, B. F., Fisher, J. L., Uhlenhake, J. C., Mellinger, D., Foster, C., Szczepiorkowski, Z. M., Webber, S. M., Schned, A. R., Harris, R. D., Barth, R. J., Jr., Heaney, J. A., Noelle, R. J. & Ernstoff, M. S. (2009) Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with Aldesleukin (Interleukin 2) and IFN-{alpha}2a therapy in metastatic renal cell carcinoma patients. *Clin Cancer Res* **15,** 4986-4992.

237. Sciubba, J. J. & Goldenberg, D. (2006) Oral complications of radiotherapy. *Lancet Oncol* **7,** 175-183.

238. Scully, C., Sonis, S. & Diz, P. D. (2006) Oral mucositis. *Oral Dis* **12,** 229-241.

239. Seinost, G., Wimmer, G., Skerget, M., Thaller, E., Brodmann, M., Gasser, R., Bratschko, R. O. & Pilger, E. (2005) Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *Am Heart J* **149,** 1050-1054.

240. Sennhenn-Kirchner, S., Freund, F., Grundmann, S., Martin, A., Borg-von Zepelin, M., Christiansen, H., Wolff, H. A. & Jacobs, H. G. (2009) Dental therapy before and after radiotherapy--an evaluation on patients with head and neck malignancies. *Clin Oral Investig* **13,** 157-164.

241. Seymour, G. J., Gemmell, E., Reinhardt, R. A., Eastcott, J. & Taubman, M. A. (1993) Immunopathogenesis of chronic inflammatory periodontal disease: cellular and molecular mechanisms. *J Periodontal Res* **28,** 478-486.

242. Seymour, G. J., Powell, R. N. & Davies, W. I. (1979) Conversion of a stable T-cell lesion to a progressive B-cell lesion in the pathogenesis of chronic inflammatory periodontal disease: an hypothesis. *J Clin Periodontol* **6,** 267-277.

243. Shefer, A., Markowitz, L., Deeks, S., Tam, T., Irwin, K., Garland, S. M. & Schuchat, A. (2008) Early experience with human papillomavirus vaccine introduction in the United States, Canada and Australia. *Vaccine* **26 Suppl 10,** K68-75.

244. Shieh, S. H., Wang, S. T., Tsai, S. T. & Tseng, C. C. (1997) Mouth care for nasopharyngeal cancer patients undergoing radiotherapy. *Oral Oncol* **33,** 36-41.

245. Silverman, S., Jr. (2007) Diagnosis and management of oral mucositis. *J Support Oncol* **5,** 13-21.

246. Siribamrungwong, M. & Puangpanngam, K. (2012) Treatment of periodontal diseases reduces chronic systemic inflammation in maintenance hemodialysis patients. *Ren Fail* **34,** 171-175.

247. Sonis, S. T. (2002) The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Crit Rev Oral Biol Med* **13,** 380-389.

248. Sonis, S. T. (2004) The pathobiology of mucositis. *Nat Rev Cancer* **4,** 277-284.

249. Sonis, S. T. (2007) Pathobiology of oral mucositis: novel insights and opportunities. *J Support Oncol* **5,** 3-11.

250. Sonis, S. T. (2010) New thoughts on the initiation of mucositis. *Oral Dis* **16,** 597-600.

251. Sonis, S. T. (2011) Oral mucositis. *Anti-Cancer Drugs* **22,** 607-612.

252. Sonis, S. T., Eilers, J. P., Epstein, J. B., LeVeque, F. G., Liggett, W. H., Jr., Mulagha, M. T., Peterson, D. E., Rose, A. H., Schubert, M. M., Spijkervet, F. K. & Wittes, J. P. (1999) Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* **85,** 2103-2113.

253. Sonis, S. T., Elting, L. S., Keefe, D., Peterson, D. E., Schubert, M., Hauer-Jensen, M., Bekele, B. N., Raber-Durlacher, J., Donnelly, J. P. & Rubenstein, E. B. (2004a) Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer* **100,** 1995-2025.

254. Sonis, S. T., O'Donnell, K. E., Popat, R., Bragdon, C., Phelan, S., Cocks, D. & Epstein, J. B. (2004b) The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. *Oral Oncol* **40,** 170-176.

255. Sonis, S. T., Peterson, R. L., Edwards, L. J., Lucey, C. A., Wang, L., Mason, L., Login, G., Ymamkawa, M., Moses, G., Bouchard, P., Hayes, L. L., Bedrosian, C. & Dorner, A. J. (2000) Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncol* **36,** 373-381.

256. South Australian Cancer Registry (2010) Cancer in South Australia 2007 – with projections to 2010. Adelaide: South Australian Department of Health.

257. Specenier, P. & Vermorken, J. B. (2007) The role of taxanes and targeted therapies in locally advanced head and neck cancer. *Curr Opin Oncol* **19,** 195-201.

258. Spitler, L. E., Grossbard, M. L., Ernstoff, M. S., Silver, G., Jacobs, M., Hayes, F. A. & Soong, S. J. (2000) Adjuvant therapy of stage III and IV malignant melanoma using granulocyte-macrophage colony-stimulating factor. *J Clin Oncol* **18,** 1614-1621.

259. Steinberg, J., Halter, J., Schiller, H., Gatto, L., Carney, D., Lee, H. M., Golub, L. & Nieman, G. (2005a) Chemically modified tetracycline prevents the development of septic shock and acute respiratory distress syndrome in a clinically applicable porcine model. *Shock* **24,** 348-356.

260. Steinberg, J., Halter, J., Schiller, H., Gatto, L. & Nieman, G. (2005b) The development of acute respiratory distress syndrome after gut ischemia/reperfusion injury followed by fecal peritonitis in pigs: a clinically relevant model. *Shock* **23,** 129-137.

261. Steiner, W. (1993) Results of curative laser microsurgery of laryngeal carcinomas. *Am J Otolaryngol* **14,** 116-121.

262. Stokman, M. A., Spijkervet, F. K., Boezen, H. M., Schouten, J. P., Roodenburg, J. L. & de Vries, E. G. (2006) Preventive intervention possibilities in radiotherapy- and chemotherapy-induced oral mucositis: results of meta-analyses. *J Dent Res* **85,** 690-700.

263. Strong, M. S. & Jako, G. J. (1972) Laser surgery in the larynx. Early clinical experience with continuous CO 2 laser. *Ann Otol Rhinol Laryngol* **81,** 791-798.

264. Sturgis, E. M. & Cinciripini, P. M. (2007) Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer* **110,** 1429-1435.

265. Sugerman, P. B. & Savage, N. W. (2002) Oral cancer in Australia: 1983-1996. *Aust Dent J* **47,** 45-56.

266. Sulaiman, F., Huryn, J. M. & Zlotolow, I. M. (2003) Dental extractions in the irradiated head and neck patient: a retrospective analysis of Memorial Sloan-Kettering Cancer Center protocols, criteria, and end results. *J Oral Maxillofac Surg* **61,** 1123-1131.

267. Sun, W. L., Chen, L. L., Zhang, S. Z., Ren, Y. Z. & Qin, G. M. (2010) Changes of adiponectin and inflammatory cytokines after periodontal intervention in type 2 diabetes patients with periodontitis. *Arch Oral Biol* **55,** 970-974.

268. Teles, R. P., Sakellari, D., Konstantinidis, A., Socransky, S. S. & Haffajee, A. D. (2009) Application of the checkerboard immunoblotting technique to the quantification of host biomarkers in gingival crevicular fluid. *J Periodontol* **80,** 447-456.

269. Thariat, J., Bolle, S., Demizu, Y., Marcy, P.-Y., Hu, Y., Santini, J., Bourhis, J. & Pommier, P. (2011) New techniques in radiation therapy for head and neck cancer: IMRT, CyberKnife, protons, and carbon ions. Improved effectiveness and safety? Impact on survival? *Anti-Cancer Drugs* **22,** 596-606.

270. Tobias, J. S., Monson, K., Gupta, N., Macdougall, H., Glaholm, J., Hutchison, I., Kadalayil, L. & Hackshaw, A. (2010) Chemoradiotherapy for locally advanced head and neck cancer: 10-year follow-up of the UK Head and Neck (UKHAN1) trial. *Lancet Oncol* **11,** 66-74.

271. Toker, H., Poyraz, O. & Eren, K. (2008) Effect of periodontal treatment on IL-1beta, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis. *J Clin Periodontol* **35,** 507-513.

272. Tonetti, M. S., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A. D., Vallance, P. & Deanfield, J. (2007) Treatment of periodontitis and endothelial function. *N Engl J Med* **356,** 911-920.

273. Tribius, S. & Bergelt, C. (2011) Intensity-modulated radiotherapy versus conventional and 3D conformal radiotherapy in patients with head and neck cancer: is there a worthwhile quality of life gain? *Cancer Treat Rev* **37,** 511-519.

274. Trotti, A., Bellm, L. A., Epstein, J. B., Frame, D., Fuchs, H. J., Gwede, C. K., Komaroff, E., Nalysnyk, L. & Zilberberg, M. D. (2003) Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol* **66,** 253-262.

275. Tsai, C., Hayes, C. & Taylor, G. W. (2002) Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dent Oral Epidemiol* **30,** 182-192.

276. Tsakos, G., Bernabe, E., D'Aiuto, F., Pikhart, H., Tonetti, M., Sheiham, A. & Donos, N. (2010) Assessing the minimally important difference in the oral impact on daily performances index in patients treated for periodontitis. *J Clin Periodontol* **37,** 903-909.

277. Vandenbrouck, C., Sancho-Garnier, H., Chassagne, D., Saravane, D., Cachin, Y. & Micheau, C. (1980) Elective versus therapeutic radical neck dissection in epidermoid carcinoma of the oral cavity: results of a randomized clinical trial. *Cancer* **46,** 386-390.

278. Vaughan, C. W., Strong, M. S. & Jako, G. J. (1978) Laryngeal carcinoma: transoral treatment utilizing the CO2 laser. *Am J Surg* **136,** 490-493.

279. Veldeman, L., Madani, I., Hulstaert, F., De Meerleer, G., Mareel, M. & De Neve, W. (2008) Evidence behind use of intensity-modulated radiotherapy: a systematic review of comparative clinical studies. *Lancet Oncol* **9,** 367-375.

280. Vera-Llonch, M., Oster, G., Hagiwara, M. & Sonis, S. (2006) Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. *Cancer* **106,** 329-336.

281. Vissink, A., Jansma, J., Spijkervet, F. K., Burlage, F. R. & Coppes, R. P. (2003) Oral sequelae of head and neck radiotherapy. *Crit Rev Oral Biol Med* **14,** 199-212.

282. Wallace, B., Minderman, H., O'Loughlin, K. & Podniesinski, E. (2012) Multiplex Bead Array for the Department of Flow and Image Cytometry.

283. Weissberg, J. B., Pillsbury, H., Sasaki, C. T., Son, Y. H. & Fischer, J. J. (1983) High fractional dose irradiation of advanced head and neck cancer. Implications for combined radiotherapy and surgery. *Arch Otolaryngol* **109,** 98-102.

284. WHO (2012) International Classification of Diseases (ICD).

285. Wilson, T. G., Jr., Glover, M. E., Malik, A. K., Schoen, J. A. & Dorsett, D. (1987) Tooth loss in maintenance patients in a private periodontal practice. *J Periodontol* **58,** 231-235.

286. Wong, C. H., Kelly, L. E. & Tripcony, L. B. (2007) Nasopharygeal carcinoma in Queensland, Australia: a review of 10 years experience. *Australas Radiol* **51,** 276-282.

287. Wong, P. C., Dodd, M. J., Miaskowski, C., Paul, S. M., Bank, K. A., Shiba, G. H. & Facione, N. (2006) Mucositis pain induced by radiation therapy: prevalence, severity, and use of self-care behaviors. *J Pain Symptom Manage* **32,** 27-37.

288. Wong, R. M., Ng, S. K., Corbet, E. F. & Keung Leung, W. (2012) Non-surgical periodontal therapy improves oral health-related quality of life. *J Clin Periodontol* **39,** 53-61.

289. Woo, S. B., Lee, S. J. & Schubert, M. M. (1997) Graft-vs.-host disease. *Crit Rev Oral Biol Med* **8,** 201-216.

290. Wood, R. M., Lander, V. L., Mosby, E. L. & Hiatt, W. R. (1989) Nutrition and the head and neck cancer patient. *Oral Surg Oral Med Oral Pathol* **68,** 391-395.

291. Worthington, H. V., Clarkson, J. E., Bryan, G., Furness, S., Glenny, A. M., Littlewood, A., McCabe, M. G., Meyer, S. & Khalid, T. (2011) Interventions for preventing oral mucositis for patients with cancer receiving treatment (review). *Cochrane Database Syst Rev***,** CD000978.

292. Worthington, H. V., Clarkson, J. E., Khalid, T., Meyer, S. & McCabe, M. (2010) Interventions for treating oral candidiasis for patients with cancer receiving treatment. *Cochrane Database Syst Rev***,** CD001972.

293. Yoshinari, N., Kawase, H., Mitani, A., Ito, M., Sugiishi, S., Matsuoka, M., Shirozu, N., Ishihara, Y., Bito, B., Hiraga, M., Arakawa, K. & Noguchi, T. (2004) Effects of scaling and root planing on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1beta in gingival crevicular fluid and gingival tissues. *J Periodontal Res* **39,** 158-167..

294. Young, M. R., Neville, B. W., Chi, A. C., Lathers, D. M., Boyd Gillespie, M. & Day, T. A. (2007) Oral premalignant lesions induce immune reactivity to both premalignant oral lesions and head and neck squamous cell carcinoma. *Cancer Immunol Immunother* **56,** 1077-1086.

295. Yuen, A. P., Ho, C. M., Chow, T. L., Tang, L. C., Cheung, W. Y., Ng, R. W., Wei, W. I., Kong, C. K., Book, K. S., Yuen, W. C., Lam, A. K., Yuen, N. W., Trendell-Smith, N. J., Chan, Y. W., Wong, B. Y., Li, G. K., Ho, A. C., Ho, W. K., Wong, S. Y. & Yao, T. J. (2009) Prospective randomized study of selective neck dissection versus observation for N0 neck of early tongue carcinoma. *Head Neck* **31,** 765-772.

296. Zackrisson, B., Mercke, C., Strander, H., Wennerberg, J. & Cavallin-Stahl, E. (2003) A systematic overview of radiation therapy effects in head and neck cancer. *Acta Oncol* **42,** 443-461.

297. Zee, K. Y. (2009) Smoking and periodontal disease. *Aust Dent J* **54 Suppl 1,** S44-50.

298. Zeitels, S. M., Dailey, S. H. & Burns, J. A. (2004) Technique of en block laser endoscopic frontolateral laryngectomy for glottic cancer. *Laryngoscope* **114,** 175-180.

Chapter 2: Influence of periodontitis on the experience of oral mucositis in cancer patients undergoing head and neck radiotherapy

Arlene Khaw[1,2], Sharon Liberali[3], Richard Logan[1], Dorothy Keefe[4], P. Mark Bartold[1,2]

[1] School of Dentistry, Faculty of Health Sciences, University of Adelaide

[2] Colgate Australian Clinical Dental Research Centre, University of Adelaide

[3] Special Needs Unit, Adelaide Dental Hospital

[4] School of Medicine, Faculty of Health Sciences, University of Adelaide

2.1 Introduction

Virtually all patients who receive head and neck radiotherapy develop some degree of oral mucositis (Sonis, 2011). Most patients consistently report oral mucositis as the most debilitating acute side effect of radiotherapy that they experience. Severe oral mucositis may necessitate an interruption in the course of radiotherapy, and can thus serve as a dose-limiting factor (Denham et al., 1999, Sonis et al., 1999, Vera-Llonch et al., 2006, Rosenthal, 2007). The pathogenesis of radiation-induced oral mucositis involves the production of a range of inflammatory molecules and proteins such as interleukin (IL)-1β, IL-6 and tumour-necrosis factor, that lead to apoptosis and tissue injury (Sonis, 2002). These factors are not only damaging, but also provide a positive feedback loop that drives the destructive process forward.

Periodontitis is a host-driven inflammatory response to a pathogenic bacterial biofilm in the subgingival environment, resulting in the progressive destruction of the tissues that support the teeth; specifically the gingiva, periodontal ligament, and alveolar bone (Kornman, 2008). This disease affects more than 50% of the population (Roberts-Thomson, 2007, Hugoson et al., 2008, Eke et al., 2012). Given its high prevalence, periodontitis can be considered an important global health problem in terms of quality of life. Despite the localised nature of periodontitis, a plethora of systemic inflammatory markers of this disease such as C-reactive proteins (CRP), IL-6, IL-1β and TNF, have been reported and it is speculated that these contribute to systemic diseases such as cardiovascular disease, diabetes mellitus and rheumatoid arthritis (Loos, 2005, Madianos et al., 2010).

Considering that radiation-induced oral mucositis and periodontitis are both characterised by the continuing presence of systemic inflammation, they may be associated through a primed inflammatory response as proposed by the “two-hit” model (Golub et al., 2006). Alternatively, both conditions may be correlated as they represent a dysregulation of

the inflammatory response (Sonis et al., 2004, Bartold et al., 2010). To date, no studies have investigated the association between these conditions.

In light of the above, we hypothesized that patients with higher severity of periodontitis will suffer from a higher severity of oral mucositis following head and neck radiotherapy. Therefore, the aim of this study is to determine whether the severity of oral mucositis is associated with the severity of periodontitis in cancer patients undergoing head and neck radiotherapy.

2.2 Materials and methods

2.2.1 Study sample

This prospective cohort study was approved by the Human Research Ethics Committee of the University of Adelaide (H-229-2011) and SA Health, Government of South Australia.

Eighty-five consecutive patients seeking oral health assessment prior to head and neck radiotherapy at the Special Needs Unit, Adelaide Dental Hospital, between February 2012 and December 2012, were assessed for their eligibility for participation in the study. Inclusion criteria included a willingness to participate, an age of 25 and above, and those receiving head and neck radiotherapy as a radical treatment for cancer. Exclusion criteria included patients who could not give informed consent (e.g. a poor understanding of English, visually and/or hearing impaired, severely ill), would need antibiotic prophylaxis before any medical/ dental procedures, recent periodontal therapy (within the previous six months), and those receiving a radiotherapy dosage of less than 50 Gy. Forty-one patients met the inclusion criteria. They were divided into two groups; with and without periodontitis (Page & Eke 2007, Eke et al. 2012). The study protocol was explained to each patient and all signed an informed consent form.

Demographic data and medical history were assessed through interview during initial consultation. The questionnaire included items on age, smoking habit (five categories), antibiotic therapy within the last six months for symptoms arising from cancer or its treatment, systemic diseases (e.g. cardiovascular disease, diabetes mellitus, rheumatoid arthritis) and medications other than antibiotics. Smoking habit was divided into five categories: non-smoker, former smoker (tobacco cessation preceded ≥ 5 years), occasional smoker (up to 10 cigarettes per day), smoker (up to 20 cigarettes per day) and heavy smoker (more than 20 cigarettes per day) (Lang and Tonetti, 2003). Information regarding the site of primary tumour, radiotherapy prescription, whether or not surgical excision of the tumour was performed and presence of concurrent chemotherapy was obtained from the Special Needs Unit and the Radiation Oncology Unit at the Royal Adelaide Hospital.

2.2.2 Scoring of radiation-induced oral mucositis

Mucosal reactions within the irradiated field were examined clinically and described weekly until the completion of radiotherapy by a single examiner, a specialist in Special Needs Dentistry (S.L.) according to the WHO system (Table 14) (Scully et al., 2006). This study was limited to "acute mucositis" which occurs during active therapy with no intention of capturing potential delayed consequences of mucositis.

Score	Criteria
0	No changes
1	Soreness with erythema
2	Erythema, ulcers, can eat solids
3	Ulcers, only liquid diet
4	Alimentation not possible

Table 14: WHO system to score the severity of oral mucositis (Scully et al., 2006)

2.2.3 Periodontal clinical measurements

Following assessment of demographic data and medical history, full-mouth periodontal variables were assessed by a single examiner who is a periodontics registrar

(A.K.) using a Michigan O periodontal probe with Williams markings at six sites/tooth. The plaque index (Table 15) (Silness and Löe, 1964) was recorded by assigning a score from 0-3 to each surface and calculating the full mouth mean plaque score. The percentage of sites which bled on gentle probing (BOP) was calculated after assessing dichotomously the presence of bleeding from the bottom of the pocket with a manual probe. Full mouth probing depths (PD) and recession of the gingival margin (REC) were recorded at the same time, with measurements rounded to the nearest millimetre. Recession (REC) was recorded as a positive value if the free gingival margin occurred apical to the cemento-enamel junction (CEJ), whereas it was recoded as a negative value if it was coronal to the CEJ. In the latter case, the examiner reinserted the probe angled 45^{o} into the site in order to detect the CEJ. Full-mouth clinical attachment levels (CAL) were calculated as PD plus REC.

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Table 15: Plaque index system (Silness and Löe, 1964)

2.2.4 Radiographic periodontal bone loss

A radiographic examination was performed by a single examiner (A.K.) who examined and categorised periodontal bone loss severity from a standardised orthopantomograph. The extent of bone loss was established using the modified Hugoson and Jordan classification method (Table 16) (Mercado et al., 2000) whereby the distance from the CEJ to the tooth apex was measured. This measurement was divided into thirds (1/3, 2/3 and greater than 2/3 of the root length) and used as a scale to assess the amount of bone loss

measured from the crest of the alveolar bone to the CEJ. The most severe bone loss was used to classify the status of each patients.

Score	Criteria
0	No discernible radiographic evidence of bone loss
1	(Mild) proximal bone loss reaching at most 1/3 of normal bone height
2	(Moderate) proximal bone loss between 1/3 and 2/3 of normal bone height
3	(Severe) proximal bone loss more than 2/3 of normal bone height

Table 16: Modified Hugoson and Jordan classification for periodontal disease (Mercado et al., 2000)

2.2.5 Gingival crevicular fluid (GCF) sampling

Gingival crevicular fluid (GCF) was sampled from at least one site per patient by a single examiner (A.K.). For periodontitis patients, the deepest site was selected, while for periodontally healthy patients, the most convenient site was selected. Only one site was sampled per patient in order to improve patient acceptance, given that these patients are often in a hurry to rush to another medical appointment. The site to be sampled was isolated with cotton rolls and dried gently with an air syringe. Supragingival plaque was carefully removed before sampling. A timed, 30-second GCF sample was collected using a paper strip (Periopaper, Oraflow). The strip was inserted into the pocket until mild resistance was felt. If the sample was contaminated with blood or saliva, it was discarded and a new sample taken. The volume for each strip was measured with a calibrated meter (Periotron 8000). For each subject, the strip was wrapped in aluminium foil, placed into a storage tube and stored at -20^{o}C pending analysis.

2.2.6 Preparation of GCF samples

One strip per patient was analysed. The strip was inserted into an Eppendorf tube containing 0.5 ml of phosphate-buffered saline. After elution for 30 minutes at room temperature, the strips were removed and samples were centrifuged at 3,000 x g for 5 minutes. The supernatant was collected and analysed.

2.2.7 Multiplexed bead immunoassay

Cytokine levels were determined using a multiplexed bead immunoassay. Fifty microlitres of GCF sample were analysed for interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, tumour necrosis factor (TNF) and interferon (IFN)-γ, using a commercially available kit (MILLIPLEX® MAP kit, Millipore, Lot #2110182) in an analyser (Luminex 200, Millipore) according to the manufacturer's instructions. Briefly, 96-well filter plates were pre-wet with assay buffer and the solution was aspirated from the wells using a vacuum manifold (Millipore). Microsphere beads coated with monoclonal antibodies against the eight different target analytes were added to the wells. Samples, controls and standards (ranging from 3.2 to 2,000 pg/ml for each analyte) were pipetted into the wells and incubated overnight at 4^{o}C. The wells were washed with washing buffer and aspirated using a vacuum manifold. Subsequently, a mixture of biotinylated secondary antibodies was added. After incubation for one hour, streptavidin conjugated to the fluorescent protein R-phycoerythrin (streptavidin-RPE) was added to the beads and incubated for 30 minutes. After washing to remove unbound reagents, sheath fluid (Luminex, Millipore) was added to the wells and the beads (≥100 per analyte) analysed in the bead analyser (Luminex 200, Millipore). The concentrations of the unknown samples (antigens in GCF samples) were estimated from a standard curve using a software program (Xponent version 3.1) and the cytokine levels were expressed as the total amount (pg) per site.

2.2.8 Statistical analysis

An initial power analysis based on an alpha value of 0.05 and statistical power of 0.80 resulted in a required sample size of 28 to detect a moderate correlation of 0.5.

Data collected from the study was entered into an Excel (Microsoft Office 2010) database and proofed for entry errors. The database was subsequently locked, imported into

SAS 9.3 (SAS Institute Inc., Cary, NC, USA), formatted and analysed. Due to the small sample size, several variables were dichotomised (Table 17).

Variables	New category (dichotomised)	Description
Smoking status	No	Scores 0,1
	Yes	Scores 2,3,4
Severity of oral mucositis	Can take solids	Scores 0,1,2
	Liquids only/ alimentation not possible	Scores 3,4
Severity of radiographic bone loss	None/ mild	Scores 0,1
	Moderate/ severe	Scores 2,3

Table 17: Dichotomisation of smoking status, severity of oral mucositis and severity of radiographic bone loss

The association between each variable and the severity of oral mucositis, the association between demographic variables and smoking status, and the severity of periodontal disease were analysed. Logistic regression was used to obtain odds ratios (ORs) and 95% confidence interval for categorical outcome variables, while linear regression was used to obtain mean estimates and 95% CI for continuous outcome variables. To determine whether the presence of periodontitis was associated with the severity of oral mucositis, two-way contingency tables were prepared and the data was analysed using the Fisher's Exact Test. Statistical significance was set at $p<0.05$.

2.3 Results

Among the 41 patients who consented to the study, five did not attend any of their review appointments, two subsequently received a radiotherapy dosage of less than 50 Gy and one declined cancer treatment. Thus, in the present analysis, data from 33 patients have been included for the oral mucositis assessment. However, of this cohort of 33, five patients were edentulous, leaving 28 patients suitable for a periodontal assessment. The mean age and standard deviation of the overall study population was 63.3 years ± 11.0 (range 44.8 to 82.9 years).

Table 18 shows the frequency of the demographic data, medical history, site of primary tumour, radiotherapy prescription, whether or not surgical excision of tumour was performed, the presence of concurrent chemotherapy, severity of oral mucositis and severity of radiographic bone loss for all 33 patients. The majority of patients were male (73%) and either non-smokers or former smokers (70%). Over half of the patients had received antibiotic therapy within the last six months (61%) for symptoms arising from cancer or its treatment such as post-surgical excision of tumour. Almost half of the patients were systemically healthy and were not on daily medications (48%), while the remaining suffered from systemic diseases, such as cardiovascular disease, and were on daily medications such as anti-hypertension drugs (52%). The primary tumour site was most commonly the oral and salivary gland (45%), followed by the pharynx (33%) and larynx and others (21%). The most frequent other site was the skin, in the form of melanoma. The majority of patients underwent conventional fractionation radiotherapy (85%) and received a total dose of 60-69 Gy (85%) for a duration of 6 weeks (79%). The patients were almost equally divided between unilateral (48%) and bilateral radiation (52%). About one-third of the patients received concurrent chemotherapy (36%), while the majority underwent surgical excision of tumour (70%). During radiotherapy, more than half of the patients experienced grade 3 or 4 oral mucositis (64%) which prevented them from eating solids or eating altogether. Only one patient showed no changes in the oral mucosa throughout the entire duration of radiotherapy (grade 0). Less than half of the patients demonstrated moderate or severe radiographic bone loss (39%).

Categorical variables	All subjects, n=33 (%)
Gender	
Male	24 (73)
Female	9 (27)
Smoking status	
No	23 (70)
Yes	10 (30)
Antibiotic therapy within the last 6 months	
No	13 (39)
Yes	20 (61)
Presence of systemic diseases (e.g. CVD, DM, RA)	
No	16 (48)
Yes	17 (52)
On daily medication (e.g. anti-HPT, OHA, NSAIDS)	
No	16 (48)
Yes	17 (52)
Primary tumour site	
Oral and salivary gland	15 (45)
Pharynx	11 (33)
Larynx and others	7 (21)
Type of radiotherapy	
Conventional fractionation	28 (85)
Intensity modulated radiotherapy (IMRT)	5 (15)
Total dose of radiotherapy	
50-59 Gy	2 (6)
60-69 Gy	28 (85)
≥70 Gy	3 (9)
Duration of radiotherapy	
5 weeks	2 (6)
6 weeks	26 (79)
7 weeks	5 (15)
Field of radiotherapy	
Unilateral	16 (48)
Bilateral	17 (52)
Concurrent chemotherapy	
No	21 (64)
Yes	12 (36)
Post-surgery	
No	10 (30)
Yes	23 (70)
Severity of oral mucositis	
Can take solids	12 (36)

Liquids only/ alimentation not possible	21 (64)
Severity of radiographic bone loss	
None/ mild	17 (61)
Moderate/ severe	11 (39)

(CVD, cardiovascular disease; DM, diabetes mellitus; RA, rheumatoid arthritis; anti-HPT, anti-hypertensive agents; OHA, oral hypoglycaemic agents; NSAIDS, non-steroidal anti-inflammatory drugs)

Table 18: Frequency of categorical variables for all 33 patients

Table 19 shows the results of logistic regression analyses for variables with a possible association with the severity of oral mucositis. Duration of radiotherapy was the only variable found to be significantly associated with the severity of oral mucositis (p-value=0.038). The severity of oral mucositis did not appear to correlate significantly with patient's age, gender, smoking status, antibiotic therapy within the last 6 months, the presence of systemic diseases, daily medications, primary tumour site, type, total dose or field of radiotherapy, concurrent chemotherapy, history of surgical excision of tumour, number of teeth, mean plaque score or clinical, radiological and immunological signs of periodontitis.

Variables	OR	95% CI	p-value
Age	1.00	0.94 to 1.07	0.90
Gender (female versus male)	6.77	0.73 to 62.86	0.093
Smoking status (non-smoker versus smoker)	3.08	0.53 to 17.80	0.21
Antibiotic therapy within the last 6 months (no versus yes)	0.50	0.12 to 2.13	0.35
Presence of cardiovascular disease (no versus yes)	0.81	0.18 to 3.60	0.78
On anti-hypertensive agents (no versus yes)	0.81	0.18 to 3.60	0.78
Primary tumour site (larynx and others versus oral and salivary gland)	0.20	0.028 to 1.42	0.099
Primary tumour site (larynx and others versus pharynx)	0.09	0.009 to 0.84	0.099

Primary tumour site (oral and salivary gland versus pharynx)	0.44	0.068 to 2.89	0.099
Type of radiotherapy (conventional versus IMRT)	1.20	0.17 to 8.43	0.85
Total dose of radiotherapy	1.22	0.99 to 1.51	0.060
Duration of radiotherapy			0.038[1]*
Field of radiotherapy (unilateral versus bilateral)	0.91	0.22 to 3.76	0.90
Concurrent chemotherapy (no versus yes)	0.22	0.04 to 1.257	0.090
Post-surgery (no versus yes)	0.44	0.095 to 2.01	0.29
Number of teeth	0.97	0.90 to 1.05	0.41
Mean Plaque Score	0.90	0.30 to 2.67	0.85
% sites CAL≥3mm	1.01	0.97 to 1.05	0.63
% sites CAL≥4mm	1.01	0.98 to 1.05	0.53
% sites CAL≥5mm	1.01	0.97 to 1.05	0.54
% sites CAL≥6mm	1.01	0.96 to 1.08	0.65
% sites PPD≥4mm	1.01	0.97 to 1.05	0.54
% sites PPD≥5mm	1.02	0.97 to 1.08	0.42
% sites PPD≥6mm	1.01	0.89 to 1.15	0.86
% sites BOP	1.02	0.97 to 1.06	0.43
CAL mean	1.27	0.53 to 3.05	0.59
CAL SD	2.35	0.27 to 20.31	0.44
PPD mean	1.34	0.41 to 4.35	0.62
PPD SD	1.20	0.22 to 6.50	0.83
Radiographic bone loss (none/ mild versus moderate/severe)	0.82	0.17 to 3.90	0.80

Cytokine level IL-1α	0.79	0.37 to 1.68	0.53
Cytokine level IL-1β (units=0.0001)	1.00	1.00 to 1.002	0.73
Cytokine level IL-2 (units=0.0001)	0.98	0.89 to 1.08	0.67
Cytokine level IL-4 (units=0.0001)	1.00	0.99 to 1.01	0.53
Cytokine level IL-6 (units=0.0001)	1.03	0.96 to 1.11	0.41
Cytokine level IL-8	0.70	0.20 to 2.42	0.57
Cytokine level TNF (units=0.0001)	1.00	0.97 to 1.04	0.96
Cytokine level IFN-γ (units=0.0001)	0.99	0.93 to 1.04	0.58

[1] Fisher's Exact Test P value: sparse cells in contingency table meant that the validity of the logistic model fit is questionable, so a two-way contingency table was used instead

* Estimates/ OR statistically significant (p-value<0.05)

Table 19: Association between various predictors and the severity of oral mucositis

To further assess whether oral mucositis could be associated with periodontitis, two-way contingency tables were prepared according to the presence or absence of periodontitis for each of the five oral mucositis scores (Table 20). For these analyses, 28 patients were included because five patients from the original cohort of 33 were edentulous. No statistically significant association could be determined although some interesting trends were noted. For oral mucositis grades 1,2 and 3, 100%, 71% and 83% respectively of the cases had periodontitis. For grade 4 oral mucositis, the incidence of periodontitis reduced to 60%.

	Without Periodontitis	With Periodontitis	Total
Oral mucositis Grade 0	0 (0%)	1 (100%)*	1
Oral mucositis Grade 1	0 (0%)	3 (100%)*#	3
Oral mucositis Grade 2	2 (29%)	5 (71%)*#	7
Oral mucositis Grade 3	2 (17%)	10 (83%)*#	12
Oral mucositis Grade 4	2 (40%)	3 (60) *#	5
Total number of subjects	6	22	28

Case definition for periodontitis was ≥2 sites with CAL≥3mm and ≥2 sites with PPD≥4mm (Page & Eke 2007, Eke et al. 2012). *p=0.73 for association between all grades of oral mucositis (grade 0-4) and presence of periodontitis. #p=0.14 for association between oral mucositis grade 1-4 and periodontitis

Table 20: Distribution of patients without and with periodontitis according to oral mucositis grading 0 to 4

2.4 Discussion

This pilot study investigated the association between the severity of oral mucositis and the severity of periodontitis in 33 cancer patients undergoing head and neck radiotherapy.

The age, gender and primary tumour site of these patients reflected the epidemiology of head and neck cancer. In this study, the mean age was 63.3 years ± 11.0, which was very close to the mean age of 450 head and neck cancer patients (61.3 years ± 12.3) in the United States who received radiotherapy (Vera-Llonch et al., 2006). Head and neck cancer was also found to affect more males (73%) than females, consistent with reports by Vera-Llonch et al. (2006) (78.6%) and Trotti et al. (2003) (81%). This study also found that the oral cavity and salivary glands was the most common primary tumour site for head and neck cancer (45%), followed by pharynx (33%) and larynx and others (21%). These proportions were very close to those estimated by the International Agency for Research on Cancer for the burden of cancer worldwide in 2008 (41.6%, 34.6% and 23.9% respectively) (Ferlay et al., 2010). This data implied that the patient group investigated was representative of the general head and neck cancer population.

A number of scoring systems have been defined to assess the severity of oral mucositis but no single scale has been uniformly employed (Scully et al., 2006, Sonis et al., 1999). In this study, we used the established guidelines proposed by the World Health Organization (WHO) in 1979 (Scully et al., 2006). This system relied on a combination of clinician-based observations (erythema and ulceration) and functional outcomes (ability to eat). Ease of use is the strength of the WHO scale. A potential confounder of the WHO scale was the need for the evaluator to determine the patient's ability to eat. Therefore, the evaluator had to be certain that the subject's inability to eat was caused by mucositis and not by another cause such as nausea (Sonis, 2011). According to these criteria, the incidence of grade 3 and 4 oral mucositis in the present study was 64%, similarly reported by Vera-Llonch et al. (2006) (63.8%). Although the grading was based on the investigator's judgement rather than a standardised scoring system, these were performed by experienced clinicians (i.e. medical and radiation oncologists).

This study found that the duration of radiotherapy was the only variable significantly associated with the severity of oral mucositis (p-value=0.038). This was in agreement with other studies which have reported that type and total dose of radiotherapy were overwhelming risk factors for the development of radiation-induced oral mucositis (Weissberg et al., 1983, Pinto et al., 1991, Horiot et al., 1992, Horiot et al., 1997, Bensadoun et al., 2001, Awwad et al., 2002, Zackrisson et al., 2003, Trotti et al., 2003, Vera-Llonch et al., 2006).

To the best of our knowledge, no studies have looked into the association between radiation-induced oral mucositis and periodontitis. Considering that both conditions are characterised by the continuing presence of systemic inflammation, they may be associated through a primed inflammatory response as proposed by the "two-hit" model (Golub et al., 2006). This model suggests that inflammation at the periodontium level which is periodontitis (first "hit") followed by radiation (second "hit") can lead to an exacerbated response in the

form of oral mucositis. The converse may also hold true in that radiation-induced oral mucositis (first "hit") exacerbates the inflammatory response of developing periodontitis (second "hit") (second "hit") (Figure 19). The "two-hit" model has been previously used to explain the pathogenesis of acute respiratory distress syndrome (ARDS) (Carney et al., 1999, Steinberg et al., 2005), as well as the association between periodontitis and systemic diseases such as rheumatoid arthritis (Ramamurthy et al., 2005, Bartold et al., 2010, Cantley et al., 2011, Ribeiro et al., 2005, Al-Katma et al., 2007, Ortiz et al., 2009, Erciyas et al., 2012). Alternatively, both conditions may be correlated as they represent a dysregulation of the inflammatory response (Bartold et al., 2010, Sonis, 2004, Sonis, 2002, Sonis et al., 2004).

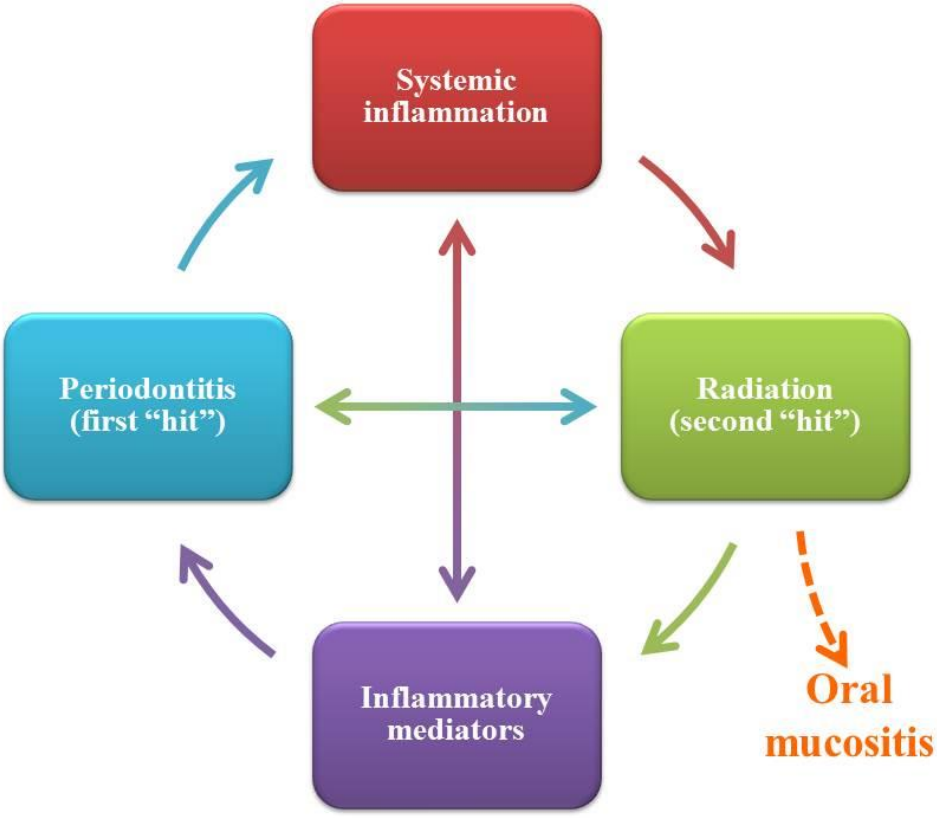


Figure 19: A hypothetical "two-hit" model demonstrating the association between radiation-induced oral mucositis and periodontitis

Data from this pilot study demonstrated some interesting trends with regard to an association between radiation-induced oral mucositis and periodontitis with more periodontitis patients present in all four grades of mucositis (grades 1-4). However, these

trends were not statistically significant. There are several explanations for this. Firstly, the severity of periodontitis may have been over- or underestimated because we recruited patients after they had undergone surgical excision of their tumour. To avoid a second surgical trauma to the patient, teeth with hopeless prognosis and teeth in close proximity to the tumour were often extracted during the resective surgery. In the future, these patients should be recruited prior to their surgery, so that a more accurate assessment of their periodontal status can be made.

Secondly, these patients were on various cancer treatment regimens. Even if the severity of periodontitis had an effect on the severity of oral mucositis, this association would have been obscured by the differences in the aggressiveness of the cancer treatment that they received. Future studies may want to focus on a particular radiotherapy protocol or exclude patients on concurrent chemotherapy, because chemotherapy itself can cause oral mucositis.

Thirdly, one could question whether the sample size was sufficient to demonstrate an association. Based on the required sample size of 28 from the initial power analysis, this study which included 33 patients, had sufficient power to show a significant association in a univariate analysis. However, this sample size was insufficient to demonstrate an association between both conditions in a multivariate model. Post factum calculations showed that 75 patients would be needed in order to demonstrate an association between both conditions, while controlling five variables such as primary tumour site, type, total dose and duration of radiotherapy, as well as concurrent chemotherapy. This would have been a difficult task to achieve given the duration of the study.

Although pilot in nature, this study demonstrated that patients seeking oral health assessment prior to head and neck radiotherapy at the Special Needs Unit, Adelaide Dental Hospital, were a good representation of the general head and neck cancer population. As such, this unit can potentially be a centre for retrieval of valuable data pertaining to the

characteristics and management of these patients. In conclusion, this study did not demonstrate an association between radiation-induced oral mucositis and periodontitis. Nonetheless, a trend towards increased pocket depth and clinical attachment levels was noted in patients with oral mucositis grades 1-4. The resultant lack of association was attributed to the extraction of teeth prior to periodontal examination, lack of uniformity of cancer treatment regimens and lack of statistical power. Hence, larger studies with a tighter inclusion criteria (e.g. similar radiotherapy protocol, without chemotherapy or surgery) are now required to follow-up on these preliminary findings.

2.5 References

1. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03: June 14 2010.
2. National Cancer Institute Common Toxicity Criteria. Version 2.0, April 30, 1999.
3. Al-Katma, M. K., Bissada, N. F., Bordeaux, J. M., Sue, J. & Askari, A. D. (2007) Control of periodontal infection reduces the severity of active rheumatoid arthritis. *J Clin Rheumatol* **13,** 134-137.
4. Awwad, H. K., Lotayef, M., Shouman, T., Begg, A. C., Wilson, G., Bentzen, S. M., Abd El-Moneim, H. & Eissa, S. (2002) Accelerated hyperfractionation (AHF) compared to conventional fractionation (CF) in the postoperative radiotherapy of locally advanced head and neck cancer: influence of proliferation. *Br J Cancer* **86,** 517-523.
5. Bartold, P. M., Marino, V., Cantley, M. & Haynes, D. R. (2010) Effect of Porphyromonas gingivalis-induced inflammation on the development of rheumatoid arthritis. *J Clin Periodontol* **37,** 405-411.
6. Bensadoun, R. J., Magne, N., Marcy, P. Y. & Demard, F. (2001) Chemotherapy- and radiotherapy-induced mucositis in head and neck cancer patients: new trends in pathophysiology, prevention and treatment. *Eur Arch Otorhinolaryngol* **258,** 481-487.
7. Cantley, M. D., Haynes, D. R., Marino, V. & Bartold, P. M. (2011) Pre-existing periodontitis exacerbates experimental arthritis in a mouse model. *J Clin Periodontol* **38,** 532-541.
8. Carney, D. E., Lutz, C. J., Picone, A. L., Gatto, L. A., Ramamurthy, N. S., Golub, L. M., Simon, S. R., Searles, B., Paskanik, A., Snyder, K., Finck, C., Schiller, H. J. & Nieman, G. F. (1999) Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* **100,** 400-406.
9. Denham, J. W., Peters, L. J., Johansen, J., Poulsen, M., Lamb, D. S., Hindley, A., O'Brien, P. C., Spry, N. A., Penniment, M., Krawitz, H., Williamson, S., Bear, J. &

Tripcony, L. (1999) Do acute mucosal reactions lead to consequential late reactions in patients with head and neck cancer? *Radiother Oncol* **52,** 157-164.

10. Dvorak, G., Reich, K., Tangl, S., Lill, C. A., Gottschalk-Baron, M., Watzek, G., Gruber, R. & Haas, R. (2009) Periodontal histomorphometry and status of aged sheep subjected to ovariectomy, malnutrition and glucocorticoid application. *Arch Oral Biol* **54,** 857-863.
11. Eke, P. I., Dye, B. A., Wei, L., Thornton-Evans, G. O. & Genco, R. J. (2012) Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* **91,** 914-920.
12. Erciyas, K., Sezer, U., Ustun, K., Pehlivan, Y., Kisacik, B., Senyurt, S., Tarakcioglu, M. & Onat, A. (2013) Effects of periodontal therapy on disease activity and systemic inflammation in rheumatoid arthritis patients. *Oral Dis*. **19**: 394-400
13. Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. & Parkin, D. M. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. **127**: 2893-917
14. Golub, L. M., Payne, J. B., Reinhardt, R. A. & Nieman, G. (2006) Can systemic diseases co-induce (not just exacerbate) periodontitis? A hypothetical "two-hit" model. *J Dent Res* **85,** 102-105.
15. Golub, L. M., Ramamurthy, N. S., Llavaneras, A., Ryan, M. E., Lee, H. M., Liu, Y., Bain, S. & Sorsa, T. (1999) A chemically modified nonantimicrobial tetracycline (CMT-8) inhibits gingival matrix metalloproteinases, periodontal breakdown, and extra-oral bone loss in ovariectomized rats. *Ann N Y Acad Sci* **878,** 290-310.
16. Horiot, J. C., Bontemps, P., van den Bogaert, W., Le Fur, R., van den Weijngaert, D., Bolla, M., Bernier, J., Lusinchi, A., Stuschke, M., Lopez-Torrecilla, J., Begg, A. C., Pierart, M. & Collette, L. (1997) Accelerated fractionation (AF) compared to conventional fractionation (CF) improves loco-regional control in the radiotherapy of

advanced head and neck cancers: results of the EORTC 22851 randomized trial. *Radiother Oncol* **44,** 111-121.

17. Horiot, J. C., Le Fur, R., N'Guyen, T., Chenal, C., Schraub, S., Alfonsi, S., Gardani, G., Van Den Bogaert, W., Danczak, S., Bolla, M. & et al. (1992) Hyperfractionation versus conventional fractionation in oropharyngeal carcinoma: final analysis of a randomized trial of the EORTC cooperative group of radiotherapy. *Radiother Oncol* **25,** 231-241.
18. Hugoson, A., Sjodin, B. & Norderyd, O. (2008) Trends over 30 years, 1973-2003, in the prevalence and severity of periodontal disease. *J Clin Periodontol* **35,** 405-414.
19. Kornman, K. S. (2008) Mapping the pathogenesis of periodontitis: a new look. *J Periodontol* **79,** 1560-1568.
20. Lang, N. P. & Tonetti, M. S. (2003) Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT). *Oral Health Prev Dent* **1,** 7-16.
21. Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *J Periodontol* **76,** 2106-2115.
22. Madianos, P. N., Bobetsis, Y. A. & Van Dyke, T. E. (2010) Chapter 3: Infection and inflammation. In: *Periodontal disease and overall health: a clinician's guide*, (eds.) R. J. Genco & R. C. Williams. Professional Audience Communications, Inc.
23. Mercado, F., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2000) Is there a relationship between rheumatoid arthritis and periodontal disease? *J Clin Periodontol* **27,** 267-272.
24. Ortiz, P., Bissada, N. F., Palomo, L., Han, Y. W., Al-Zahrani, M. S., Panneerselvam, A. & Askari, A. (2009) Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. *J Periodontol* **80,** 535-540.
25. Page, R.C., Eke, P.I. (2007) Case definitions for use in population-based surveillance of periodontitis. *J Periodontol.* **78** (7 Suppl):1387-99.

26. Pinto, L. H., Canary, P. C., Araujo, C. M., Bacelar, S. C. & Souhami, L. (1991) Prospective randomized trial comparing hyperfractionated versus conventional radiotherapy in stages III and IV oropharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* **21,** 557-562.

27. Ramamurthy, N. S., Greenwald, R. A., Celiker, M. Y. & Shi, E. Y. (2005) Experimental arthritis in rats induces biomarkers of periodontitis which are ameliorated by gene therapy with tissue inhibitor of matrix metalloproteinases. *J Periodontol* **76,** 229-233.

28. Ribeiro, J., Leao, A. & Novaes, A. B. (2005) Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol* **32,** 412-416.

29. Roberts-Thomson, K. F. (2007) Chapter 5. Oral health status. In: *Australia's Dental Generations: The National Survey of Adult Oral Health 2004–06. Canberra: Australian Institute of Health and Welfare*, (eds.) G. D. Slade, A. J. Spencer & K. F. Roberts-Thomson, pp. 81-142.

30. Rosenthal, D. I. (2007) Consequences of mucositis-induced treatment breaks and dose reductions on head and neck cancer treatment outcomes. *J Support Oncol* **5,** 23-31.

31. Scully, C., Sonis, S. & Diz, P. D. (2006) Oral mucositis. *Oral Dis* **12,** 229-241.

32. Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy ii. correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* **22,** 121-135.

33. Sonis, S. T. (2002) The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Crit Rev Oral Biol Med* **13,** 380-389.

34. Sonis, S. T. (2004) The pathobiology of mucositis. *Nat Rev Cancer* **4,** 277-284.

35. Sonis, S. T. (2011) Oral mucositis. *Anti-Cancer Drugs* **22,** 607-612.

36. Sonis, S. T., Eilers, J. P., Epstein, J. B., LeVeque, F. G., Liggett, W. H., Jr., Mulagha, M. T., Peterson, D. E., Rose, A. H., Schubert, M. M., Spijkervet, F. K. & Wittes, J. P. (1999) Validation of a new scoring system for the assessment of clinical trial research

of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer* **85,** 2103-2113.

37. Sonis, S. T., O'Donnell, K. E., Popat, R., Bragdon, C., Phelan, S., Cocks, D. & Epstein, J. B. (2004) The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. *Oral Oncol* **40,** 170-176.
38. Steinberg, J., Halter, J., Schiller, H., Gatto, L. & Nieman, G. (2005) The development of acute respiratory distress syndrome after gut ischemia/reperfusion injury followed by fecal peritonitis in pigs: a clinically relevant model. *Shock* **23,** 129-137.
39. Trotti, A., Bellm, L. A., Epstein, J. B., Frame, D., Fuchs, H. J., Gwede, C. K., Komaroff, E., Nalysnyk, L. & Zilberberg, M. D. (2003) Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiother Oncol* **66,** 253-262.
40. Vera-Llonch, M., Oster, G., Hagiwara, M. & Sonis, S. (2006) Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. *Cancer* **106,** 329-336.
41. Weissberg, J. B., Pillsbury, H., Sasaki, C. T., Son, Y. H. & Fischer, J. J. (1983) High fractional dose irradiation of advanced head and neck cancer. Implications for combined radiotherapy and surgery. *Arch Otolaryngol* **109,** 98-102.
42. Zackrisson, B., Mercke, C., Strander, H., Wennerberg, J. & Cavallin-Stahl, E. (2003) A systematic overview of radiation therapy effects in head and neck cancer. *Acta Oncol* **42,** 443-461.